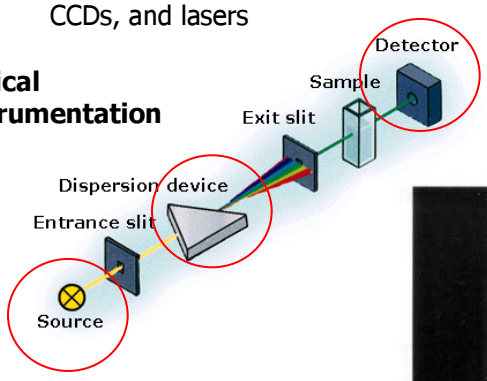
# Welcome to Lecture 7

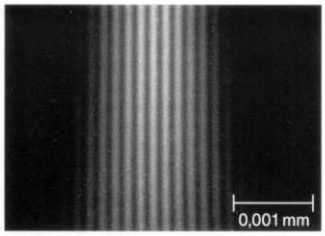
**Last time:**

- Electromagnetic radiation and properties of light
- Spectrometers, spectroscopes, photomultipliers, CCDs, and lasers

**Optical instrumentation**

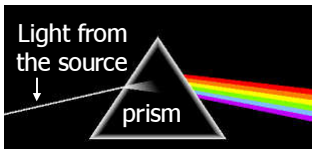


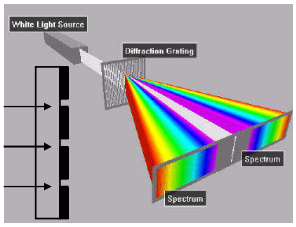

$e^-$ diffraction:

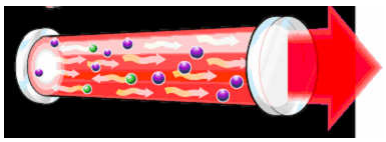



# At the last lecture

Generating a spectrum:



Laser – monochromatic, coherent light

Photomultiplier tube

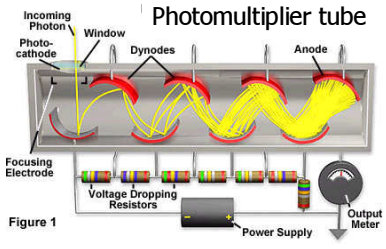
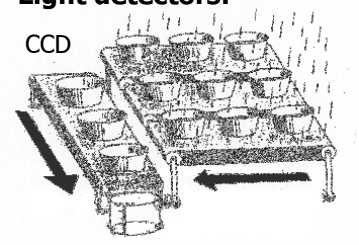



Figure 1

**Light detectors:**

CCD

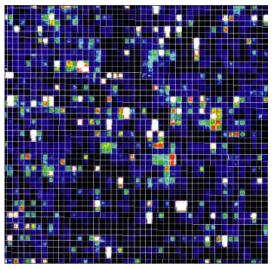


# Plan for today

**Fluorescence**: one of the most important and widely used techniques in modern biology (not just biosensors)


- Fluorescence microscopes
- Flow cytometers

**Genechips**: a massively parallel analysis of e.g. gene expression



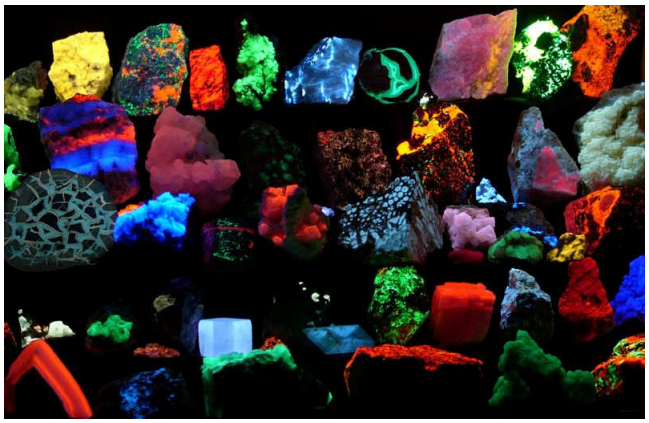
Take a look at a few cool biosensors

**After the break**:

- How colors work
- Homework

# Fluorescence

Fluorescence is a process of light emission by a substance which is being subjected to light irradiation



Fluorescence is named after the mineral fluorite, composed of calcium fluoride, which often exhibits this phenomenon

# Terminology

- **Photoluminescence** – a general term; often used to describes fluorescence and phosphorescence
- **Phosphorescence** – is physically a very different process in which energy accumulated in a substance as a result of illumination is released very slowly and continuously – so-called "glow in the dark" materials
- **Thermoluminescence** – photoluminescence (predominantly phosphorescence) stimulated by the application of heat
- **Chemiluminescence** - fluorescence that results from chemical energy during a chemical reaction (the mechanism is very similar to that used by fireflies)
- **Triboluminescence** - fluorescence that results from scratching or abrading a substance, e.g. when two pieces of quartz are rubbed together light is produced

# Phosphorescence vs fluorescence

The hands of this watch are painted with a phosphorescent ink

**Light**

**dark (1 min)**

**dark (10 min)**

The posters in the room are painted with fluorescent ink

**Normal light**

**UV light**

Only visible when the paintings are illuminated at excitation wavelength

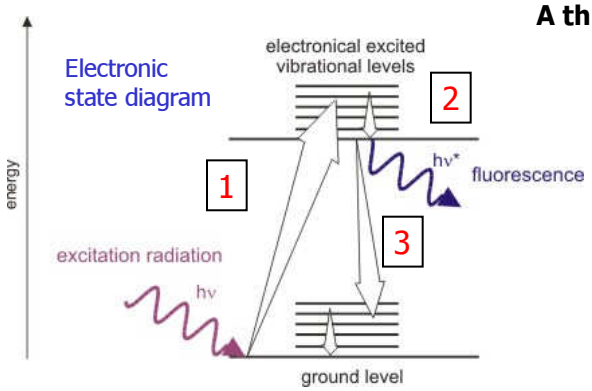
# Physical principle

Fluorescence occurs when a molecule relaxes to its ground state after being electronically excited

Excitation: $S_0 + hv \longrightarrow S_1$

Relaxation: $S_1 + hv \longrightarrow S_0$

where *hν* is a generic term for photon energy; h is Planck's constant and v is frequency of light



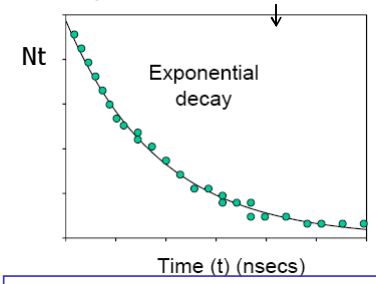

**A three stage process:**

1. The adsorption of light energy (photon) by fluorophore creating an excited unstable state $S_1$
2. Some dissipation of energy as heat; life time of $S_1$<10nsec
3. The emission of a photon and return to the ground state

# Fluorescence lifetime

The fluorescence lifetime refers to the time the molecule stays in its excited state before emitting a photon; typically 1-10 nsec

No of photons emitted at time t




τ is independent of the initial intensity of the emitted light – useful for many applications

Fluorescence typically follows first-order kinetics

$$[S_1] = [S_1]_0 e^{-t/\tau}$$ where

$S_1$ is the remaining concentration of excited state molecules at time t

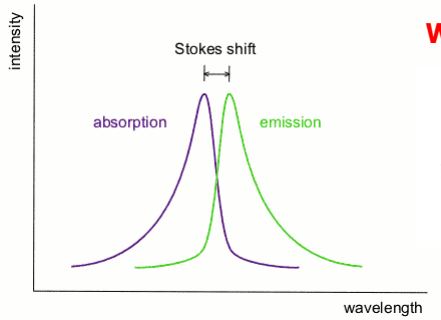
$[S_1]_0$ is the initial concentration of molecules after excitation

t is time and τ is the fluorescence lifetime $\tau = \sum_i k_i$ where

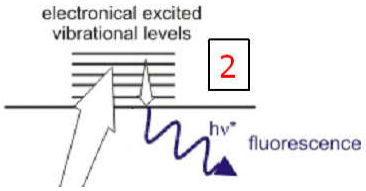
ki are the rates for each fluorescence decay pathway

# Stokes shift

Stokes shift is the difference in wavelength between positions of the adsorption and emission maximum in the spectrum




**Where does the energy go?**




Energy dissipation during the excited-state lifetime

In essence, Stokes shift is the energy difference between the absorbed and emitted photons

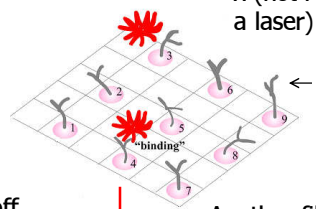
# Instrumentation

**Essential elements of a fluorescence detection system:**

1) Excitation light source
2) A fluorophore
3) Wavelength filters to isolate emission photons from excitation photons
4) Detector to register emission photons and produce a recordable output

Light source (can be a laser)

A filter to generate light of appropriate λ (not required for a laser)




