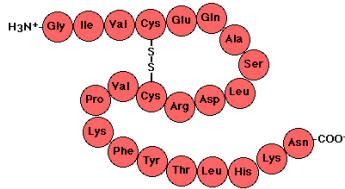






# Primary structure

is **the sequence** of amino acids in a peptide chain



## Why is it important?

### INFORMATION!

- The simplest of protein structures
- A linear arrangement of amino acids
- The structure that is formed by peptide bond
- Describes the amino acids in the structure
- Contains AA sequences
- Sequence of amino acids joined by H-bonds (and/or ionic in some answers)



# Last time

- Chemical bonds
- Redox reactions
- Electrolytic cells and electrodes
- The Nernst equation

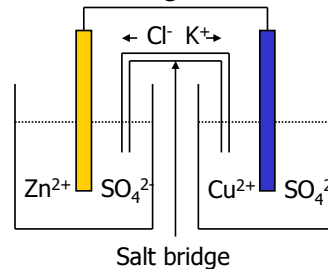
## Why is this important?

**Electrodes are electro-chemical transducers** i.e. interface between chemical and electrical energy

Some biosensors we discussed and others we will talk about are effectively bio-electrolytic cells e.g.

**Potentiometry** for enzyme reactions that generate or consume H<sup>+</sup>

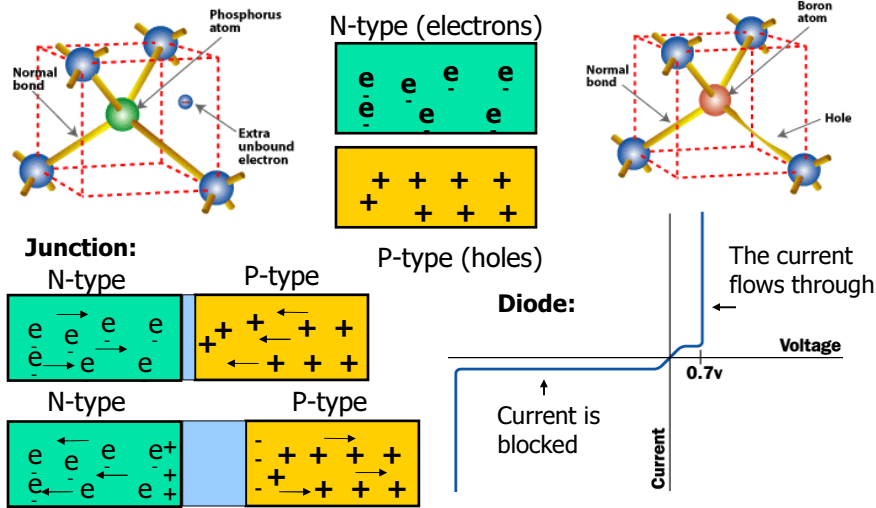
$$E_{\text{cell}} = E_{\text{cell}}^{\circ} - \frac{RT}{nF} \ln \frac{[A_{\text{red}}] \times [B_{\text{ox}}]}{[A_{\text{ox}}] \times [B_{\text{red}}]}$$



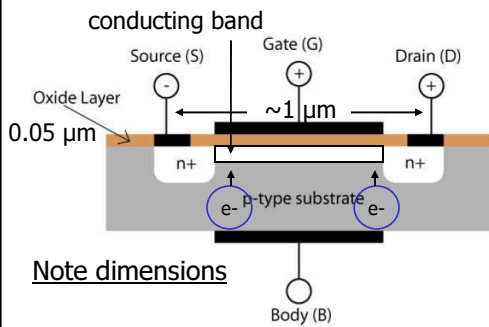
**Amperometry** for enzymes that generate or consume O<sub>2</sub> and/or H<sub>2</sub>O<sub>2</sub> – cholesterol and GOX sensors

# Also last time

We briefly talked about semi-conductors



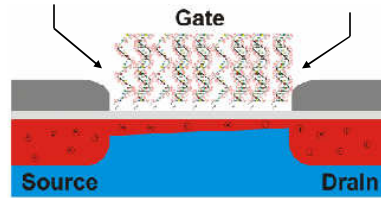
# MOSFET



Note dimensions

Application of a positive potential to the gate creates a strong electric field across the thin p-layer. It attracts e- and create a conducting channel, so that current can flow from source to drain electrodes

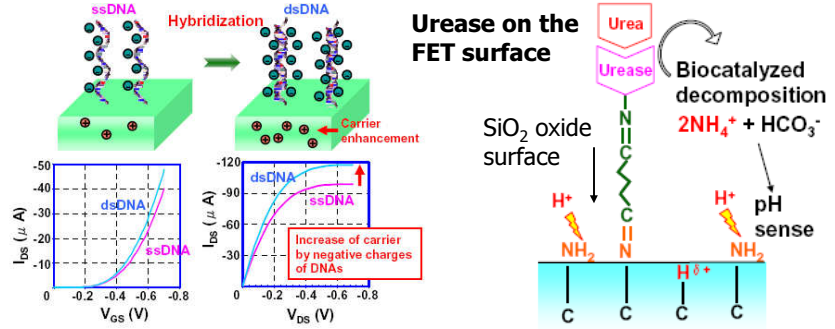
Creating a "reaction vessel" on top of the insulation layer converts MOSFET into an ion sensitive device - SENSOR



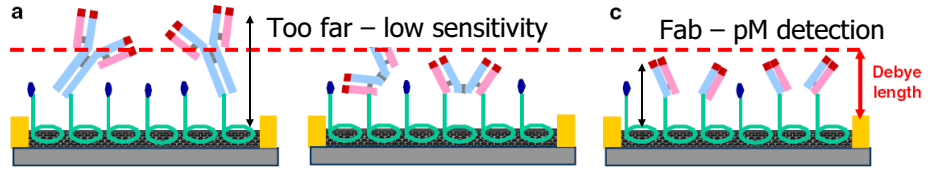
Because the device is so tiny, redistribution of charge on the surface of the oxide layer results in a change in the conducting band, and hence the electric current

**Attaching an appropriate bioreceptor (Enz, DNA, Abs) – makes a BIOSENSOR**

## For example



### Ultrasensitive carbon nanotube-FET (CNT-FETs) - even smaller!



## Plan for today

### Spectroscopy and optical transducers

- Electromagnetic radiation and properties of light
- Spectrometers and it's components
- Light detectors and semiconductors

And as last time we will start with the basics

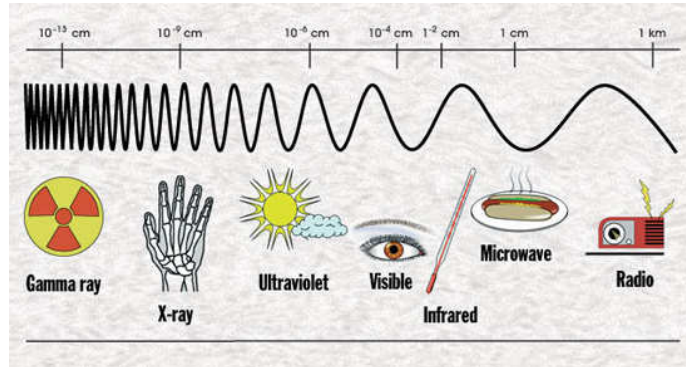


# The electromagnetic spectrum

Higher frequency ( $\nu$ ),  
Shorter wavelength ( $\lambda$ )

$$\nu = \frac{c}{\lambda}$$

Lower frequency ( $\nu$ ),  
Longer wavelength ( $\lambda$ )

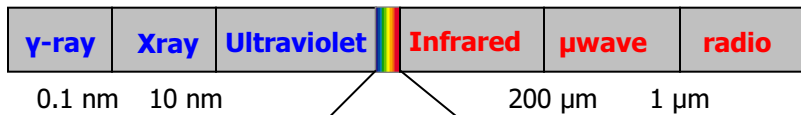


Higher energy (E) Lower energy (E)

\*where c is the speed of light, and  $\lambda$  is its wavelength



# The visible spectrum



Shorter wavelength: **400 nm** **Visible spectrum is only a TINY part** Longer wavelength: **750 nm**



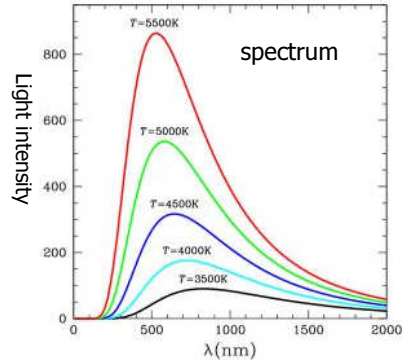
**750 THz** **400 THz**  
Higher frequency ( $\nu$ ) Lower frequency ( $\nu$ )

$4.97 \times 10^{-19} \text{ J}$       **Energy**       $2.65 \times 10^{-19} \text{ J}$   
 $E = h\nu$ , where h is Planck's constant and  $\nu$  is frequency

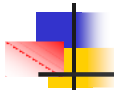


## Light emission by matter

- When a material is heated, it loses the excess energy by several mechanisms, including emission of electromagnetic radiation
- If it is hot enough, we can see the "glow" as visible light is emitted

