Nanoscopy

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SoSe 2015

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   - Stokes shift
   - Franck-Condon Principle
   - Quantum Yield
   - Concurring Processes
   - Fluorescence Lifetime
   - Photobleaching and Quenching

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   - Gas discharge lamp
   - High power LED
   - Laser
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4. Light detection
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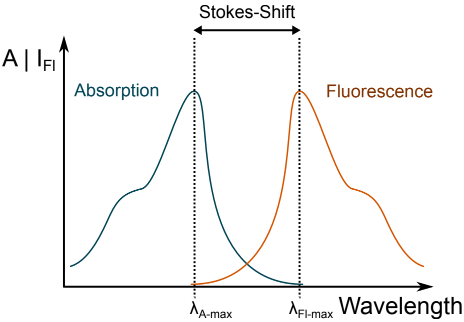
   - Semiconductors: CCDs and CMOS

Absorption- and emission spectra of fluorophores

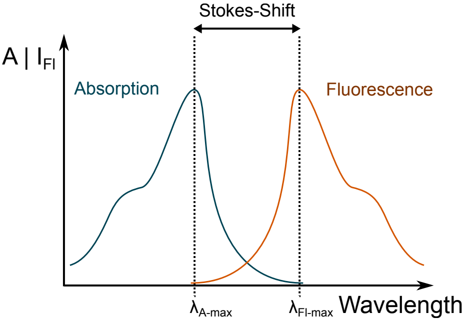
Absorption and emission of fluorescing molecules as a function of wavelength.



- Parameters to extract: **Central wavelength** of absorption an emission; **Stokes shift**
- **Mirror rule**: Spectra look like mirror images of one another

Stokes shift

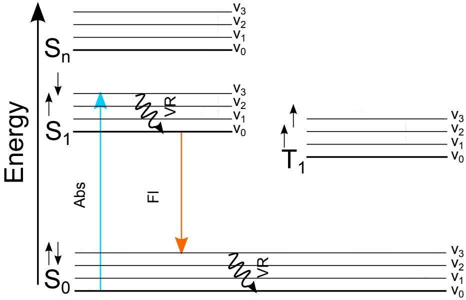
The difference between the **central wavelength of absorption** and the **central wavelength of emission** is termed the **Stokes shift**.



- Larger Stokes shifts allow for better chromatic filtering of signals.
- Dichroic Mirrors can separate the excitation light pathway from the emission light pathway.

Stokes shift explained

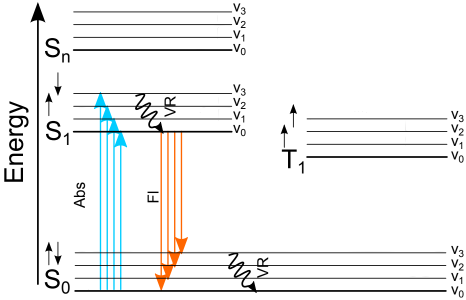
The **Stokes shift** can easily be explained on a **Jablonski diagramm** considering **Kasha's Rule**.



- A Jablonski diagramm displays different possible energy levels of a molecule and the transitions between them.
- **Kasha's Rule**: Fluorescence occurs from the lowest vibrational energy level of the first excited singlet state S_1.

Stokes shift explained

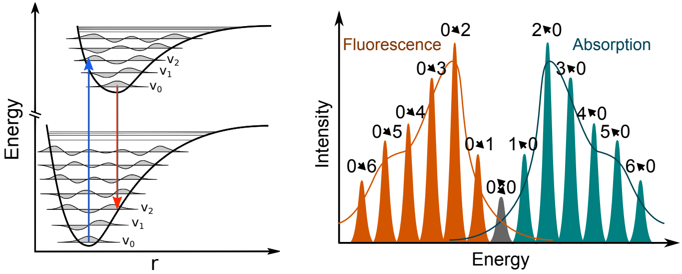
The **Stokes shift** can easily be explained on a **Jablonski diagramm** considering **Kasha's Rule**.



- **Kasha's Rule**: Fluorescence occurs from the lowest vibrational energy level of the first excited singlet state S_1.
- The **Mirror Rule** can be explained by transitions to different vibrational energy levels, considering the **Franck-Condon Principle**.