Sample with fluorophore

As in spectrometers lenses and mirrors are used to direct light

Cut off

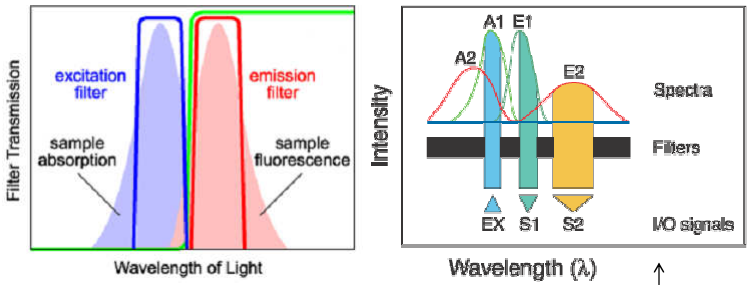
Through

Another filter to cut off the excitation light

Often a CCD for better sensitivity → ← Detector

# Fluorescent filters

- Fluorescence instruments with broadband light source use optical filters to control the spectra of the excitation and emission light
- Filters make it possible for the sample to only "see" the light within the absorption band, and for the detector to see the light exclusively within the emission band

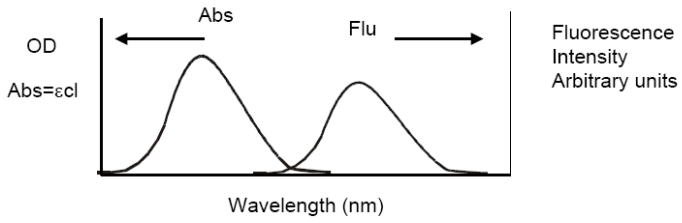



Filters also allow simultaneous detection of several fluorophores: emission E1 and E2 can be quantitatively isolated by optical filters

# Measurements

Fluorescence intensity is measured in arbitrary units

Florescent signal depends on sensitivity of the instrument; hence it must be related to measurements done with an appropriate standard solution i.e. calibration is needed

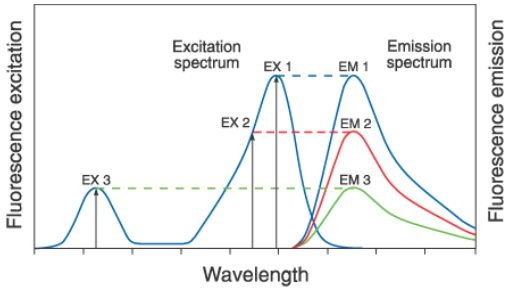



**Typical problems:**

- Light scattering e.g. turbid samples
- Background fluorescence e.g. impurities in reagents (ultra-pure are used) and intrinsic autofluorescence e.g. cells

# Important!

Excitation of a fluorophore at different wavelengths does **NOT change the emission spectrum**



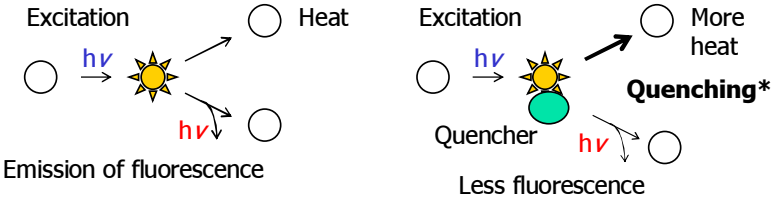

**but it does affect the emission intensity**

**Sensitivity**: Down to ~10 nM i.e. about 100 times more sensitive than absorption spectroscopy

Fluorescence allows the detection of emission photons against low background (excitation photons are cut off), while in absorption spectrometry transmitted light is measured relative to high incident light of the same wavelength

# Quenching

**Not all the excited fluorophores return to So by emission**




**Quenching -** Decrease in the fluorescence intensity of a fluorophore due to e.g.

- Collisions with other molecules
- Chemical reactions in the excited state
- Energy transfer (will discuss later in details)

**Note:** Quenching can be used to generate signals in biosensors

*Very dependent on temp; $O_2$ is a common quencher

# Quantum yield

- A molecule in its excited state, $S_1$, can relax by various competing pathways, e.g. undergo "non-radiative relaxation" in which the excitation energy is dissipated as heat (vibrations) to the solvent
- Relaxation of an $S_1$ state can also occur through interaction with a second molecule through fluorescence quenching e.g. $O_2$ is an extremely efficient quencher of fluorescence

**Fluorescence Quantum Yield**: $\Phi = \frac{\text{N photons emitted}}{\text{N photons adsorbed}}$

The maximum yield is obtained, when every adsorbed photon results in a photon emitted, ie $\Phi = 1.0$ or 100%

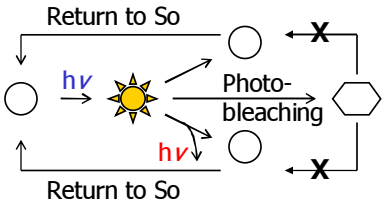
In practice, compounds with quantum yields of 0.10 are still considered quite fluorescent

Typically, the quantum yield does not dependent of the wavelength of exciting radiation

# Photobleaching

**The fluorescence process is cyclical:**

- the same fluorophore can be repeatedly excited and detected
- generation of many detectable photons is fundamental to the high sensitivity of fluorescence detection techniques



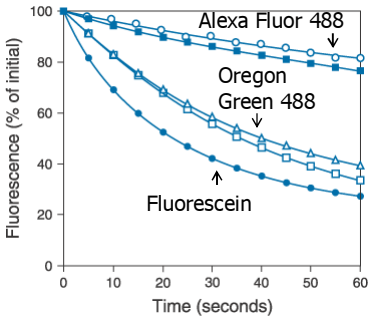

**Photobleaching**: loss of fluorescence due to light-induced chemical damage to fluorophore (especially under under high-intensity illumination)

**Effective remedies**: use less photolabile fluorophores and maximize detection sensitivity (i.e. to reduce the excitation intensity) by employing better detectors such as CCDs

# Photobleaching

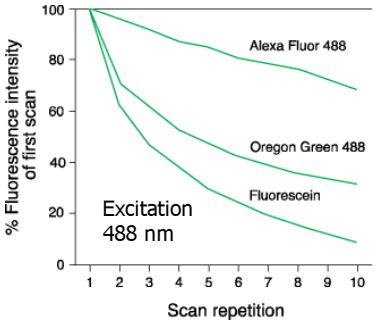
**Photostability of green-fluorescent antibody conjugates**

Under continuous illumination:




Fluorescent goat anti–mouse IgG antibody conjugates were used to detect mouse anti–human IgG

Ten consecutive argon-ion laser scans:




Fluorescent biotin-conjugated anti-CD44 antibodies

Source: Invitrogen

# The choice of fluorophore

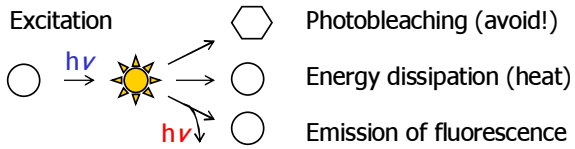
**Critical for the development of sensitive and selective sensor**

- High quantum efficiency and high extinction coefficient – high intensity of fluorescence
- A large Stokes shift – easy separation of excitation and emission signals
- Insensitivity to quenching and photobleaching
- Excitation and emission wavelength should be appropriate for the light source/detector to be used
- Suitable fluorophore functionality to allow for labeling through covalent attachment, if needed
- Minimal perturbation of biological function

**Good news: all this is well-known in the literature**

# Advantages of fluorescence

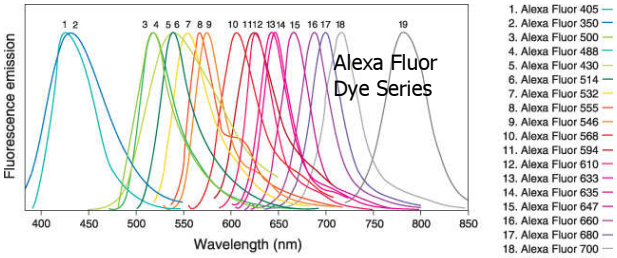
- High sensitivity
- Extra selectivity – only the fluorophore is detected i.e. reduction in the background signal due to non-specific adsorption, etc
- Well-established biochemical assays (available and tested reagents, protocols, etc) and hardware
- Can be easily adapted to the analysis of analytes at or near the interface with transducers (we will talk about this later)



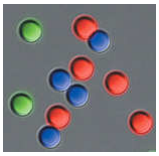

# Fluorescent reagents

Usually absorbtion spectrum is in the (near)ultraviolet range, and the emitted light is in the visible range, although it depends on the properties of a particular fluorophore

**Low MW dyes:**




**Polymeric particles:**



**Semiconductors**: UV light induced fluorescence in vials containing Cadmium selenide (CdSe) quantum dots

# Anthrax biosensor

QTL BIODETECTION

**BIOSENSOR 2000**

**Handheld Detector for Biological Agents**

**Patented Technology**

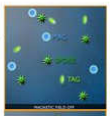
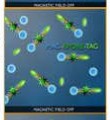
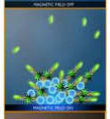
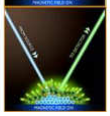
| | |
|---|---|
| Assay type: | Immunoassay |
| Time to answer: | 5 minutes |
| Operating range: | 36° - 104° F (2° - 40° C) |
| Sensitivity: | Anthrax < *10,000 spores*<br>Ricin < *1 ng*<br>SEB < *1 ng*<br>Botulism < *10 ng* |
| Features: | Wet or dry samples<br>Positive and negative control cartridges<br>Non-destructive test, sample is retained<br>Chemistries can be integrated to detect other bio-agents<br>24/365 Support<br>1 hour training |