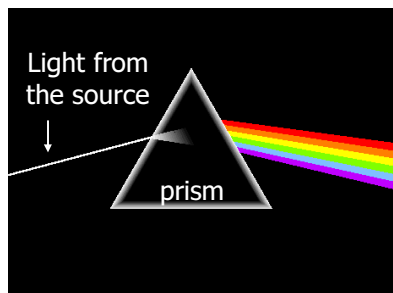


- This is a typical representation of the results of spectroscopic experiment
- The measured light intensity is plotted along the y-axis and the wavelength at which it was observed - on the x-axis



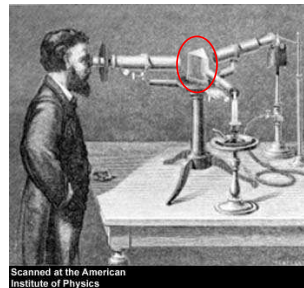
## Generating a spectrum

To generate a spectrum the light from a source should be broken up to its spectral component ("dispersed")



- For light dispersion triangular prisms can be used
- The dispersion occurs because the angle of refraction depends on wavelength

**With a prism we can make a measuring device**

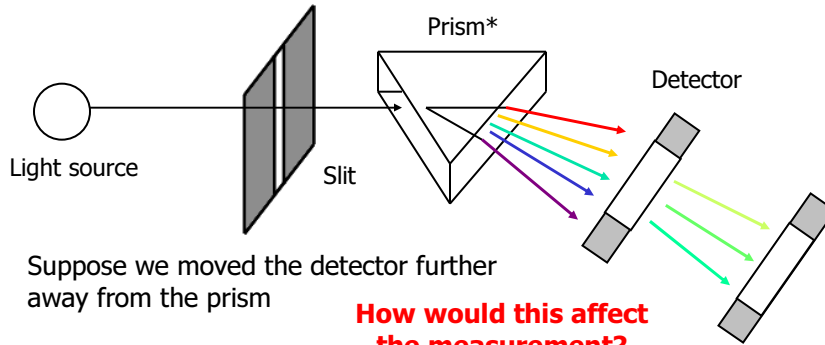


The first instrument used to observe the component wavelengths of a glowing material was the spectroscope



# The Spectrograph

An instrument that separates incoming light into a spectrum



Suppose we moved the detector further away from the prism

**How would this affect the measurement?**

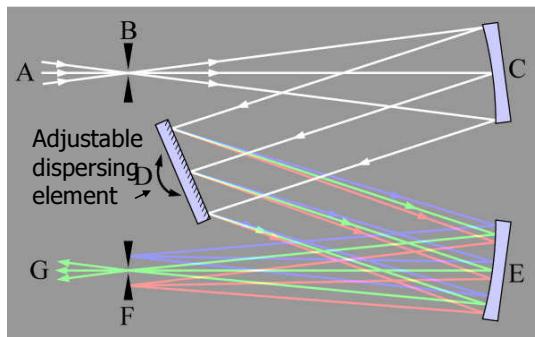
**Resolution will increase** – ever wondered why some old, high quality instruments were so bulky ☺?

\*A polychromator is an optical device for dispersing light in different directions to isolate parts of the spectrum: output multiple beams over a range of wavelength



# The spectrophotometer

**A key part of a spectrophotometer is a monochromator** - an optical device made to transmit only a narrow band of light, while cutting off the rest of the wavelength spectrum



A – light source; B – entrance slit; C – collimator (curved mirror); D – prism or grating; E – another mirror; F – exit slit; G – light with selected  $\lambda$

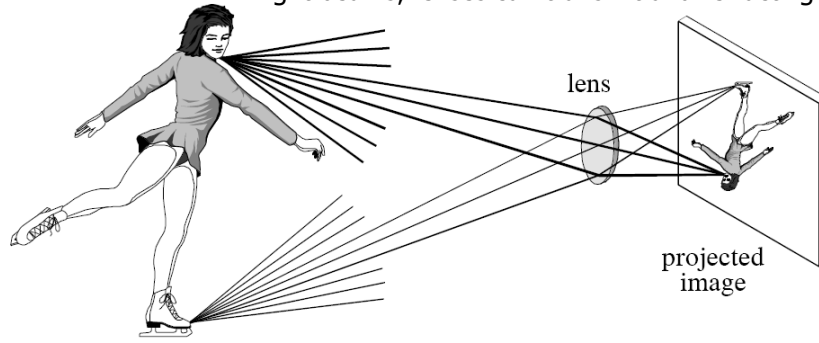
- Monochromators can also use a prism or grating (diffraction) to spatially separate the colors of light
- A collimator is a device that focuses light at infinity i.e. nearly parallel beams; typically it is a curved mirror/lens

**Longer path – better resolution**

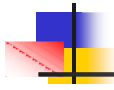


# Lenses

A lens is an optical device which converges (or diverges) incoming light beams; lenses can transmit and refract light

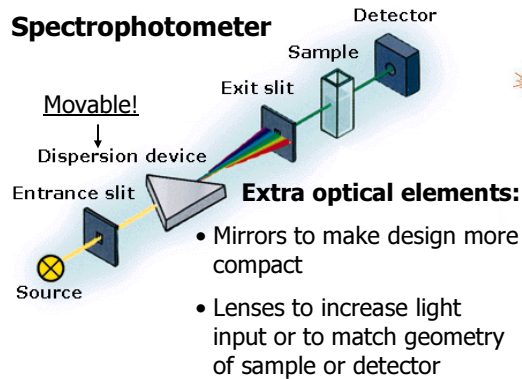


A lens gathers light expanding from a point source, and force it to return to a point at another location. This allows lenses to project images onto a surface



# Instrumentation comparison

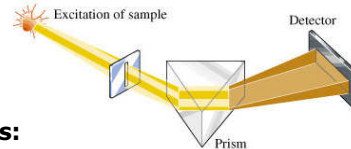
## Spectrophotometer



### Spectrometer advantages

- Higher resolution for the same price
- Easier sample quantification
- Simpler to miniaturize

## Spectrograph



### Spectrograph advantages

- Spectrum collected in one exposure
- Multiple scans can be done for better signal to noise
- More information collected per unit time



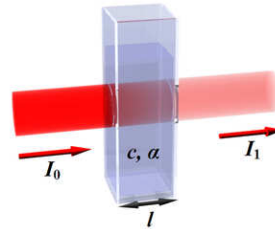


## Doing the measurement

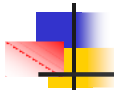
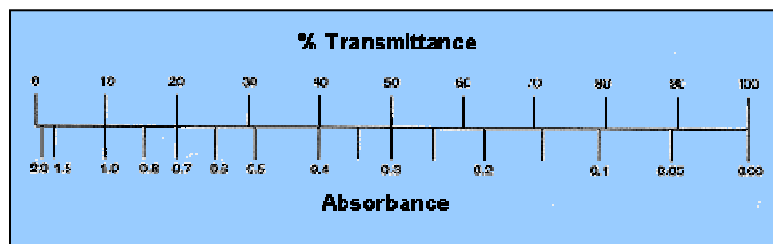
**Transmittance,  $T = I / I_0$**

**% Transmittance,  $\%T = 100 T$**

**Absorbance,  $A = \log_{10} I_0 / I$**

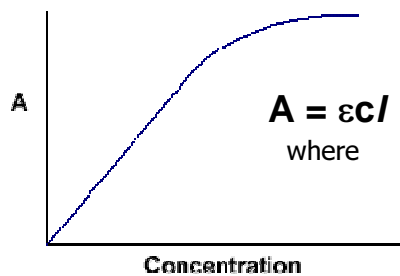


**Note the log relationship between A and I:** At optical density  $A=1$  only 10% of light passes through



## The Beer-Lambert law

describes a relationship between the absorption of light and the concentration of the light adsorbing substance(s) in a sample

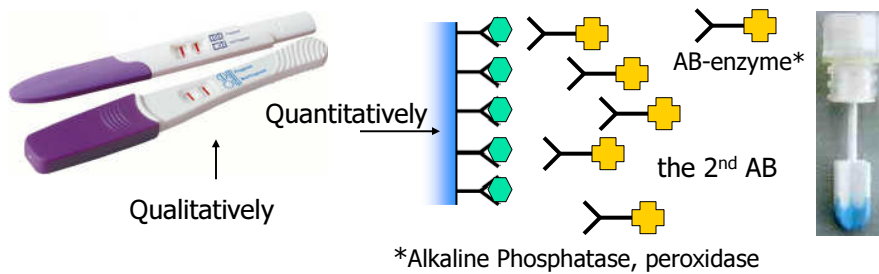


A - absorbance  
 $\epsilon$  - the molar absorptivity with units of  $L \text{ mol}^{-1} \text{ cm}^{-1}$   
l - the path length of the sample  
c - the concentration of the compound in solution, expressed in  $\text{mol L}^{-1}$