Franck-Condon Principle

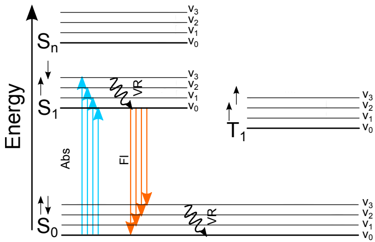
The **Franck-Condon Principle** explains that mirrored transitions have similar probabilities.

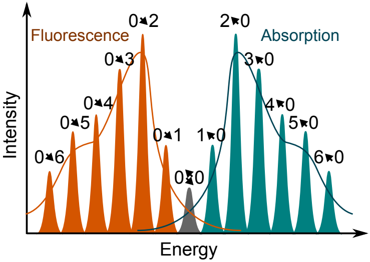


- Electronic transitions occur instantly in relation to the movement of nuclear movement inside the molecule.
- Maximum overlap indicates the most probable transition.

Mirror Rule

Franck-Condon Principle describes the probabilities of electronic transitions to different vibrational energy levels.





- Transitions with a high probability contribute to higher intensities in absorption an emission spectra.
- In liquid or solid state materials the sharp bands of the spectrum are broadened inhomogeneously. Well known shape of fluorophore spectra.

Quantum Yield

The **Quantum Yield** is the ratio of emitted photons and absorbed photons of a fluorescent molecule

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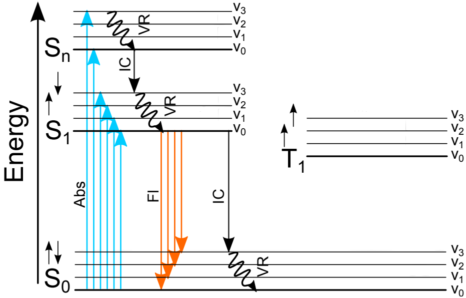


$$\Phi_f = \frac{k_r}{k_r + k_{nr}}$$

- Φ_f is calculated by the rate constant k_r for **radiative processes** and the rate constant k_{nr} for **non radiative processes**.
- Inner conversion **(IC)** is a non radiative process concurring to fluorescence.

Concurring Processes

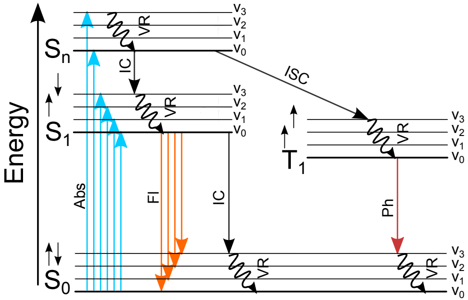
There are several **concurring processes to fluorescence**.

Rate constants of different transition pathways

Fluorescence	$S_1 \rightarrow S_0$	k_f	$10^{7} - 10^{9}$	s^{-1}
Internal Conversion	$S_n \| T_n \rightarrow S_1 \| T_1$	k_{ic}	$10^{10} - 10^{14}$	s^{-1}
	$S_1 \rightarrow S_0$		$10^{6} - 10^{7}$	s^{-1}
Vibrational Relaxation	$S_{1;v=n} \rightarrow S_{1;v=0}$	k_{vr}	$10^{10} - 10^{12}$	s^{-1}
Singlet-Singlet-Absorption	$S_1 \rightarrow S_n$	k_{exc}	10^{15}	s^{-1}
Intersystem Crossing	$S_1 \rightarrow T_1$	k_{isc}	$10^{5} - 10^{8}$	s^{-1}
	$S_n \rightarrow T_n$			
	$T_n \rightarrow S_n$			
Phosphorescence	$T_1 \rightarrow S_0$	k_p	$10^{2} - 10^{3}$	s^{-1}
Triplet-Triplet-Absorption	$T_1 \rightarrow T_n$	k_{exc}	10^{15}	s^{-1}

Fluorescence Lifetime

The average time a molecule spends in its first excited singlet state before spontaneous fluorescence emission occurs is termed **fluorescence lifetime**



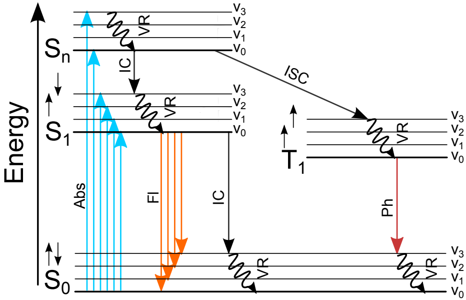
$$\tau_f = \frac{1}{k_r + k_{nr}}$$

$$I(t) = I_0 exp\left(\frac{-t}{\tau_f}\right)$$

- The fluorescence lifetime τ_f ist the inverse sum of rate constants for radiative and non radiative relaxations ($k_r; k_{nr}$)
- The initial fluorescence intensity of fluorescent molecules I_0 exhibits an exponential decay, with the lifetime as decay constant.