# Anthrax biosensor: specification

**Instrument**

| | |
|---|---|
| **Weight** | 6 lbs (2.7kg) |
| **Size** | 11" x 10" x 5" (27 x 25 x 14 cm) |
| **User interface** | LCD Screen, 4 button interface<br>On-screen operation instructions<br>Visual and audible alarms |
| **Housing** | Fully deconable<br>Waterproof and dustproof<br>IP 67 certified |
| **Power** | Battery (50+ tests)<br>AC or vehicle supply |
| **Data Storage** | 100 test results including:<br>◆ target type<br>◆ cartridge serial # |
| **Connectivity** | Wireless or serial port |

# Anthrax biosensor: operation

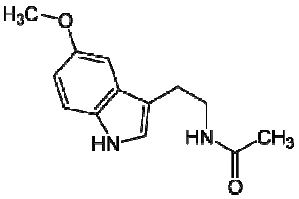
| STEP | | BENEFIT |
|---|---|---|
| 1. **MIX** Sample is mixed with the QTL Sensing Solution. |  | The QTL Bioassay incorporates highly fluorescent particles in the assay that have superior brightness relative to many commercially available materials. |
| 2. **BIND** Sensing materials bind to target during incubation. |  | Several QTL Bioassays incorporate proprietary antibodies that show high binding along with superior specificity relative to their commercially available counterparts. |
| 3. **MAGNETIZE** All bound and unbound magnetic material is pulled to surface. |  | This step effectively concentrates the sample for subsequent detection. |
| 4. **WASH** All remaining sensing and non-target material is washed away. |  | False positives are virtually eliminated by removing potential interferants from the sample solutions. |
| 5. **READ** Concentrated sample (pellet) is illuminated and emits a signal if target is present. |  | QTL's exclusive Dynamic Surface Generation technology effectively binds, concentrates and isolates the sample before detection - resulting in superior sensitivity to other immunoassays. |

# Cool biosensors

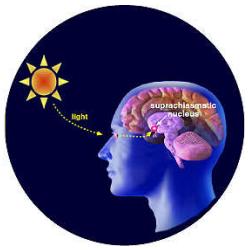
- A biosensor for monitoring water quality in spaceships
- Sleep-o-meters to warn drivers: to detect rising melatonin
- Allergy-meters (histamines and peanut allergy)
- Mood and ambitiousness meters
- A biosensor to detect ovulation
- Low progesterone biosensor in early pregnancy
- The reason for baby crying and breast milk quality sensors
- Early detection of cancer
- A biosensor for detecting metastases in cancer patients
- Biosensors for other medical conditions e.g. heart attack, cholesterol plaques epilepsy, detection of malaria, TB, etc

# Melatonin

Melatonin is a hormone found in most animals, including humans. It regulates the circadian rhythms and some other physiological functions; produced in the brain by the pineal gland from Trp




Circulating levels of melatonin vary in a daily cycle: when the brain is stimulated by daylight, melatonin production is suppressed i.e. **light out - melatonin on**




**The idea:** by measuring rising/high level of melatonin we can warn drivers to stop and have a cup of coffee

**Let's explore a few possible designs for such a sensor**

# Whole cell melatonin sensor

Lower vertebrates (e.g. fish, amphibians) have special pigmented cells capable of quick color changes

These cells are responsible for rapid color changes triggered by hormonal stimulation: important for sexual signaling camouflage, UV light protection

**and they also respond to melatonin stimulation**

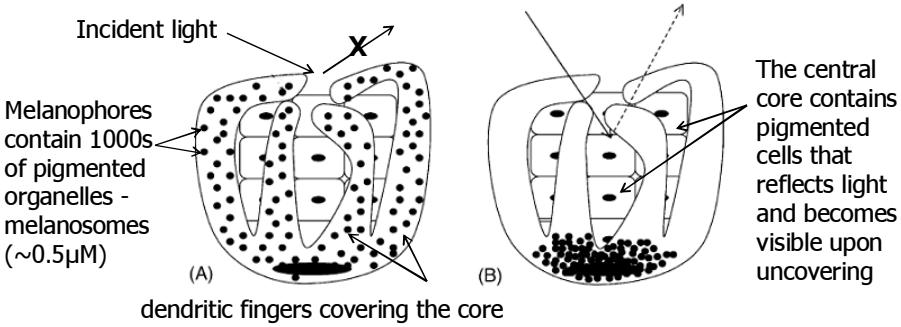

Pigmentary patterns of the pencilfish:

A: the daytime pattern; the three characteristic longitudinal stripes

B: the nighttime pattern

# Melanophores

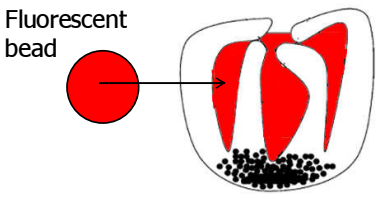
Dark pigmented cells found in the skin of some frogs

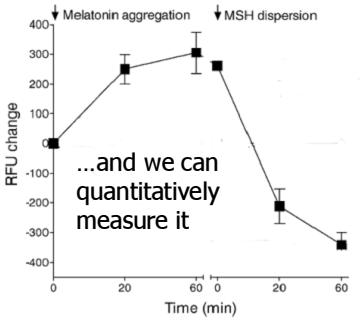



- Due to dark color and size melanosomes efficiently absorb and scatter light
- Quick color changes of the skin by hormones is possible due to melanosome dispersion (A) or aggregation (B) that covers and uncovers the central core – this can be induced by melatonin

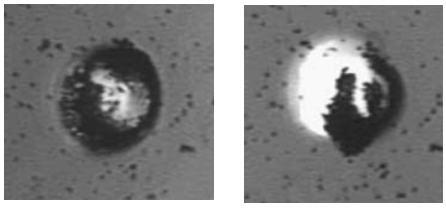
# Melatonin biosensor

Suppose now we grow Melanophores on fluorescent beads




Then on melatonin stimulation there will be a change in surface exposure…



Resting melanophore

Melatonin-induced aggregation

In Xenopus frog melatonin induces aggregation, and α-melanocyte stimulating hormone (MSH) dispersion – both acting through GPCRs

Biosensors and Bioelectronics 21 (2005) 111–120

# Is this practical?

Probably not for use in the car, but there are plenty of other methods e.g. electrochemical detection and ELISA (monoclonal Abs with Kd ~$10^{-11}$M were obtained)

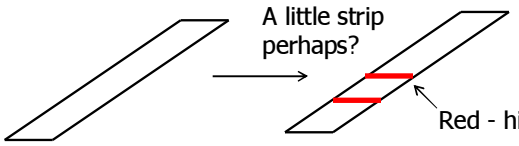
Plasma concentration is ~50 pM

**But would you like to prick your finger to find out whether you are sleepy or not?**

**Good news**: This may not be necessary ☺

As many other plasma metabolites melatonin is also present in saliva

**Sleep-o-meter?**


**Why did I draw two bands?**

# Is a sleep-o-meter feasible?

Technically - yes, but there are other problems

**Any ideas what these might be?**

**Individual Differences in the Amount and Timing of Salivary Melatonin Secretion**

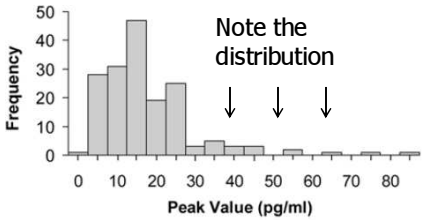
The melatonin profiles were derived from saliva samples from 170 healthy people aged 18–45 years

| | |
|---|---|
| Sex | 85 men, 85 women |
| Hormonal birth control | 16 hormonal birth control, 69 no hormonal birth control |
| Menstrual phase[a] | 34 follicular, 33 luteal, 2 unknown |
| Race | 104 White, 32 Asian, 23 Black, 11 Other |
| Ethnicity | 14 Hispanic, 156 non Hispanic |
| Education | 3 high school, 68 some college, 44 college graduate, 44 higher degree, 11 unknown |
| Employment | 35 unemployed, 116 student and/or part time work, 19 full time work |
| Living condition | 37 alone, 133 not alone |
| Bed partner | 41 bed partner, 129 no bed partner |
| Season | 40 spring, 88 summer, 20 fall, 22 winter |
| Eyeglasses | 61 wore eyeglasses, 68 no eyeglasses, 41 unknown |
| Contact lenses | 55 wore contacts, 74 no contacts, 41 unknown |
| Sunglasses | 93 yes sunglasses, 14 no sunglasses, 63 unknown |
| Caffeine in saliva | 25 had caffeine in saliva, 145 had no caffeine in saliva |

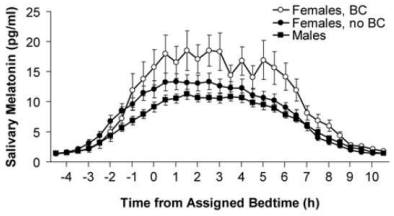
Burgess HJ, Fogg LF (2008) PLoS ONE 3(8): e3055

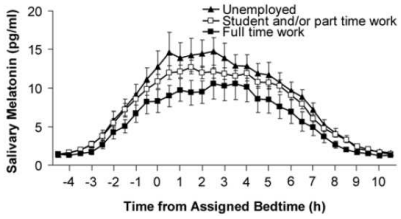
# The findings

- Sex and menstrual phase were not significantly associated with any of the melatonin parameters, but hormonal birth control was associated with a higher peak value
- Increased weight was associated with a lower peak value and a lower AUC
- Race, ethnicity (NIH classification), education level and living condition were not associated with any melatonin parameter




- However, there were effects of employment status - Increased levels of employment were associated with reduced AUC