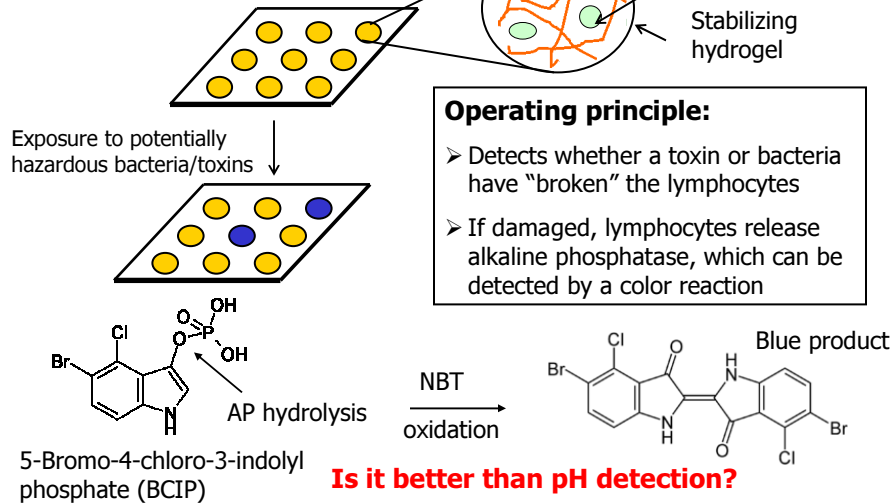
## Colorimetric reagents

- Most organic molecule absorb light in the UV region of the spectrum. Hence, finding a wavelength clear of interfering transitions in the UV region is often difficult or impossible
- Many "colorimetric" reagents have been developed to react specifically with particular functional groups to form colored compounds or substrates to give a colored product in an enzymatic reactions so that their absorptions in the visible spectral region are clear of interference e.g.



## Lymphocytes sense toxins

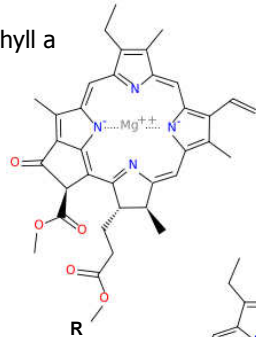
This setup allows multiple parallels assays



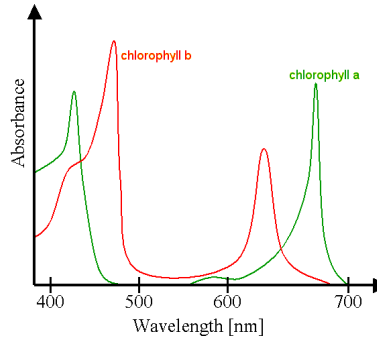
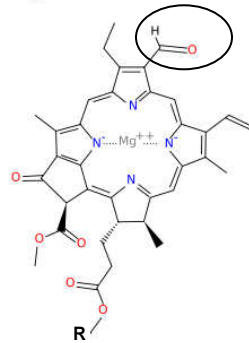


## Sensitivity to substitutions

Chlorophyll a



Chlorophyll b



Absorbance spectra of free chlorophyll a (green) and b (red) in a solvent

The spectra are different due to a slight move of  $Mg^{2+}$  - different interactions with the porphyrin



## The nature of light

- When a matter absorbs energy it can emit light and there is a fundamental relationship between the energy and **wavelength**
- The light can be split into constituent **wavelength** components e.g. with the help of a prism
- We can measure the properties of light using a relatively simple device and record a spectrum – dependence of light intensity on **wavelength**

**But what is a wave?**



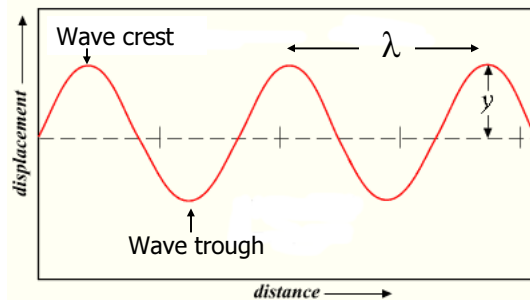
## What is a wave?

- A **pulse** is a single disturbance moving through a medium from one location to another location
- A **medium** is a substance or material comprising a collection of interacting particles and it is the interactions of one particle with an adjacent one that allows the disturbance to propagate
- When such a disturbance is repeated periodically (i.e. propagates through space and time) it is referred to as a **wave**

**A wave is an energy transport phenomenon** - individual particles in the medium are only displaced temporarily and then return to their prior positions

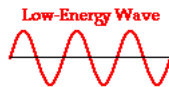


## Basic properties

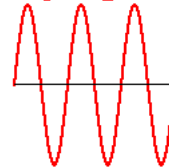


$\lambda$  – wavelength

$y$  – amplitude



High-Energy Wave

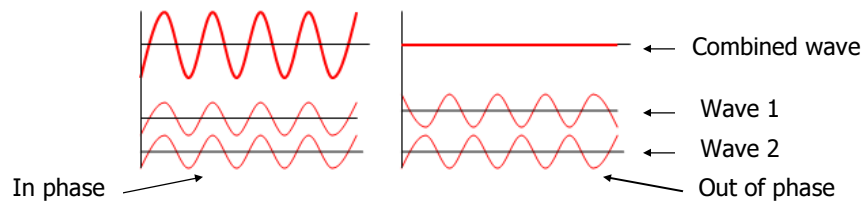


The amplitude of a wave is related to the energy it transports



## Superimposition principle

The principle of superposition states that displacement at any point is equal to the sum of the displacements of different waves at that point

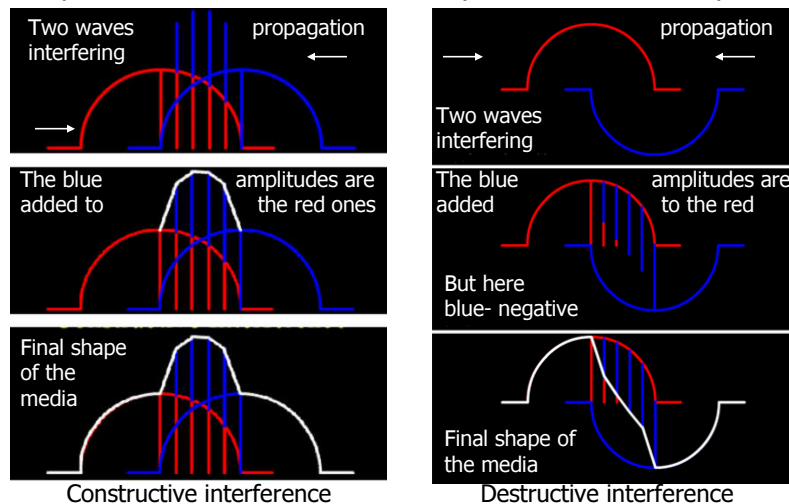


- **constructive interference:** when the crest (trough) of one wave is superimposed upon the crest (trough) of another wave
- **destructive interference:** when the crest of one wave is superimposed upon the trough of another wave



## Interference

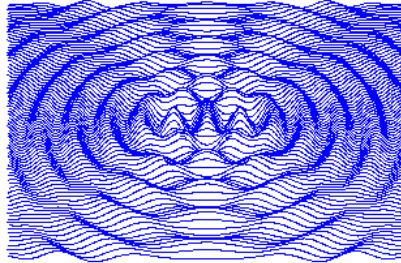
Interference occurs when one wave passes through another - the shape of the medium is determined by the sum of their amplitudes



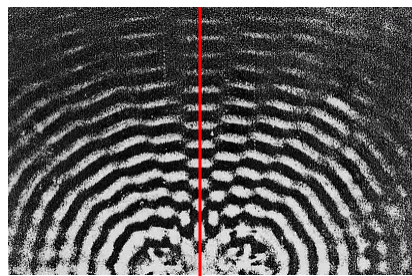


## Visualizing interference

in 3D



in 2D



What would it look like in 1D?

in 1D

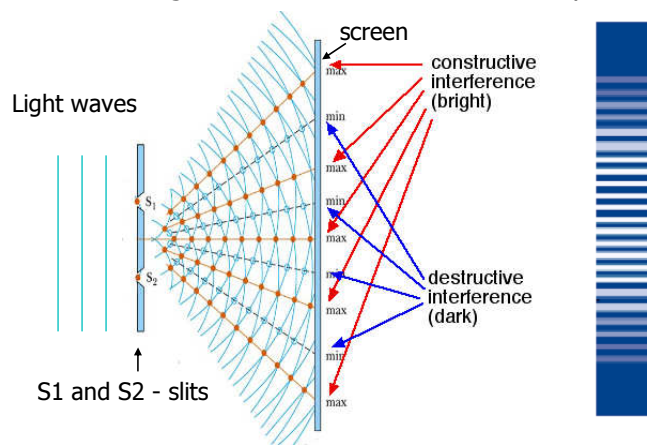


Does light behave as mechanical waves?