Photobleaching

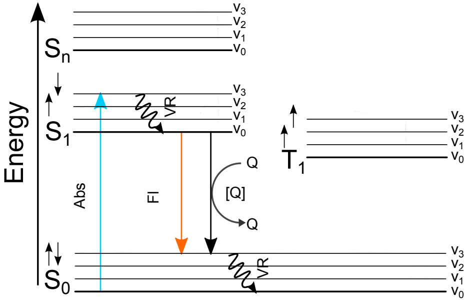
Photobleaching is a non reversible process in which the fluorescent molecule loses its ability to emit fluorescence photons



- There are several pathways for photobleaching i.e.:
- Ionization of the molecule.
- Population of the triplet state.

Quenching

Collisional quenching



Stern-Volmer-Equation

$$\frac{F_0}{F} = 1 + K[Q]$$

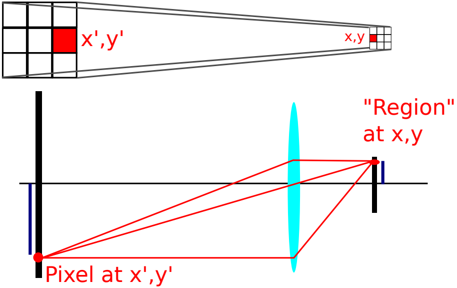
- The Stern-Volmer-Equation describes the dependency of quenched fluorescence intensity F and the quencher concentration $[Q]$.
- If the molecule is sensitive to the quencher the Stern-Volmer constant K takes large values.
- A possible quencher in aqueous solutions is molecular oxygen.

Recapitulation: Fluorescence microscope

Recapitulation: Magnification / Pixels

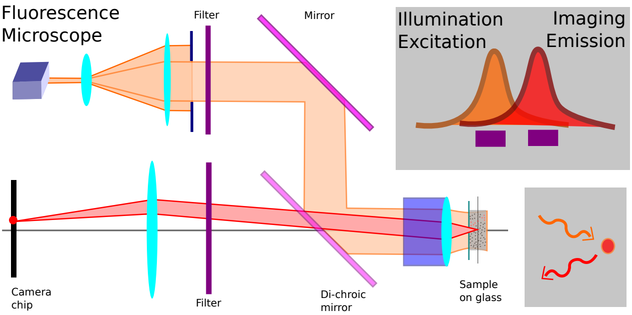
$$M(x, y) = I \cdot S(x, y)$$

- Sample $S(x, y)$ reacts to illumination I, measured as $M(x, y)$.
- **Magnification** links rectangular pixels ($d = 50 \ldots 150\,\mu\mathrm{m}$) to areas on the samples focal plane (e.g. $d' = 75\,\mu\mathrm{m}$ and $f = 60\times$ to $d = 125\,\mathrm{nm}$).
- Think *effective pixel size*.



Camera pixel: Rectangular area collecting photons, thus integrating intensity. Maps to a (usually and ideally) regtangular area on the sample.

Widefield flourescent microscopy

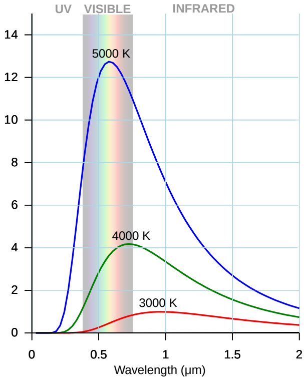


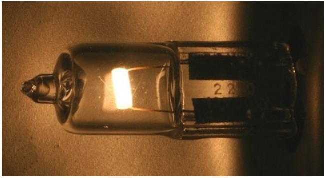
- Fluorophores capture photon, hold it for some nanoseconds, emit it at a longer wavelength (in any direction, with any polarization)
- Fluorophores have an excitation and an emission spectrum.
- Lamp/Laser filter: Illuminate the excitation spectrum
- Camera filter: Image the emission spectrum
- Ideally: Little to no overlap (with good filters)