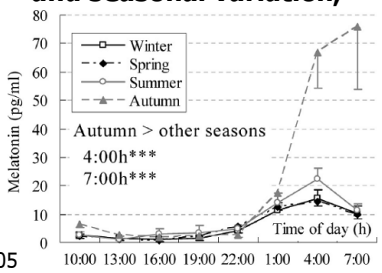

# And there is more

## Laughter elevates the levels of breast-milk melatonin and seasonal variation,

Table 1
Effect of laughter on the levels of breast-milk melatonin

| | Melatonin (pg/ml) | | |
|---|---|---|---|
| | Healthy mothers | | |
| | | Viewing | |
| | No viewing | Control DVD | Humorous DVD |
| 2200 h | 5.2 (0.3) | 5.3 (0.3) | 7.1 (0.4)* |
| 2400 h | 8.4 (0.4) | 8.2 (0.4) | 10.2 (0.6)* |
| 0200 h | 15.6 (0.8) | 15.9 (0.9) | 20.8 (1.0)* |
| 0400 h | 12.2 (0.7) | 12.5 (0.8) | 16.5 (0.8)* |
| 0600 h | 5.5 (0.4) | 5.1 (0.4) | 6.3 (0.4)* |

Values are shown as mean (SE). *Significant increase P<0.05




- Forty-eight infants aged 5–6 months
- Their mothers viewed either an 87-min humorous DVD (Charlie Chaplin) or non-humorous weather information at 22.00
- After viewing, breast milk was collected

Kimata (2007) Psychosomatic Res 62, 699– 702

Eight Japanese women: Six showed big effect, two - none

**and stress, and mood, and disease** e.g. significant down-regulation in melatonin secretion in depressive patients during the acute phase of illness

# Sleep-o-meter

Mood and ambitiousness meters – same problem? A genetic ambition meter would probably work better, if there is a gene ☺

**Business argument?**

- There are about 200mln drivers in the U.S. (my estimate)
- Selling a packet of saliva sensors to 5% of them once a year – 10 mln customers
- If the company can make $2-3 on the sale – $20-30mnl pa in U.S. alone (more $$$ can be made internationally)

**If you think, you got a good idea**

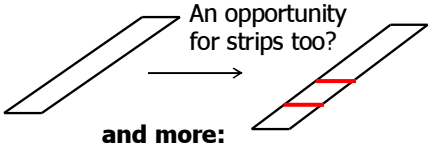
- Patent – Poly can help
- Capital – Poly can help and there are grants available
- Lab space – Poly can help e.g. Technology Incubator

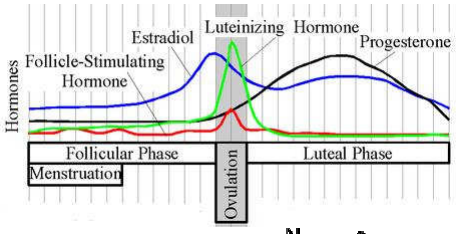
# Non-invasive sampling

Evaluation of saliva collection devices for the analysis of steroids, peptides and therapeutic drugs

Michael Gröschl[a,*], Henrik Köhler[a], Hans-Georg Topf[a], Thomas Rupprecht[b], Manfred Rauh[a]

- **Low progesterone biosensor** in early pregnancy - saliva or urine
- **A biosensor to detect ovulation**
  LH can be detected in urine




**and more:**

- **Allergy-meters based on detection of histamine**

  Mediator of inflammation – released by basophiles (leukocytes) and mast cells in response to allergen


Also in saliva

Journal of Pharmaceutical and Biomed Analysis 47 (2008) 478–486

# Allergy-o-meter

**Detection of histamine:** ✓ Fluorescence e.g. o-phthaldialdehyde

✓Electrochemical e.g. histamine oxidase

✓Other proteins from parasites e.g. ticks

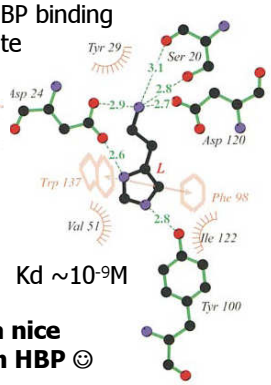
✓ Antibodies: ELISA and other methods

- Unlike insects, many ticks remain attached to their hosts for a long time (days to weeks) sometimes increasing their body weight >100 times
- Ticks' saliva contains high-affinity histamine-binding proteins (HBPs) which help them to continue feeding
- HBPs sequester histamine at the wound site, outcompeting histamine receptors for the ligand, thereby overcoming their hosts' inflammatory responses

Paesen et al (1999) Mol Cell 3, 661–671

tick

HBP binding site




Kd ~$10^{-9}$M

**Can make a nice sensor from HBP** ☺

# Breast milk sensors


Alcohol in breast milk

**What do we want to measure?**

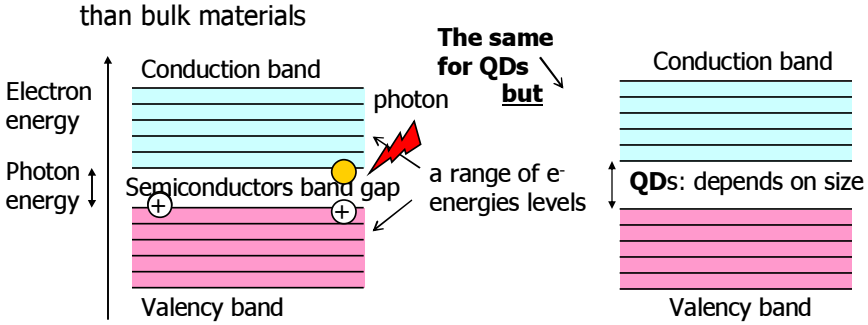
- Bacterial contamination
- Pesticides and pollutants

**Mastitis** – mammary gland inflammation that often occur on breast feeding

- ✓ The most common bacteria causing mastitis (~30%) is called *Staphylococcus aureus*
- ✓ Mastitic milk is known to degrade $H_2O_2$ at a higher rate due to increased catalase activity – electrochemical or optical sensor?
- ✓ Lactate dehydrogenase (LDH) activity is increased – electrochemical?
- ✓ Elevated C-reactive protein (CRP), a biomarker of inflammation, in milk – colorimetric ELISA?
- ✓ The increased excretion of lactose in urine is a predictive biomarker of developing mastitis – lacZ biosensor?

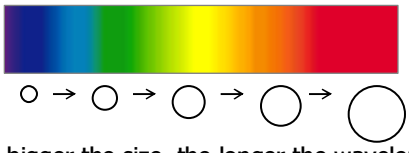
# Quantum dots

- Quantum dots are fluorescent semiconductor nanocrystals composed of inorganic materials e.g. CdSe, ZnS, InP; typical size is 2-20nm in diameter (10s or 100s of atoms)
- At this size materials' properties are often different from that displayed in bulk
- Unique properties – small nanocrystals behave more like atoms than bulk materials



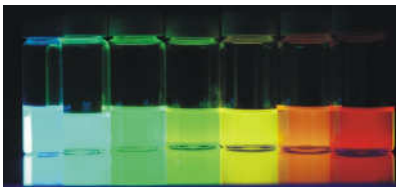

# Quantum dots

For the same material the maximum of fluorescent emission of QDs depends on the crystal size

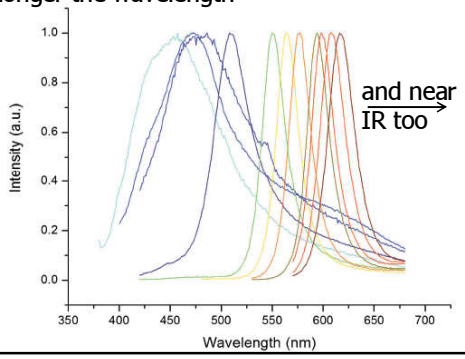


The bigger the size, the longer the wavelength

2.3nm → 5.5nm

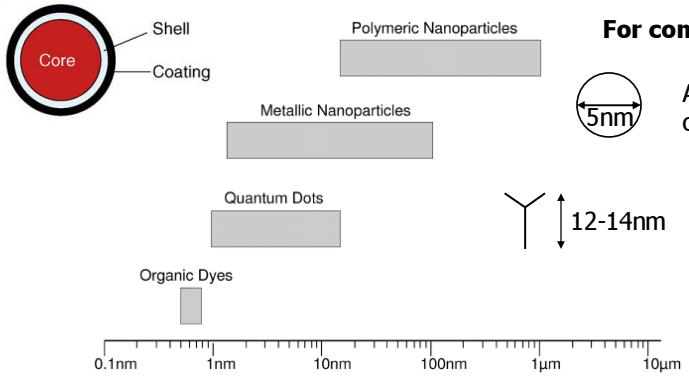


UV-excited CdSe quantum dots



# Nanotechnology

Size range of commonly used fluorescent nanoprobes




**For comparison:**

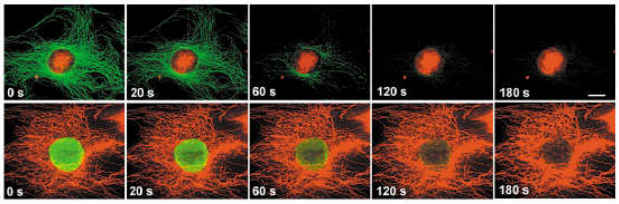
5nm — A globular protein of ~50KDa MW

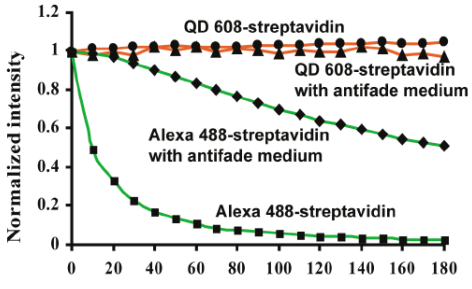
12-14nm — Antibodies

- Tunable emission
- Excellent quantum yield
- Excitation in UV – very large Stokes shift and opportunity for multi-probe/color analysis