## Double-slit experiment

Diffraction is the constructive and destructive interference of two beams of light that results in a characteristic pattern



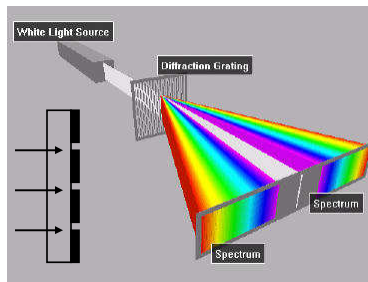
**Interference pattern:** The bright lines are constructive interference and the dark lines - destructive interference



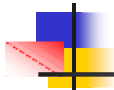
## Diffraction grating

A diffraction grating is an optical device which can disperse light into spectrum - just like a prism

It has a regular pattern of closely-spaced parallel grooves or slits, typically several thousand per cm, that produce interference patterns in a way that separates the components of the incoming light

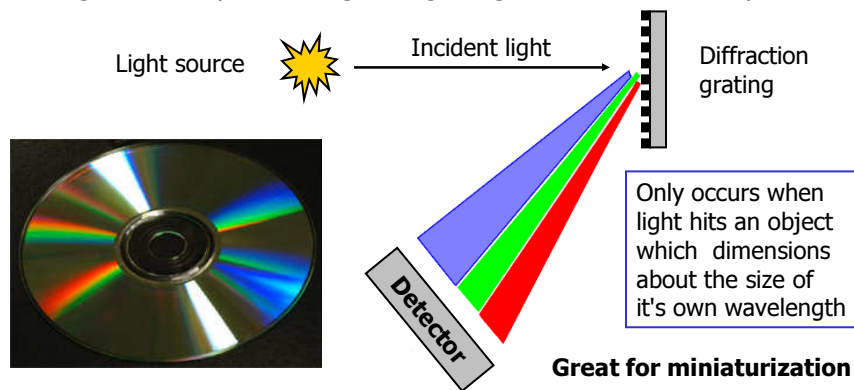


- A transmission grating has grooves on top of a transparent material (e.g. glass), so that when a beam of light passes through it is partly split into so-called sets or orders with spectra on either side of it
- The orders of spectra increase in dispersion and faintness with distance from the direct beam



## Diffraction grating

A **reflection grating** has grooves assemble onto a reflective coating. Its advantage over a transmission grating is that it produces a spectrum extending from ultraviolet to infrared, since the light doesn't pass through the grating material – no adsorption

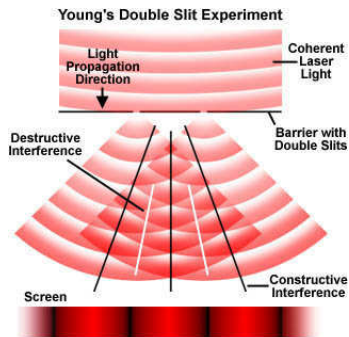




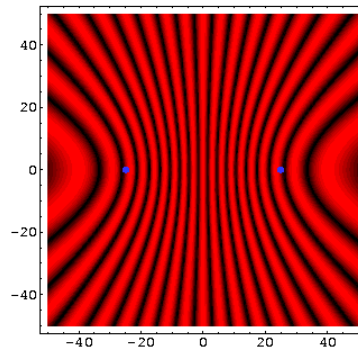
# Light vs sound

Clearly the same phenomenon

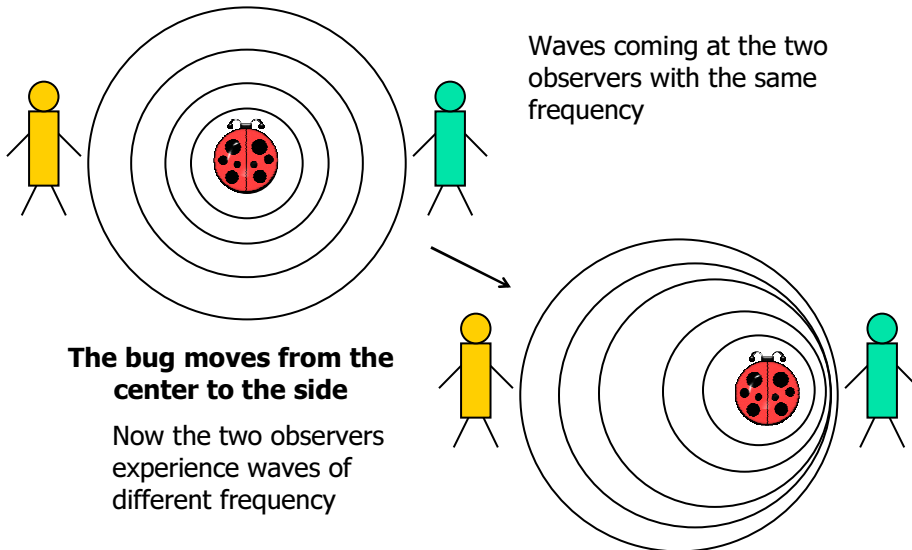
Diffraction of light



Diffraction of mechanical waves



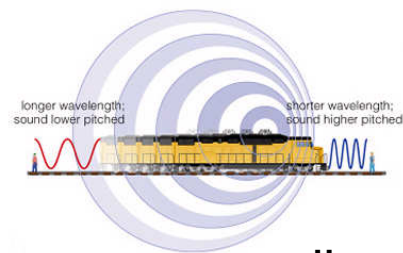
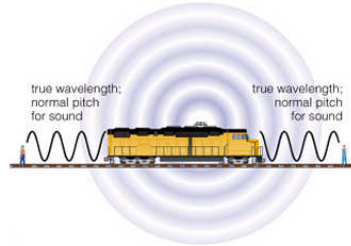
# The Doppler effect



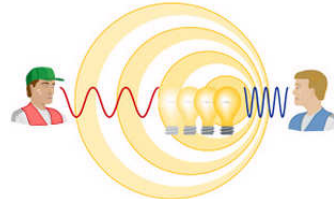




## Light is just like sound...



The Doppler effect can also be observed with light



Sound and light waves seem to behave in exactly the same way...

**Why do you see the sun but do not hear it?**

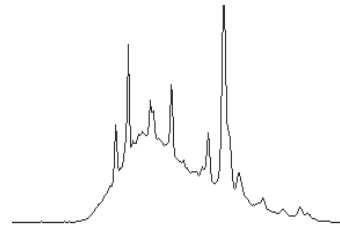
Sound doesn't travel in vacuum but light does

**How can a wave propagate in vacuum?**



## Light is different

- This spectrum was generated on a telescope focused on a star in the constellation Cygnus
- It has the general shape of a typical spectrum, but also contains sharp "spikes" (peaks) that are clearly from some other origin
- The explanation of these features were the focus of research of many prominent physicists in the early XXth century



After comparison to spectra of various materials, most of the "mysterious" peaks\* occurred at the same wavelength in the spectrum of excited hydrogen

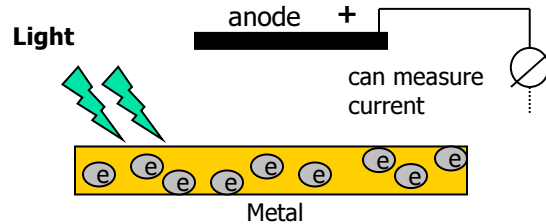
\*The positions of these peaks can be calculated from a simple formula  $E = k(1/n^2 - 1/m^2)$ , where  $n$  and  $m$  are integers. The H atom appeared to have a series of "quantized" energy states...





## Photoelectric effect

When a metallic surface is exposed to electromagnetic radiation the light is absorbed and electrons are emitted

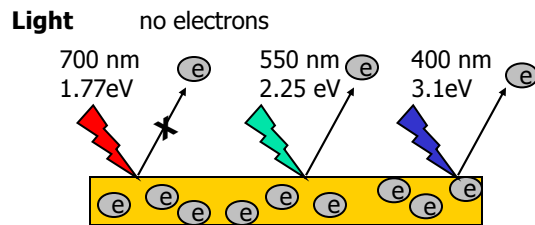


- Photoelectric effect can be observed with a variety of metals (and other materials) and in a range of radiation frequencies
- For a given metal and frequency of incident light radiation, the rate of photoelectrons ejection was directly proportional to the light intensity



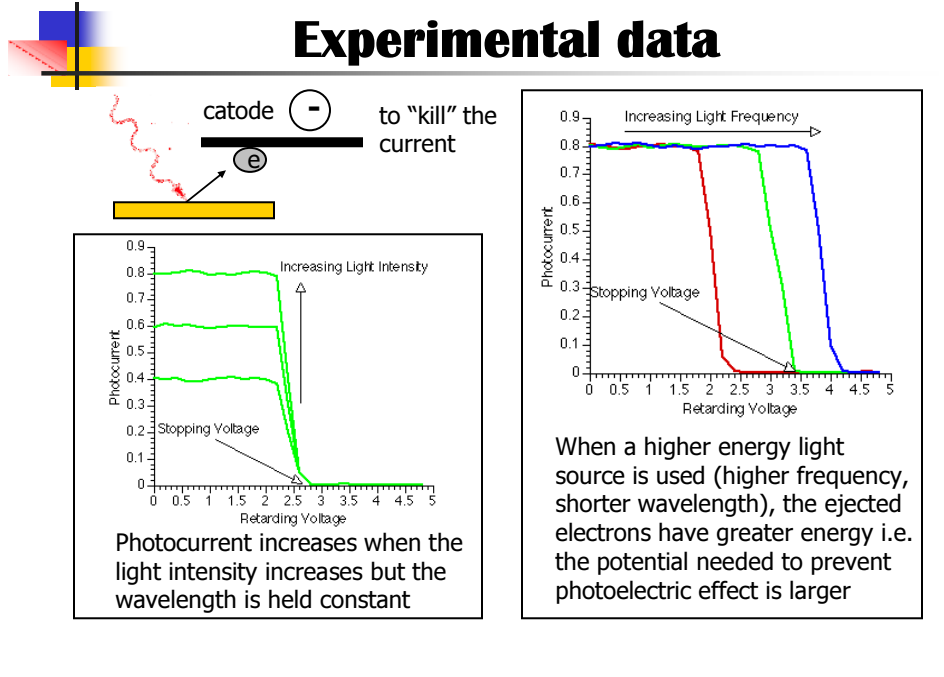
## But there was something odd...