Light sources

Light sources: Incandescent light bulb

- Patent 1845, Osram halogen in 1959
- Heated tungsten, approx. black body spectrum, halogen for higher temperature
- Light: No coherence, not at all monochrome
- Filtering to a narrow-band emission throws away lots of the spectrum
- Historically cheap and easy source, today rivaled by (cheap) high power LEDs

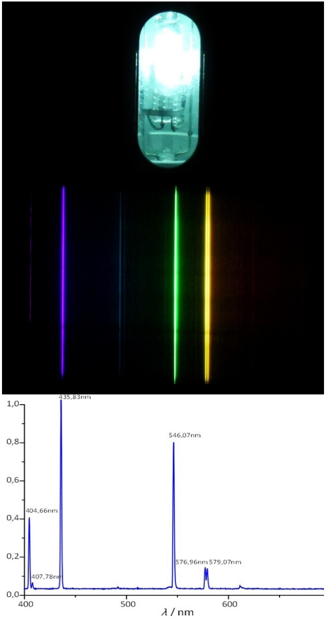




Wikimedia/Spektrum, Wikimedia/Halogen Lamp

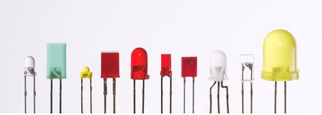
Light sources: (mercury) gas-discharge lamp

- Mercury lamps: discovered 1705, first applications 1901, referred to as *burners*
- Light generated by electrical discharge that ionizes gas
- No coherence, spectral lines given by gas, spectral broadening when using high pressure
- Filtering to narrow-band emission is quite effective if close to spectral line.
- Today still in use, again rivaled by (quality) high power LEDs and Lasers.



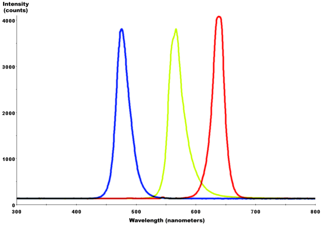
Wikimedia/Hg-Spektrum

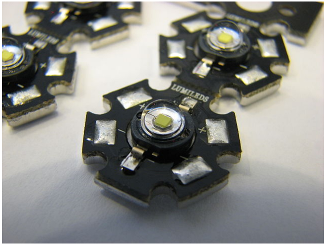
Light sources: High Power LEDs (Light-emitting diode)



Wikimedia/LEDs

- Prototype 1962, blue LEDs 1994, Nobel Prize 2014
- Semiconductor diode with band gap photon emission
- Light: No coherence, wave length set by band gap, spectral peak somewhat broad (compared to lasers)
- Filtering gives a narrower band if required
- High power LEDs became available only some years ago
- Today: Go-to light source (that is cheaper than a laser)





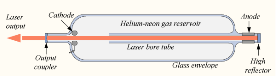
Wikimedia/LED-Spectrum

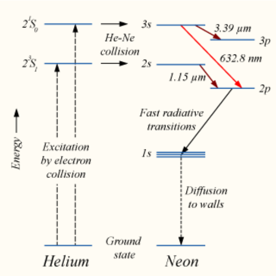
Wikimedia/High Power LED

Light sources: LASER / principal (light amplification by stimulated emission of radiation)

- Patent 1960, semiconductor laser diodes since approx. 1990
- Laser principle: Stimulated emission in a system with multiple energy states
- Different types, most important:
  - Gas (e.g. He/Ne)
  - solid state/crystals
  - semi-conductor/diode

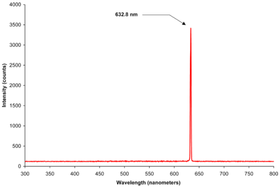
Also, pumping one type (crystal) with another (diode).

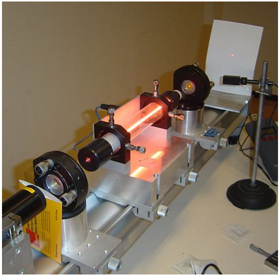




Wikimedia/HeNe diagram
Wikimedia/HeNe states
Wikimedia/HeNe spectrum

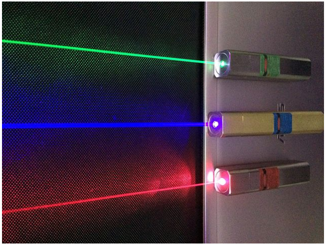
Wikimedia/HeNe laser

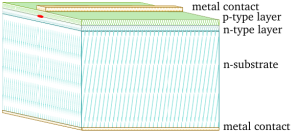




Light sources: LASER / application

- Laser diodes: Similar to LEDs, but withstand higher local currents and (might) have a more complex band structure.
- Laser light (in general): Very coherent, single wave length, narrow band
- Filtering still useful, especially for semi-conductor lasers
- **Coherent light** is very versatile when designing optical systems: Think gratings, interference patterns.
- Today: Generally laser diodes (cheap, powerful), other types for special requirements (good coherence, multiple spectral lines).