# Photobleaching of QDs

Photostability comparison between QDs and Alexa 488



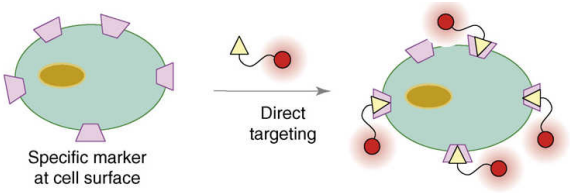




Top row: Nuclear antigens were labeled with QD 630 (red), andmicrotubules were labeled with Alexa 488 conjugated to IgG (green)

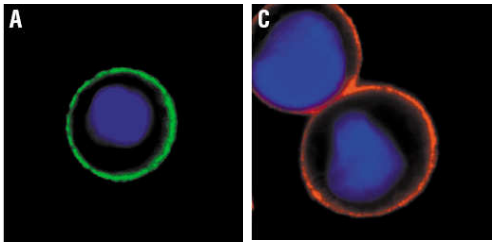
Bottom row: Microtubules were labeled with QD 630– (red), and nuclear antigens with Alexa 488 IgG conjugate

# Targeting QDs

Labeling of cancer cells with Ab-QD conjugates




HER-2* on the surface of human breast cancer cells




Wu et al (2003) Nature Biotech 21, 41

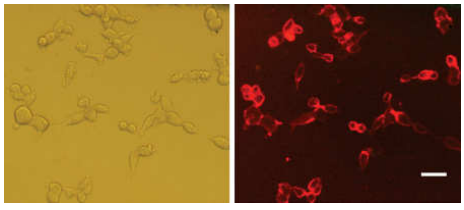
QD 535 and QD 630 (emission maximum 535 and 630nm, respectively) conjugated to IgG

*HER-2 is a cancer marker over-expressed on the surface of some breast cancer cells

**Biosensor for cancer detection?**

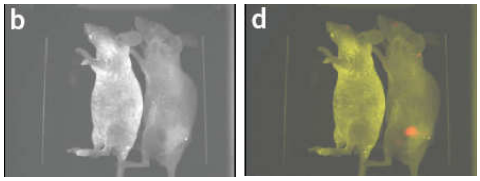
# QDs in cancer imaging

**QD-PSMA* Ab binding activity in cultured prostate cancer cells**

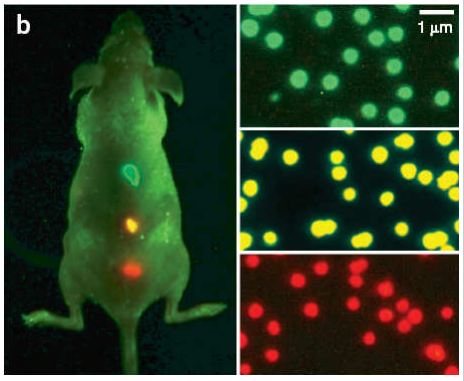


Bright field Fluorescence

**QD-PSMA Ab conjugates in live animals tumor xenografts**




**Multi-color QD imaging of cancer**

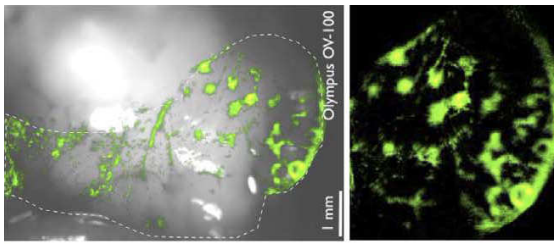



*Prostate-Specific Membrane Antigen expressed on the surface of prostate cancer but not normal cells

Gao et al (2004) Nature Biotechnology 22, 969

# Early detection of cancer

- Fluorescent imaging of early stage Pancreatic Ductal Adeno-carcinoma (PDAC)
- Poor prognosis due metastasis found at the time of diagnosis
- Oligopeptides* highly selective for plectin-1 (biomarker of PDAC)



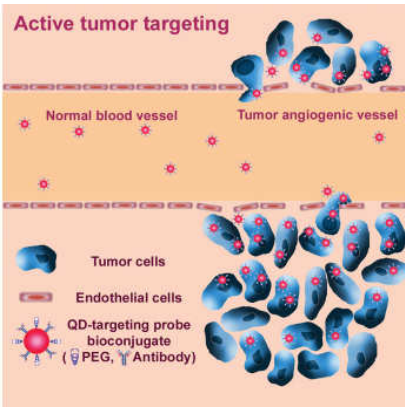

Low-magnification view of pancreatic fluorescence shows distribution of the label in distinct areas of the pancreas; dotted line outlines the pancreas

Kelly et al (2008) PloS v5, April 2008

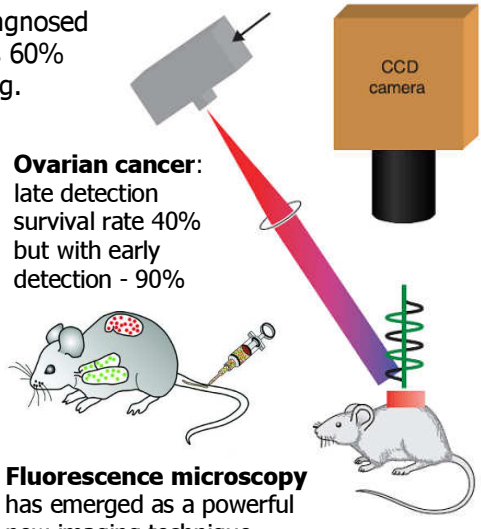
*identified using phage display

# Metastasis

By the time patients are diagnosed with many types of cancers 60% already have metastases e.g.




**Ovarian cancer**: late detection survival rate 40% but with early detection - 90%




**Fluorescence microscopy** has emerged as a powerful new imaging technique

# Molecular Imaging and cancer

In cancer diagnostics precise imaging technology is critical; it can provide more information than sensors:

- Where is the tumor located?
- How large is it?
- Are there metastases and, if so, where?
- Any critical anatomical changes that may influence the treating strategy

Molecular imaging provides an opportunity to transform the management of cancer, e.g.

- Early detection
- Molecular information of cancerogenesis
- Evaluation of treatment efficacy

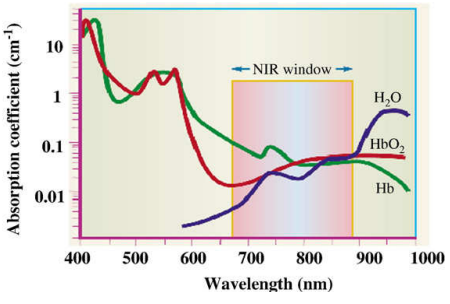
# NIR Imaging

Light in UV–Vis spectral range is strongly absorbed by tissues, thus limiting penetration

Near-infrared light of 650 to 900 nm achieves the highest tissue penetration due to minimal absorbency of the surface tissue in this spectral region

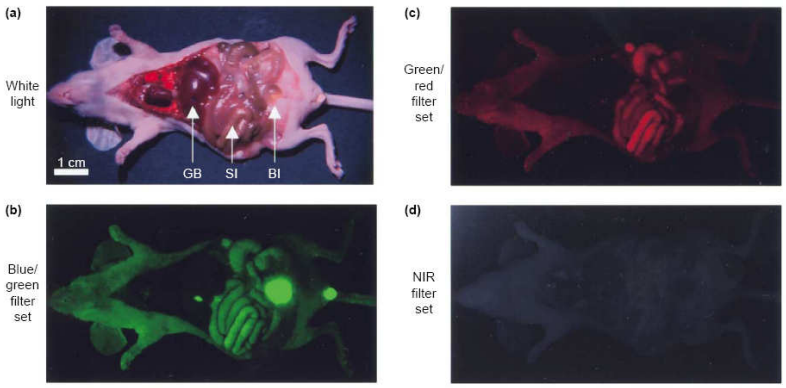
QD emission spectrum can be adjusted to near IR

Auto-fluorescence of tissues - another problem in optical imaging is also reduced in NIR




Hemoglobin and water, the main absorbers of visible light and IR light in tissues have their lowest absorption coefficients in the NIR region

# Tissue auto-fluorescence




Tissue autofluorescence was then imaged using three different sets of excitation/emission filters: (b) blue/green (460–500 nm/505–560 nm); (c) green/red (525–555 nm/590–650 nm); and (d) NIR (725–775 nm/790–830 nm). Fluorescence images have identical normalization. Arrows mark the location of the gallbladder (GB), small intestine (SI) and bladder (BI)