For any particular metal the photoelectric effect was only observed above a certain threshold frequency light...



and the energy of the emitted electrons increased with the frequency of the light but not with the intensity of radiation  
**as would be predicted by the wave theory of light**

## Experimental data



## The explanation

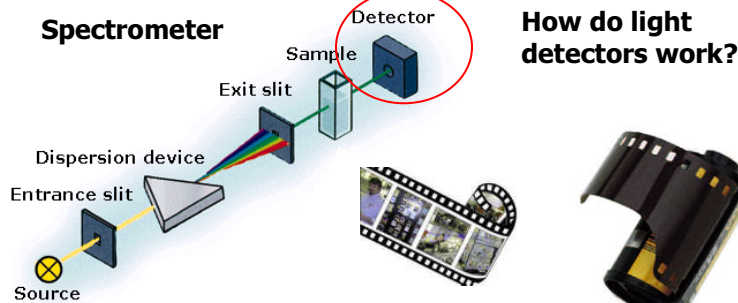
- This paradox was resolved by Einstein (1905) who proposed that light is composed of discrete "quanta" of energy - **photons**, rather than being a continuous wave
- Einstein also postulated that the energy of the ejected electrons should increase linearly with the frequency of light:

$$E = h \times \nu$$

where  $h$  is Planck's constant and  $\nu$  is frequency

- The photoelectric effect helped to establish the then-emerging concept of the **dualistic nature of light** i.e. at different times and under different conditions light can display characteristics of **either waves or particles**
- And there were numerous technological implications too...

# Detectors



Most spectrometers in use before 1960 relied on this form of photodetection

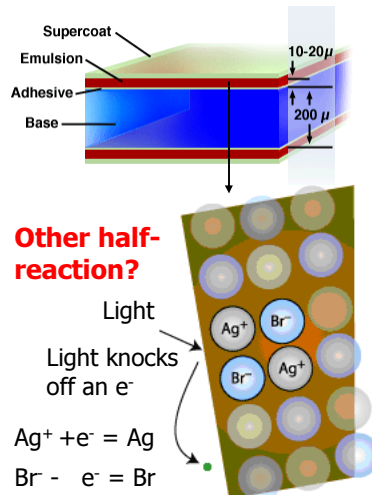
**A photographic film**  
– the simplest detector

## Detectors: photographic film

**A photographic film transduces light into chemical energy**



- It is a plastic film coated with silver salt crystals, the size of which determines the film's sensitivity to light
- Upon light exposure, silver is photo-reduced to very fine grains of Ag metal and the development process simply washes away the gel medium and unexposed silver salts
- Most spectrometers before 1960 used this form of photodetection - the dark lines on the film had to be converted to a spectral plot by eye or using a film scanner
- The quantum efficiency is only ~10%



## But the world has changed



There are much cooler photo-detector in use today

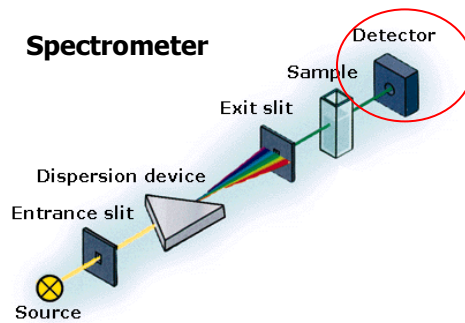
What do camera cell phones have to do with biosensors?

**All this and much more...  
after the break**



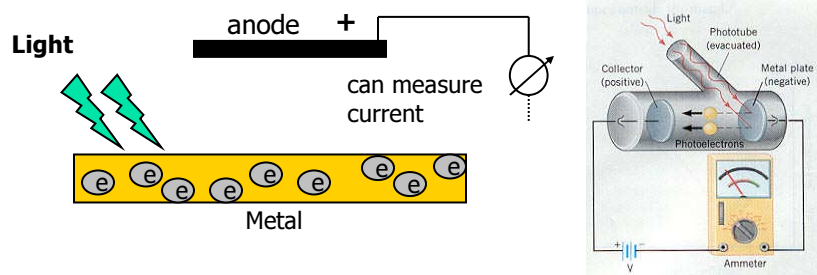
## Light detectors

How do modern photodectors work?





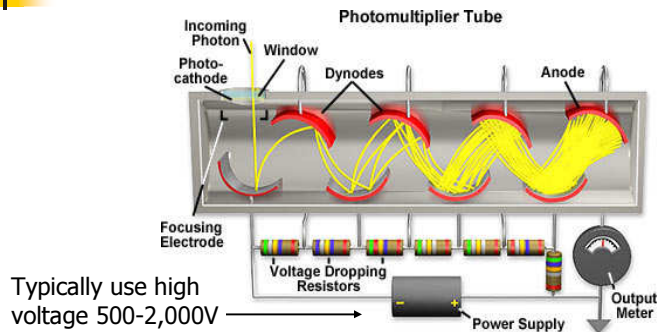
## Phototubes



- Phototubes utilize the photoelectric effect to detect light
- Photons with sufficient energy eject electrons from the surface of thin metallic films and the resulting current, which is proportional to the light intensity (the number of photons), is then measured



## Photomultipliers



In practice, phototubes often contain cascaded metal electrodes at high potential differences - the accelerated photoelectrons knock off additional electrons at each stage, thus amplifying the photocurrent

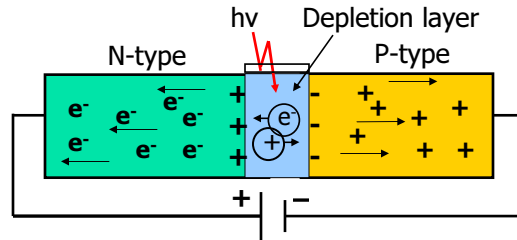
Tubes with 8-10 stages can reach quantum efficiencies of 90%

**Great but bulky and power hungry**



# Photodiode

Photodiodes are photodetectors that convert light into either current or voltage, depending upon the mode of operation



Similar to regular diodes but there is a "window" to allow light to reach the photo sensitive part of the device

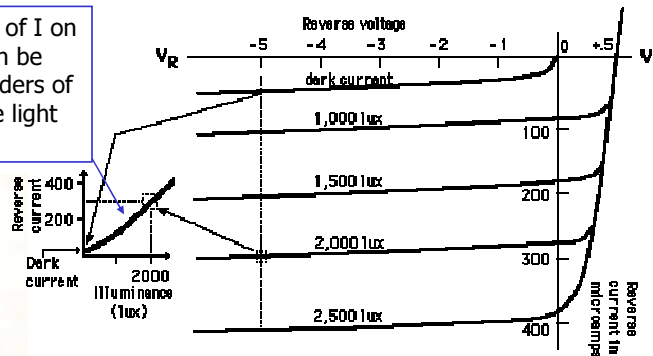
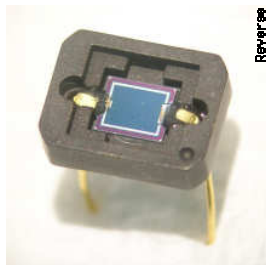
Light absorbed in the depletion region generates electron-hole pairs, which contribute to photocurrent



# Operation mode

**Photoconductive mode:** a reverse voltage (i.e. voltage in the direction where the diode is not conducting) is applied and the resulting photocurrent is measured

The dependence of  $I$  on light intensity can be linear over six orders of magnitude of the light power



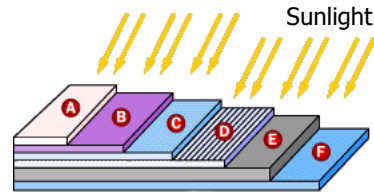
A photodetector can be constructed from a transistor too ("phototransistor") - are more sensitive



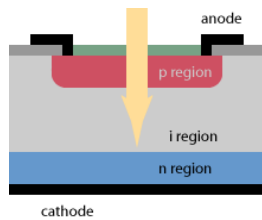


## Just for your information

**Photovoltaic mode:** the photodiode can generate voltage too; this is, by the way, how solar panels work



A: cover glass  
B: antireflective coating  
C and F: electrodes  
D: N-type  
E: P-type



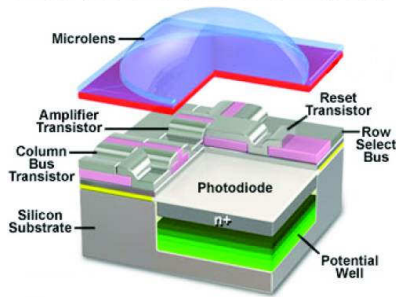
**PIN (p-i-n) photodiodes:** To increase performance an intrinsic (un-doped) layer can be incorporated between n and p layers – same principle but electron-hole pairs are generated in the i-layer