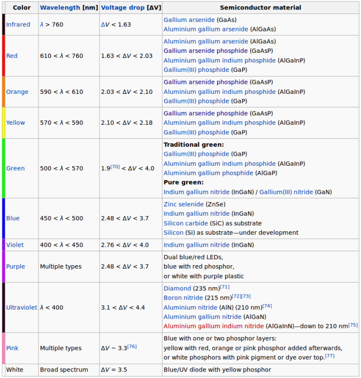




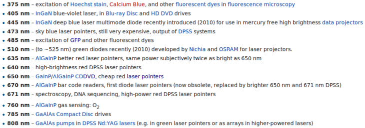
Wikimedia/Lasers

Wikimedia/Laser diode

Spectrum of LEDs and Laser diodes



Color	Wavelength [nm]	Voltage drop [ΔV]	Semiconductor material
Infrared	$\lambda > 760$	$\Delta V < 1.63$	Gallium arsenide (GaAs) Aluminium gallium arsenide (AlGaAs)
Red	$610 < \lambda < 760$	$1.63 < \Delta V < 2.03$	Aluminium gallium arsenide (AlGaAs) Gallium arsenide phosphide (GaAsP) Aluminium gallium indium phosphide (AlGaInP) Gallium(III) phosphide (GaP)
Orange	$590 < \lambda < 610$	$2.03 < \Delta V < 2.10$	Gallium arsenide phosphide (GaAsP) Aluminium gallium indium phosphide (AlGaInP) Gallium(III) phosphide (GaP)
Yellow	$570 < \lambda < 590$	$2.10 < \Delta V < 2.18$	Gallium arsenide phosphide (GaAsP) Aluminium gallium indium phosphide (AlGaInP) Gallium(III) phosphide (GaP)
Green	$500 < \lambda < 570$	1.9[70] $< \Delta V < 4.0$	**Traditional green:** Gallium(III) phosphide (GaP) Aluminium gallium indium phosphide (AlGaInP) Aluminium gallium phosphide (AlGaP) **Pure green:** Indium gallium nitride (InGaN) / Gallium(III) nitride (GaN)
Blue	$450 < \lambda < 500$	$2.48 < \Delta V < 3.7$	Zinc selenide (ZnSe) Indium gallium nitride (InGaN) Silicon carbide (SiC) as substrate Silicon (Si) as substrate—under development
Violet	$400 < \lambda < 450$	$2.76 < \Delta V < 4.0$	Indium gallium nitride (InGaN)
Purple	Multiple types	$2.48 < \Delta V < 3.7$	Dual blue/red LEDs, blue with red phosphor, or white with purple plastic
Ultraviolet	$\lambda < 400$	$3.1 < \Delta V < 4.4$	Diamond (235 nm)[71] Boron nitride (215 nm)[72][73] Aluminium nitride (AlN) (210 nm)[74] Aluminium gallium nitride (AlGaN) Aluminium gallium indium nitride (AlGaInN)—down to 210 nm[75]
Pink	Multiple types	$\Delta V \sim 3.3$[76]	Blue with one or two phosphor layers: yellow with red, orange or pink phosphor added afterwards, or white phosphors with pink pigment or dye over top.[77]
White	Broad spectrum	$\Delta V = 3.5$	Blue/UV diode with yellow phosphor