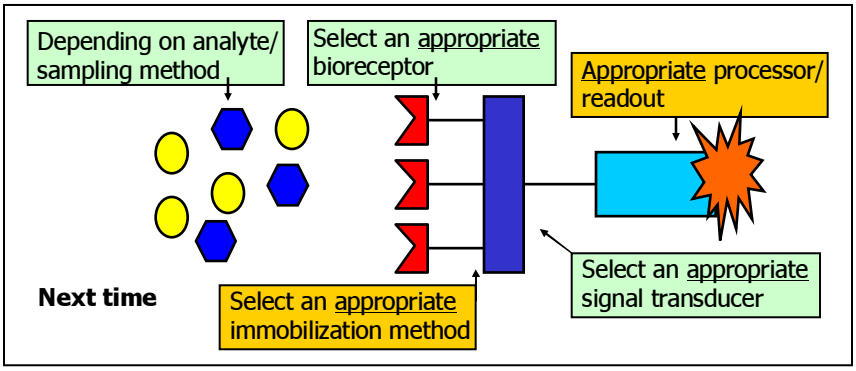
Current Opinion in Chemical Biology 2003, 7:626–634

# Your cool biosensors

- A biosensor for monitoring water quality in spaceships
- Sleep-o-meters to warn drivers: to detect rising melatonin
- Allergy-meters (histamines and peanut allergy)
- Mood and ambitiousness meters
- A biosensor to detect ovulation
- Low progesterone biosensor in early pregnancy
- The reason for baby crying and breast milk quality sensors
- Early detection of cancer
- A biosensor for detecting metastases in cancer patients
- Biosensors for other medical conditions e.g. heart attack, cholesterol plaques epilepsy, detection of malaria, TB, etc

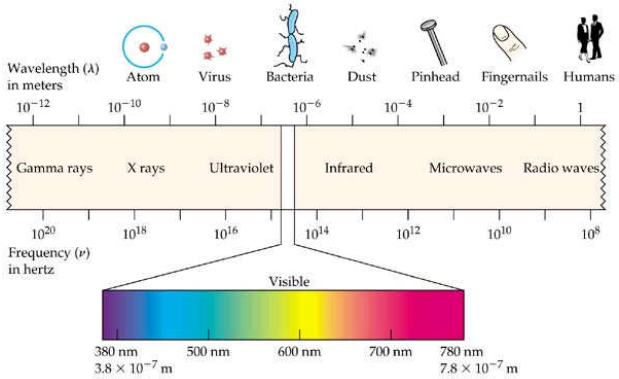
**Issues**: Real [medical] need and human variability

# What does it take to make it?




- Glucose biosensors
- Label-free biosensors e.g. SPR and other
- Energy transfer biosensors
- Nucleic acid-based sensors
- Biochips and lab-on-the-chip technology
- AFM and microcantilevers

# Colors



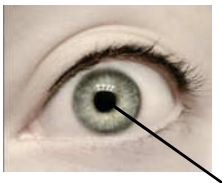

**What wavelength is black light?**

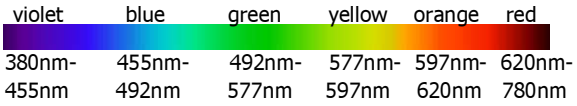
black  black

Linguistics: it has no physical meaning

# How colors work

**What color light does this apple adsorb?**






White light

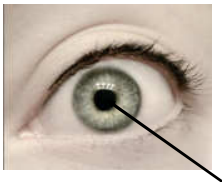
Adsorbed: violet, blue, green and yellow

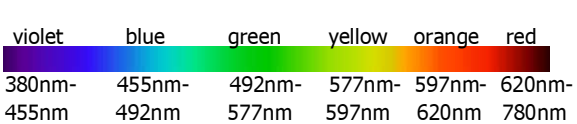
Reflected: red

**OK, let us try another one**

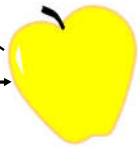
# How colors work

**What color does this apple adsorb?**






White light



Adsorbed: blue and violet

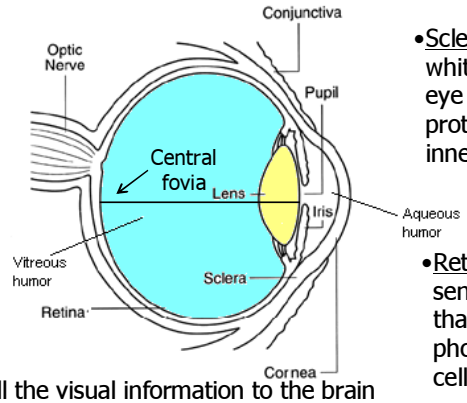
Reflected: red, orange, yellow, green

**Would you believe me if I told you that this apple may actually adsorb only blue and violet light?**

**Why?**

# Eye structure

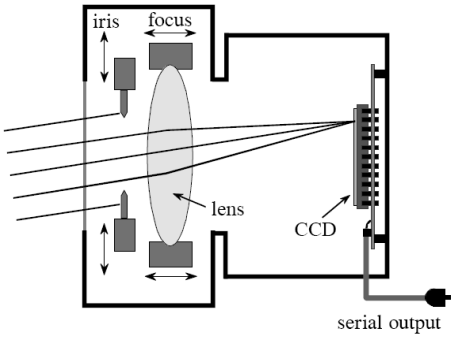
- Cornea: the transparent layer at the front of the eye
- Lens: focuses light onto the retina; can change shape to improve focus
- Iris: controls the amount of light that entering the eye; changes size depending on the light intensity

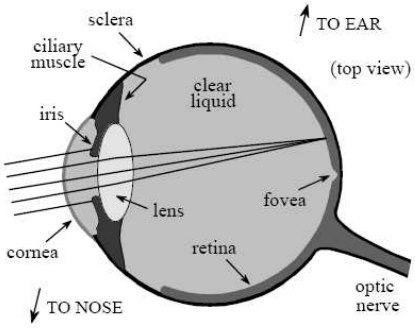



- Sclera: the outer white part of the eye that protects the inner structures
- Retina: the light sensitive layer that contains photoreceptive cells
- Optic nerve: transfers all the visual information to the brain
- Conjunctiva: a transparent vascular membrane lining the inside of the eyelids and extending over the sclera in front
- Aqueous humor: a fluid that circulates in the front part of the eye; provides nourishment and helps maintaining the eye pressure
- Vitreous humor: the clear gel in the centre of the eye; maintains the shape

# Eye vs camera

The same basic design ☺

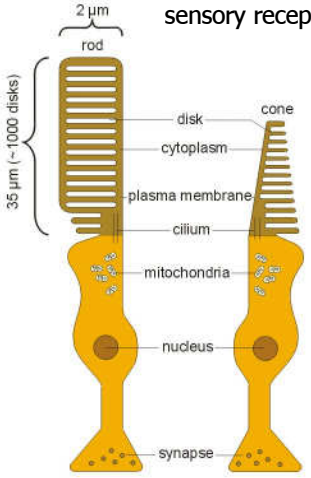


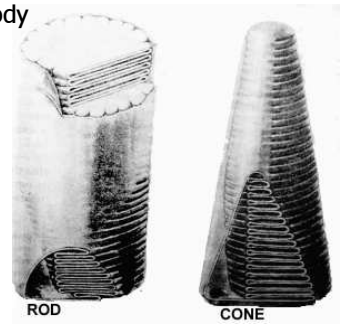



# Photoreceptor cells

Vision is due to the absorption of light by photoreceptor cells on the retina

Vertebrates have two kinds of photoreceptor cells of distinctive shape and function – **rods** and **cones**; together they account for ~70% of all sensory receptors in the body



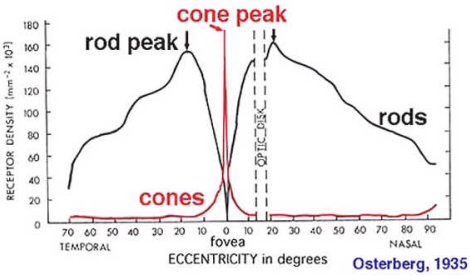

The rods and cones are "polarized" i.e. have distinctly different architecture and function at each end. The outermost part consists of numerous stacks of folded membrane with light-sensitive receptors

# Rods and cones

Cones require a relatively high level of light to be stimulated; so they only function in bright light (during the day) and are responsible for color vision

Rods are much more sensitive to light; they function in dim light and do not distinguish color i.e. rods "see" at night but only in black and white

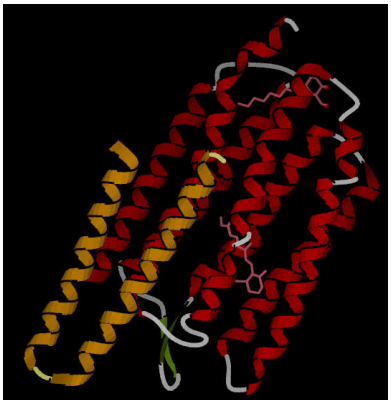
- Rods and cones have different functions in vision and their relative numbers are partly linked to whether an animal is more active during the day or night
- A human retina contains about 125 mln rods and 6 mln cones
- The rods and cones are arranged in such a way as to produce the best possible combination of night and day vision



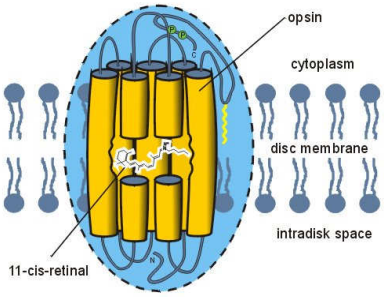

- The fovea (central part) is responsible for sharp central vision – critical for any human activity, where visual details are of primary importance

# Rhodopsin

Rhodopsin is a protein in the photoreceptor cells membrane that catalyses the only light sensitive step in vision