## CMOS sensors

### Complementary metal-oxide-semiconductor (CMOS)

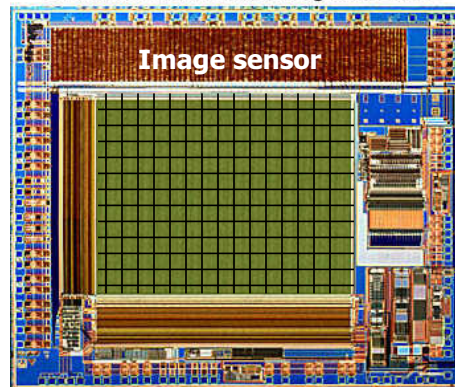
Anatomy of the Active Pixel Sensor Photodiode



Based on FET (field-effect transistors)

All we need to do is to arrange them in a grid

Active Pixel Sensor CMOS Integrated Circuit

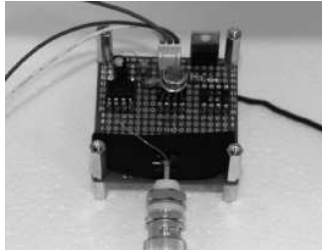


More than 90% of camera phones sold today rely on CMOS image sensing technology



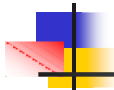
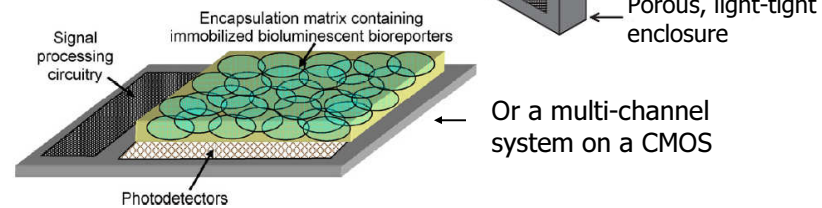
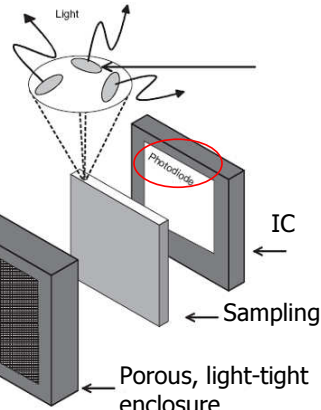
## What does it have to do with biosensors?

Detection of airborne bacteria



Photodiode sensor

Detection of salicylates

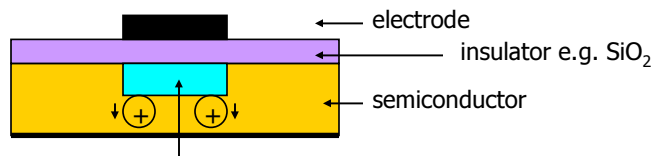


## MOS detectors

CMOS sensors - consume little energy, integrate seamlessly into modern electronic device, but can be noisy – low quality image

### MOS: Metal-Oxide-Semiconductor

Electrode is attached on top of the  $\text{SiO}_2$  (dielectric) layer on the semiconductor substrate surface



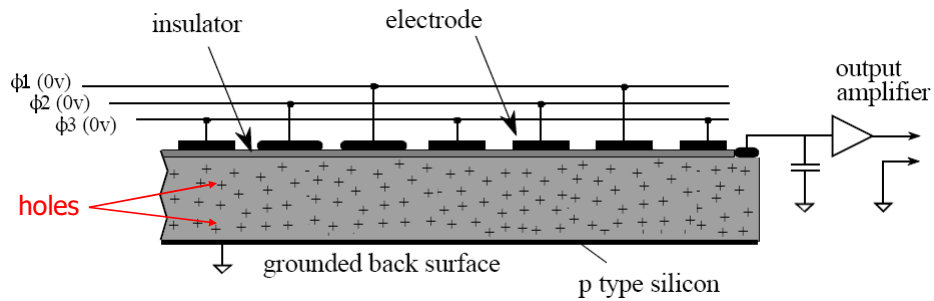
When voltage is applied a depletion layer is formed in the region near the interface of  $\text{SiO}_2$  and semiconductor interface

Arranging these guys in a grid makes a CCD sensor which are also used widely i.e. digital cameras, spectrometers, etc, etc, etc



## Charge coupled device (CCD)

Basically CCD is a thin wafer of p-type silicon covered with an insulating layer on top and an array of electrodes in a repetitive pattern. The electrodes are connected in groups of 3, so that 3 separate voltages can be applied.

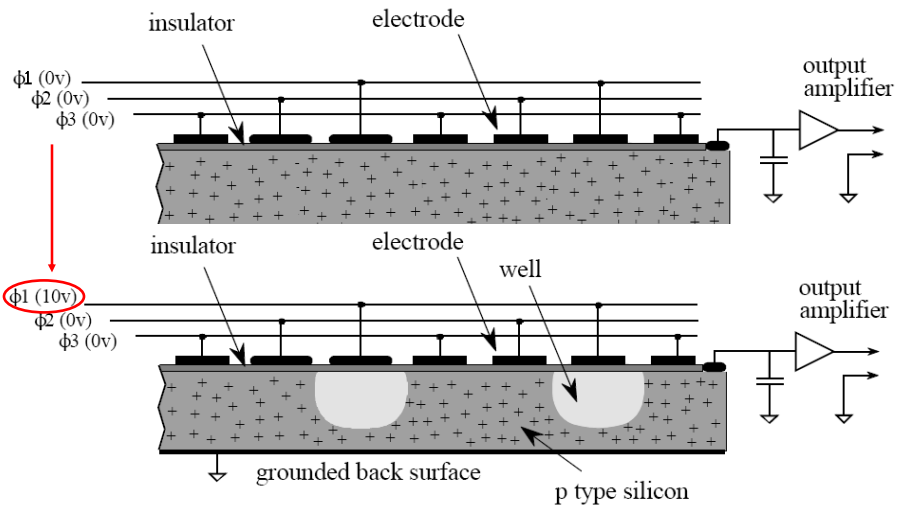


- In p-type there is an excess of positive charge carriers (holes) that can be pushed around by applying voltage



## How CCDs work 1

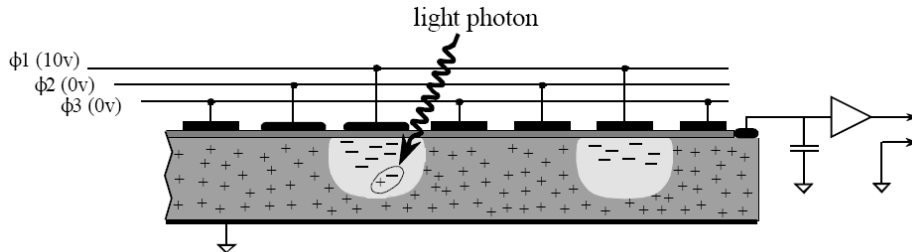
When a positive voltage is applied to an electrode, the holes are pushed away and depleted areas (wells) appear under the electrode



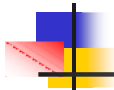


## How CCDs work 2

Each well in the CCD is a very efficient light sensor

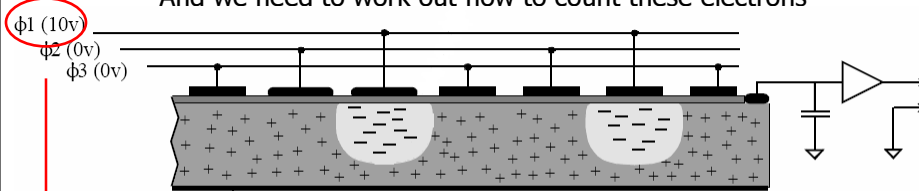


- When a photon of light strikes the silicon, its energy is converted into one  $e^-$  and one hole. The hole moves away, leaving the electron stuck in the well, where it is held by the positive voltage on the electrode
- If CCD is exposed to light for some time (integration period), the pattern of light striking is transferred into a pattern of charge within the CCD wells