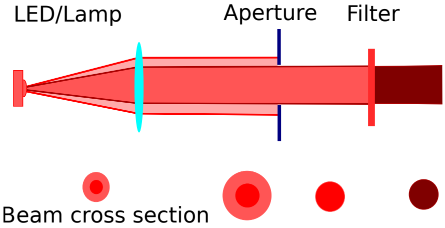


- **375 nm** – excitation of Hoechst stain, Calcium Blue, and other fluorescent dyes in fluorescence microscopy
- **405 nm** – InGaN blue-violet laser, in Blu-ray Disc and HD DVD drives
- **445 nm** – InGaN deep blue laser multimode diode recently introduced (2010) for use in mercury free high brightness data projectors
- **473 nm** – sky blue laser pointers, still very expensive, output of DPSS systems
- **485 nm** – excitation of GFP and other fluorescent dyes
- **510 nm** – (to ~525 nm) green diodes recently (2010) developed by Nichia and OSRAM for laser projectors.
- **635 nm** – AlGaInP better red laser pointers, same power subjectively twice as bright as 650 nm
- **640 nm** – high-brightness red DPSS laser pointers
- **650 nm** – GaInP/AlGaInP CDDVD, cheap red laser pointers
- **670 nm** – AlGaInP bar code readers, first diode laser pointers (now obsolete, replaced by brighter 650 nm and 671 nm DPSS)
- **671 nm** – spectroscopy, DNA sequencing, high-power red DPSS laser pointers
- **760 nm** – AlGaInP gas sensing: O_2
- **785 nm** – GaAlAs Compact Disc drives
- **808 nm** – GaAlAs pumps in DPSS Nd:YAG lasers (e.g. in green laser pointers or as arrays in higher-powered lasers)

Wikipedias List of LED (left) and LASER (right) wavelength and materials.

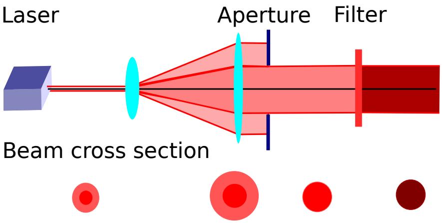
- Today: Variety of semiconductor materials to obtain different wavelength

Illumination/Source: Lamps and LEDs



- Light emission under some angle
- Lens (system) to obtain a parallel beam
- Aperture to block out low intensity outer regions
- Filter to narrow spectrum (LED typ. 25 nm FWHM)
- Ideal result: A monochrome, parallel beam with uniform intensity

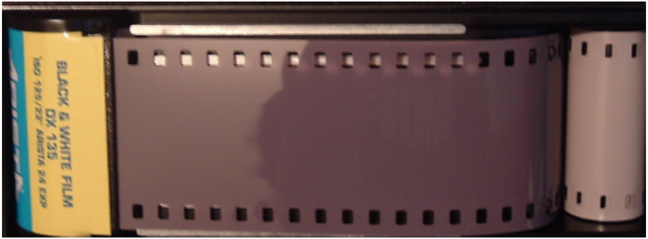
Illumination/Source: Lasers



- LASER emits almost parallel light
- Lens (system) to widen beam
- Aperture to block out low intensity outer regions. A nice profile is Gaussian, a diode can be much worse.
- Maybe: filter to clean up / correct spectrum. Native laser diode spectrum depends on technical details (current control, mechanical construction).
- Ideal result: A monochrome, coherent, parallel beam with uniform intensity

Light detection
Photons to electrons, (digital) read-out

History: Film

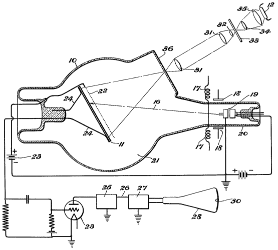


Wikimedia: Film

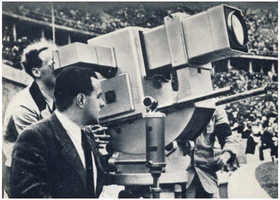
- Uses a photo-induced chemical reaction
- Today made obsolete by semiconductor devices and computer processing
- Question: How was live TV done before semiconductors?

History: Iconoscope

- Photo-sensitive coating on charged plate
- Photons allow local discharge of electrons
- Current of a read-out electron beam measures remaining charge

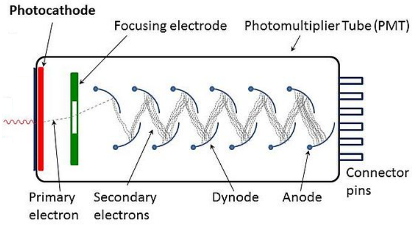


From US patent 2021907 (Wikimedia)



The "Olympic Cannon" (Wikimedia)

Point detectors: Photo multiplier tubes (PMTs)



Wikimedia: PMT

Wikimedia: PMT photo

- Photo effect yields one electron
- Cascade effect yields a few thousand electrons after some stages
- Read-out electronics has a much easier job measuring these
- Avalanche photo diodes: semi-conductor version of this effect

Camears / Light detection with semiconductors
→ Next lecture