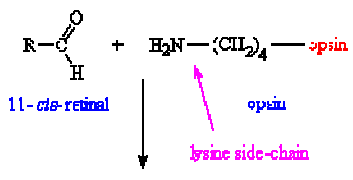
It is a GPCR with typical seven trans-membrane domains

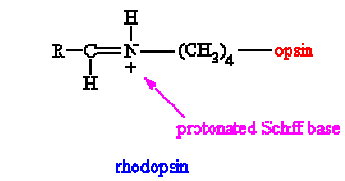


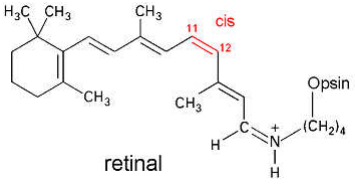

The photoreceptor cells are, in effect, signal transducers - they transduce the absorption of light into an electrical signal

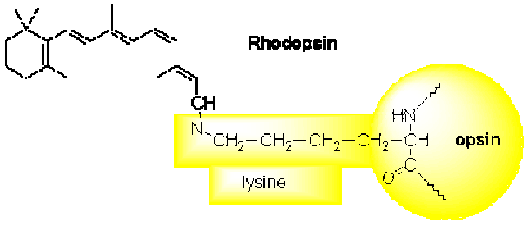
# Retinal

- The chromophore, 11-cis-retinal, seats in a pocket of the Rhodopsin approximately half way into the membrane
- It is covalently attached to the protein via Schiff base



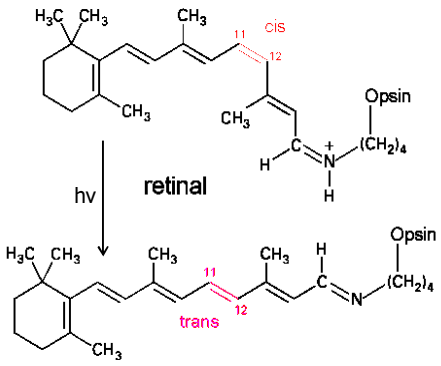



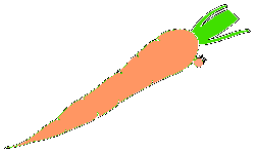



# Chemistry of photoreception

**Retinal cis-trans isomerization**



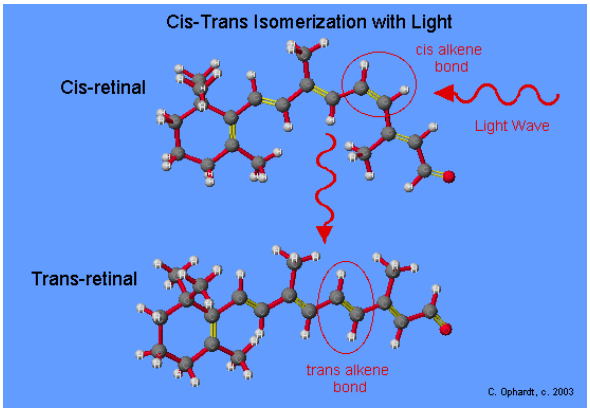

- Vitamin A (retinol) is a precursor of retinal
- A diet deficient in vitamin A can result in night blindness



The isomerization of retinal leads to a conformational change in rhodopsin that triggers a sequence of reactions which eventually lead to a nerve impulse going to the brain.

# Retinal isomerization

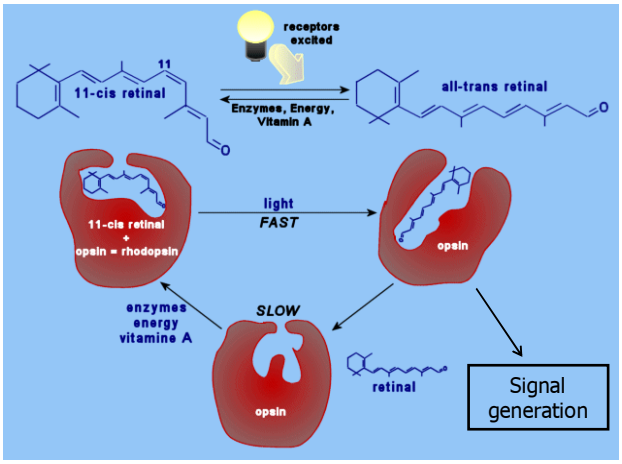
- In the cis-retinal, the hydrogens (light gray) are on the same side of the double bond (yellow).
- In the trans-retinal, the hydrogens are on opposite sides of the double bond. In this isomer all double bonds are trans




Note how the change in the shape of the molecule as a result of this isomerization - from an overall bent structure to a more or less linear one

This is critical for the subsequent chain of signal transduction events

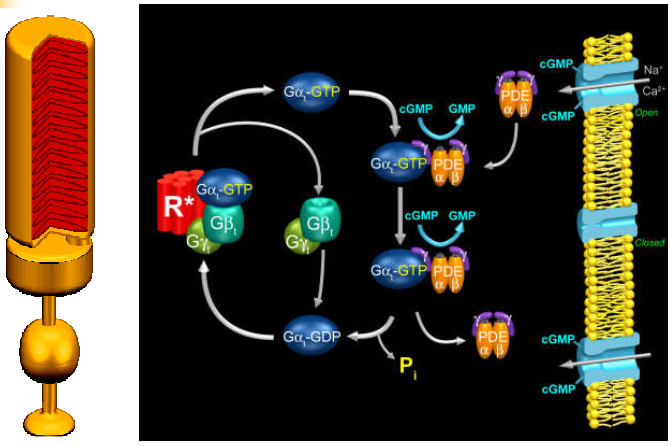
# Rhodopsin cycle




**Briefly**:

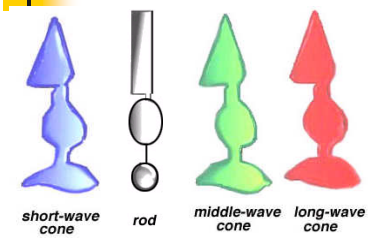
- Conformational change triggers a cascade of enzymatic reactions
- Retinal dissociates from Rhodopsin and is regenerated enzymatically
- As soon as cis-retinal binds back, Rhodopsin is ready to get excited again

# Signal transduction




G-protein Transducin is activated by a conformational change in Rh – exchange of the bound GDP for GTP and dissociation of activated α-subunit. Gα activated cGMP Phosphodiesterase (PDE), which hydrolysis cGMP, an intracellular second-messenger. Decrease in [cGMP] leads to the closure of cGMP-regulated $Na^+$ and $Ca^{2+}$ ion channels - hyperpolarized membrane potential i.e. signal transduction

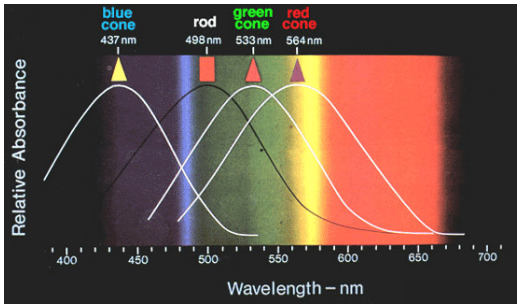
# This chemistry is "color blind"

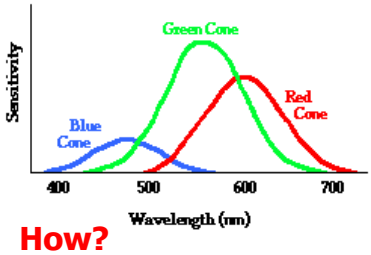



**How do we see colors then?**

In fact, we have three types of cones – blue, green and red

Also, the sensitivity of the three types of cons is different too…

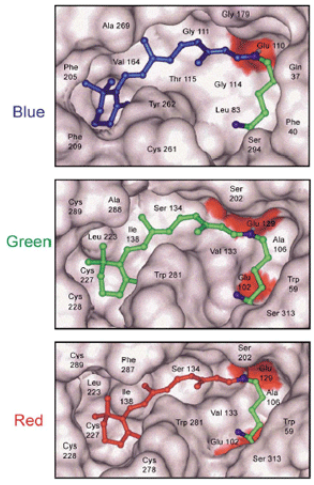


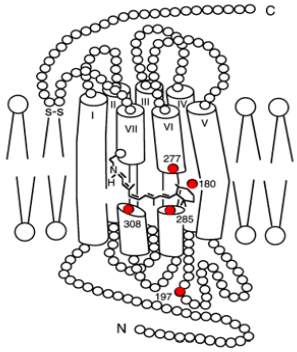




**How?**

# Rhodopsin and color vision

Most mammals with color vision has three different cones of long (L red), medium (M green) and short (S blue) wavelength sensitivity



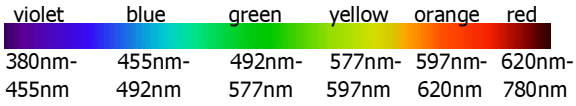




Five sites involved in the differentiation of MWS and LWS pigments.