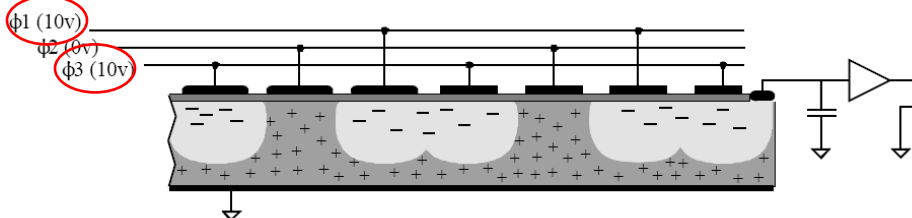


## How CCDs work 3

And we need to work out how to count these electrons



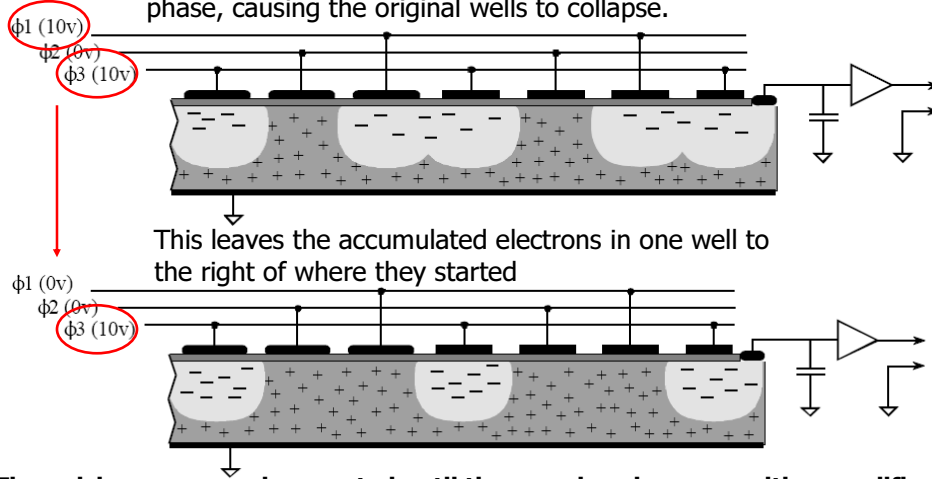
This is achieved by pushing electron to the output side of the grid to an amplifier. A positive voltage is placed on the two neighboring lines of electrodes resulting in each well expanding to the right





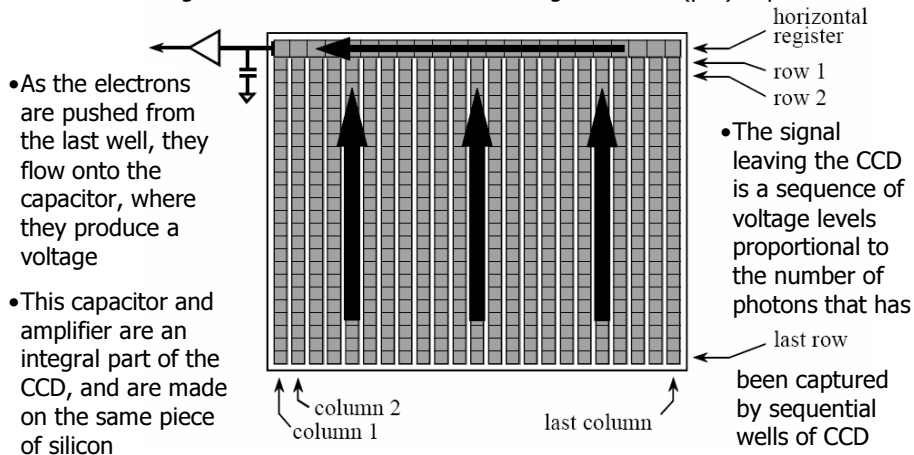
## How CCDs work 4

The next step is to remove the voltage from the first phase, causing the original wells to collapse.



## CCD architecture

The imaging wells of the CCD are arranged in columns. During readout, the charge from each well is moved up the column into a horizontal register. The horizontal register is then readout into the charge sensitive (pre)amplifier.





## CCD output

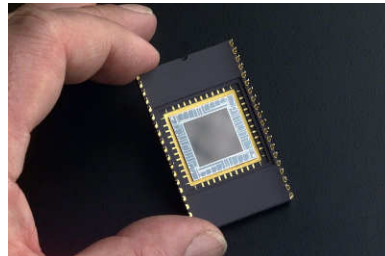
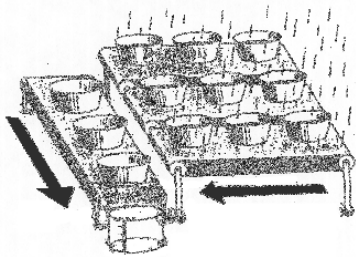
		Column															
		150				155				160				165			
Row	50	183	183	181	184	177	200	200	189	159	135	94	105	160	174	191	196
	51	186	195	190	195	191	205	216	206	174	153	112	80	134	157	174	196
	52	194	196	198	201	206	209	215	216	199	175	140	77	106	142	170	186
	53	184	212	200	204	201	202	214	214	214	205	173	102	84	120	134	159
	54	202	215	203	179	165	165	199	207	202	208	197	129	73	112	131	146
	55	203	208	166	159	160	168	166	157	174	211	204	158	69	79	127	143
	56	174	149	143	151	156	148	146	123	118	203	208	162	81	58	101	125
	57	143	137	147	153	150	140	121	133	157	184	203	164	94	56	66	80
	58	164	165	159	179	188	159	126	134	150	199	174	119	100	41	41	58
	59	173	187	193	181	167	151	162	182	192	175	129	60	88	47	37	50
	60	172	184	179	153	158	172	163	207	205	188	127	63	56	43	42	55
	61	156	191	196	159	167	195	178	203	214	201	143	101	69	38	44	52
	62	154	163	175	165	207	211	197	201	201	199	138	79	76	67	51	53
	63	144	150	143	162	215	212	211	209	197	198	133	71	69	77	63	53
	64	140	151	150	185	215	214	210	210	211	209	135	80	45	69	66	60
	65	135	143	151	179	213	216	214	191	201	205	138	61	59	61	77	63

Essentially a grid of number corresponding to # of photons in each well/pixel



## CCD: summary

**CCD imaging is performed in a three-step process:**



1. Exposure to light which converts light energy into an electronic charge at discrete wells (pixels)
2. Charge transfer to move the packets of charge in wells along the structure
3. Charge-to-voltage conversion and output amplification



# Photomultipliers and CCDs

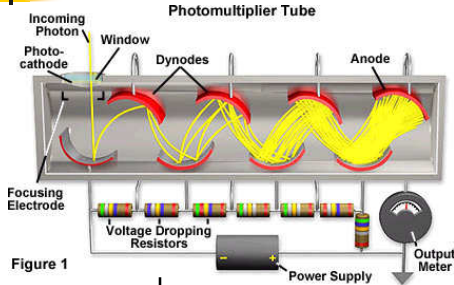
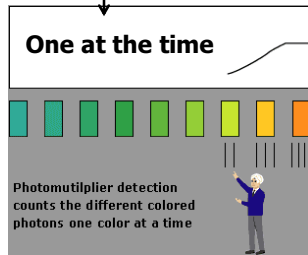


Figure 1



**What is the difference between photomultipliers and CCDs?**

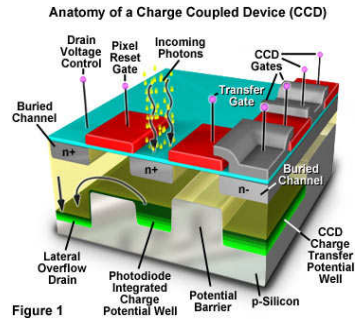


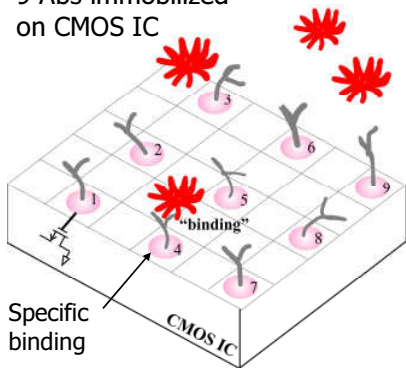
Figure 1

**while CCD can capture millions simultaneously**



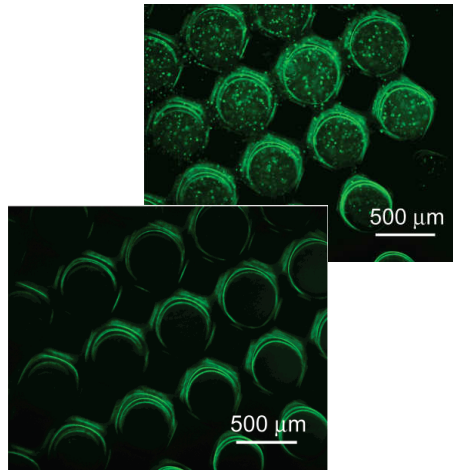
# CMOS/CCD biosensors

9 Abs immobilized on CMOS IC



Before (top) and after (bottom) exposure to a toxin

Whole mammalian cell biosensors for the optical monitoring of cell viability



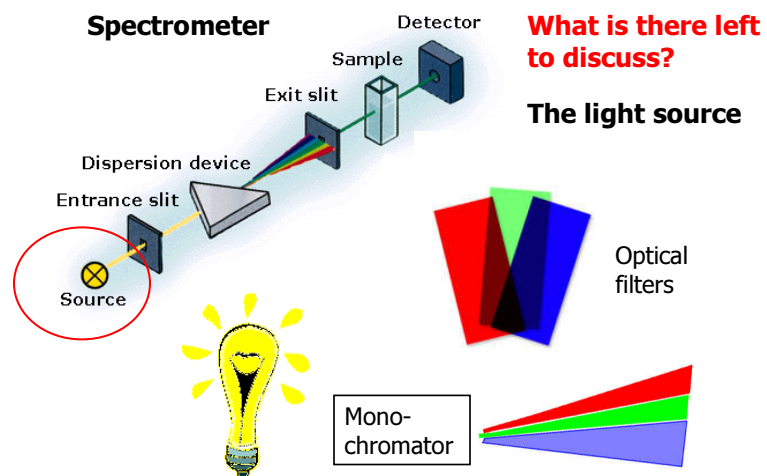


## Why is it important?

- Miniaturization/portability
- High throughput – massively parallel analysis is possible
- Cost of large scale manufacturing
- Signal digitization/ease of integration with modern electronics