The wavelength sensitivity is due to minor differences in the opsin sequence affecting side chain groups in the retinal pocket

# Color vision

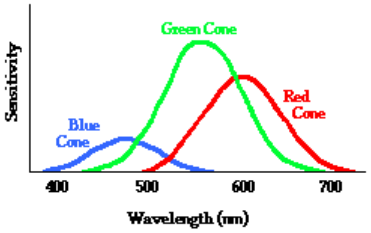
- When we look at a light source containing the full range of frequencies within the visible spectrum (white light), all three types of cones are actively sending messages to the brain. The brain detects the messages and interprets this to mean "white light" i.e. "white color"




- When we close the eyes and no light gets through all three types of cones are "silent" and the message to the brain - "no light" or "black color"

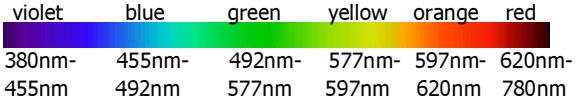
# It gets a little more complicated

- Each cone is sensitive to a range of wavelengths, e.g. the red is not only activated by wavelengths of red light, but also by orange, yellow and some green too
- The green cone is most sensitive to wavelengths of light associated with the color "green" (max at 533nm). Yet it can also be activated by wavelengths of light associated with the yellow and blue colors




- Similarly, the blue con can see "green"

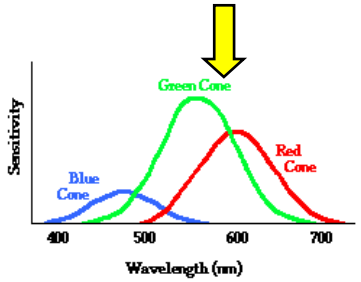
**How do we know which color is which then?**



# …and confusing ☺

Suppose light in the yellow range (~577-597 nm) enters the eye. It would activate both the green and the red cones and they would send signals to the brain

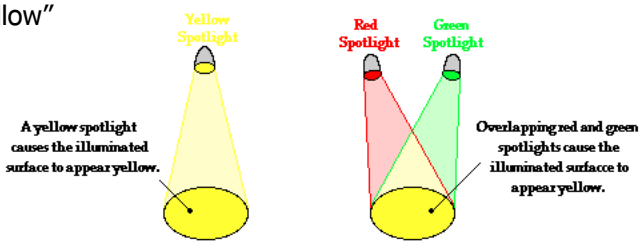
The brain recognizes the activation of red and the green cones and somehow interprets this to mean that the object is yellow. Thus, for the brain yellow color is a result of simultaneous stimulating of the red and the green cones by yellow light




**If so, what would happen if two overlapping red and green lights entered our eye?**

# Humans don't get the difference

Red light would mostly activate the red cones and green light - the green cones, each sending their usual electrical messages to the brain. But the brain knows that these two signals combined mean "yellow"

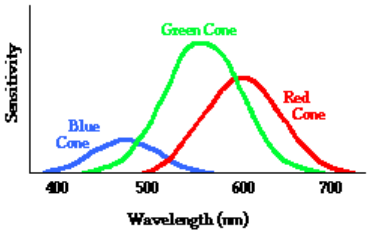



The human eye-brain system there is no difference – it responds in exactly the same way to yellow light as to mixture of red and green

**The brain has no means of distinguishing between the two!**

# What is “color’ then?

- Hence, color is just a mental response to light of a particular frequency or set of frequencies entering in the eye
- The correspondence of specific range of frequencies to a specific color is nothing more than a “name” we, humans, gave it




| violet | blue | green | yellow | orange | red |
|---|---|---|---|---|---|
| 380nm-455nm | 455nm-492nm | 492nm-577nm | 577nm-597nm | 597nm-620nm | 620nm-780nm |

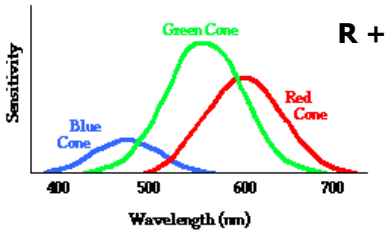
If we could talk to bees (they see further in UV) understanding each other would be pretty difficult ☺

# Color vision: the human thing

- Physically, there is no such thing as yellow light but there is light with a wavelength of about 590 nm which we, humans, call yellow. There is also light with a mixture of wavelengths of about 700 nm and 530 nm which together appears as yellow to us too. So, what is “yellow”?
- Technically it is inappropriate to refer to light as being “colored”. It is simply a wave (photons ☺) with a specific wavelength or a mixture of wavelengths. An object which is emitting or reflecting light into our eyes only appears to have a specific color as a result of the brain response to a particular frequency range
- The color of objects that we see is largely due to the way these objects interact with light - reflecting or transmitting it into our eyes - **It is NOT in the object itself, it is in our MINDS**

# Color perception

If white is not a "color" but presence of the whole visible spectrum, perhaps we can make "white" in some other way?




**R + G + B = W**

In fact, we can by simply combining red, green and blue

- "White" can be produced by combining only three distinct frequencies of light, provided that they are widely separated on the visible light spectrum
- Any three colors (light frequencies!), when combined with the right, intensity are called **primary colors of light**

# Addition of colors

And by mixing together (adding) these primary colors with varying intensities we can get a wide range of other colors

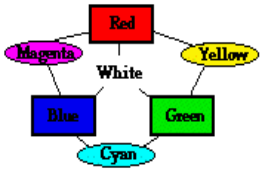
R + G = Y  R + B = M  G + B = C  R + G + B = W

Yellow (Y), magenta (M) and cyan (C) are secondary colors

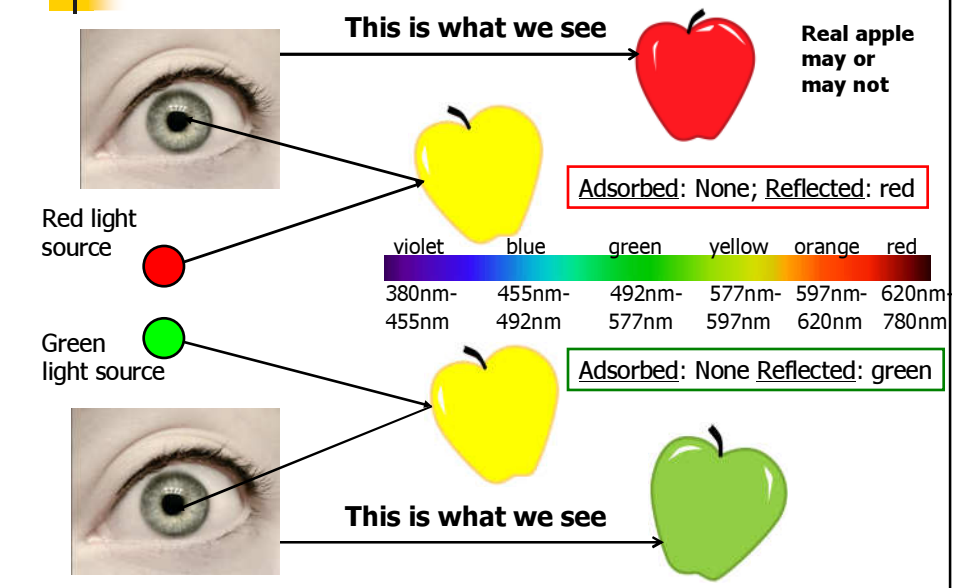
The addition of three primary colors with varying degrees of intensity will produces countless other colors – TV sets, printers

Any two colors which when mixed together in equal intensities produce white are called **complementary colors**

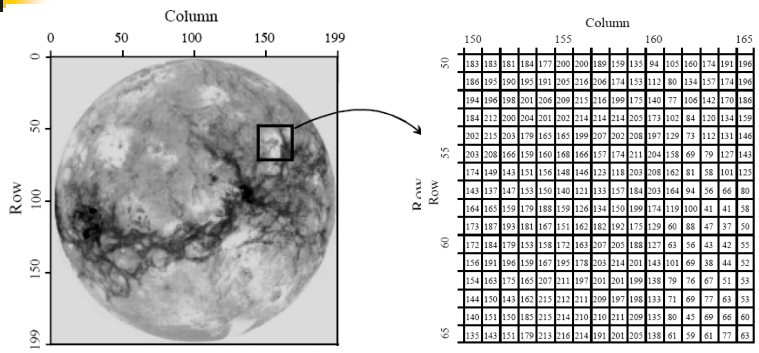
For example, red and cyan: **R + C = R + (B + G) = White**



# Object adsorbing blue light




# Are CCDs color blind?



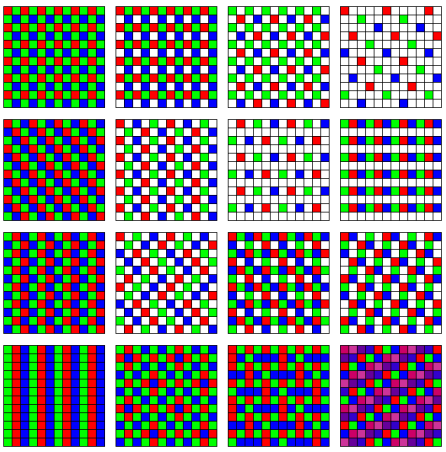

CCDs are pretty good at counting photons but the photon's wavelength (color) is not "transferred" to the electrons

**If so, how can we use CCDs to take color photo?**

# CCDs are color blind

But it is not too difficult to make them see like humans ☺

**Bayer filter**



The last step in the production of color CCDs is to give every pixel EITHER a red OR a green OR a blue "coating" – so-called Bayer filter

You would then expect the CCD to provide three values but, in fact, they often don't using software instead - color space interpolation algorithm

Make sure you understand how it works – there will be a question with colored apple at the next quiz ☺

# In conclusion

**We have discussed:**

- Fluorescence
- Talked about some cool sensors
- Nanotechnology and imaging (briefly)
- Color vision

**Have fun and see you next week**