## And finally...

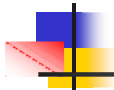
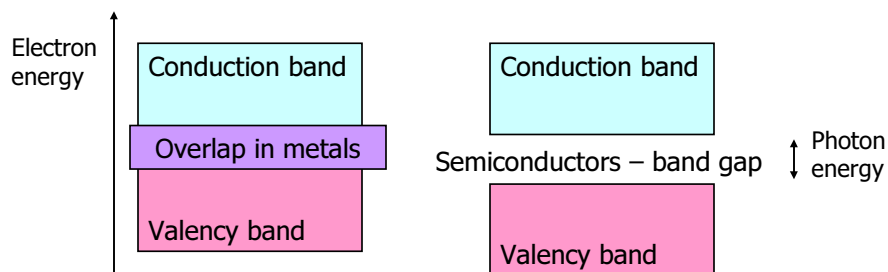






## Light emitting diode

- A LED is a semiconductor diode that emits light when an electrical current is applied in the forward direction
- Like a normal diode, a LED has a p-n junction where electrons and holes recombine – the energy released is emitted in the form of a photon
- Wavelength of the emitted light (typically very narrow) depends on the material (silicon is not used)



## Lasers

"Laser" stands for **light amplification by stimulated emission of radiation**; it is a device that controls the way that energized atoms release photons



Lasers can now be found in an amazing range of products from CD players to Starwar defense systems

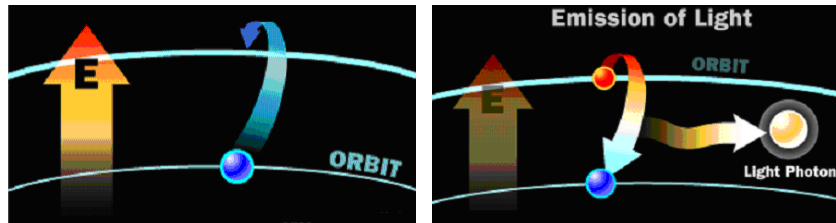
**Laser light is different** from other light sources:

- The laser light is monochromatic
- The laser light is coherent i.e. it is "organized" in such a way that photons move in step with the others
- The laser light is directional i.e. a powerful and "concentrated" beam



## Pumping

In a laser, the medium is “pumped” by very intense flashes of light or electrical discharges to create a large proportion of excited-state atoms. In general, the atoms are excited to a level that is two or three levels above the ground state



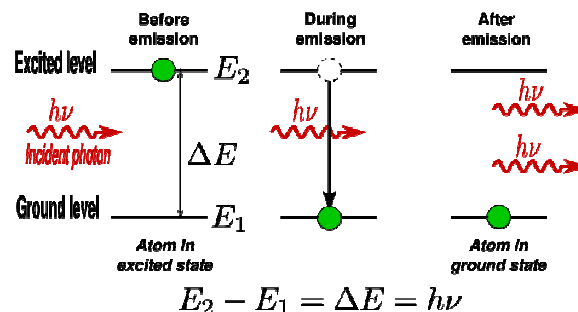
When electrons in the excited atoms return to the lower energy level, the light is emitted

But there is an interesting twist – **stimulated emission**



## Stimulated emission

The energy of emitted photons depends on the energy difference between the excited and the ground state. When an emitted photon encounters another atom that has an electron in the same excited state, **stimulated emission** occurs



The first photon induces atomic emission in such a way that photon emitted from the second atom has exactly the properties (frequency, phase, polarization, direction) as the incoming photon



## Mirrors

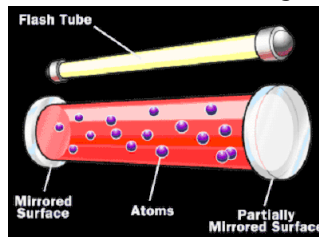
- The other key feature of lasers is a **pair of mirrors**, one at each end of the lasing medium. Photons, with a specific wavelength and phase, reflect off the mirrors and travel back and forth through the lasing medium to cause the emission of more photons of the same wavelength and phase creating a cascade effect
- One end of the laser often has a "half-silvered" mirror - it reflects some light and lets some light through, depending on intensity
- **The light that makes it through is the laser light**



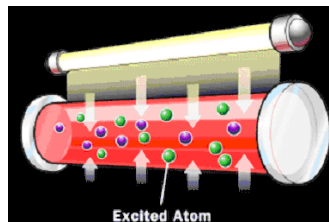
## How lasers work

- A ruby laser (694 nm) consists of a flash tube, a ruby rod and two mirrors (one half-silvered)
- The ruby rod is the lasing medium and the flash tube pumps it

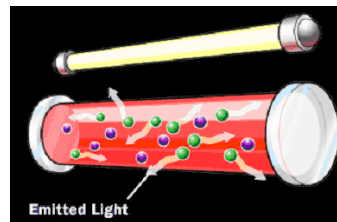
1. The laser in its non-lasing state



2. The flash tube fires light into the ruby rod which excites atoms in the ruby

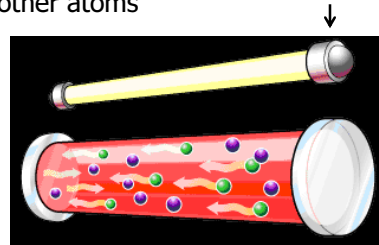
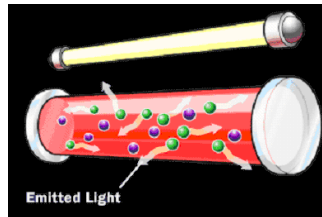


3. Some of these atoms emit photons



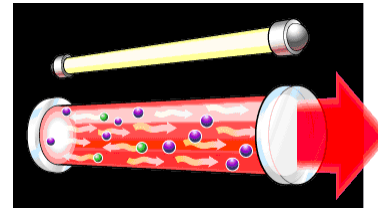
## How lasers work

The photons running in a direction parallel to the ruby's axis bounce back and forth off the mirrors and, as they pass through the crystal, stimulate emission in other atoms

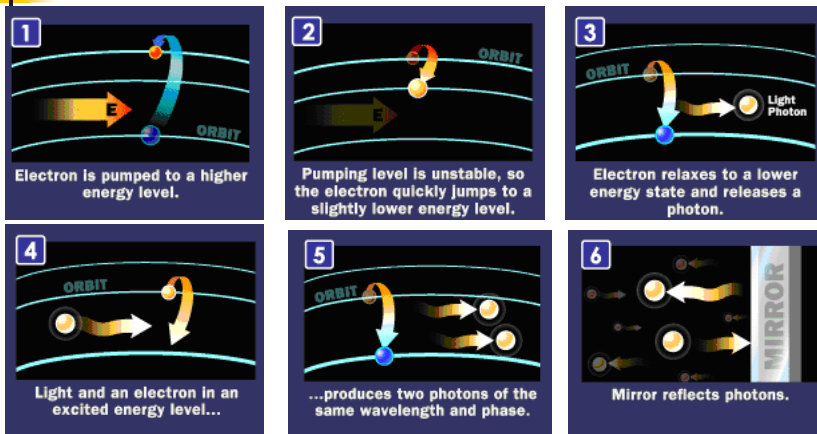


Once there is enough photons monochromatic, single-phase light leaves the ruby through the half-silvered mirror

**This is laser light**



## A three-level laser



A burst of energy excites electrons from their ground state to a higher state. The electrons then drop into a longer lived state with slightly less energy (the level down), where they can be stimulated to quickly shed the excess energy - **a laser burst** - returning to the stable ground state



## Types of lasers

- Solid-state lasers have lasing material distributed in a solid matrix e.g. the ruby laser
- Semiconductor lasers or diode lasers (not solid-state lasers) are electronic devices - generally small and use low power e.g. CD players
- Gas lasers e.g. helium and helium-neon have a primary output of visible red light. CO<sub>2</sub> lasers emit energy in the far-infrared, and are used for cutting hard materials
- Dye lasers use complex organic dyes, such as rhodamine 6G, in liquid solution or suspension as lasing media. They are tunable over a broad range of wavelengths



## Types of lasers

<b>Laser Type</b>	<b>Wavelength (nm)</b>
Argon fluoride (UV)	193
Krypton fluoride (UV)	248
Xenon chloride (UV)	308
Nitrogen (UV)	337
Argon (green)	514
Argon (blue)	488
Helium neon (green)	543
Helium neon (red)	633
Rhodamine 6G dye (tunable)	570-650
Ruby (CrAlO <sub>3</sub> ) (red)	694
Nd:Yag (NIR)	1,064
Carbon dioxide (FIR)	10,600

