



'ELITEAM'- ESTABLISHMENT OF THE ELI INSTITUTE AT THE
UNIVERSITY OF SZEGED: FOUNDATION OF INTERDISCIPLINARY
RESEARCH IN THE FIELD OF LASERS AND THEIR APPLICATIONS

LASERS in BIOPHYSICS

Why is the laser light unique?

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HUNGARIAN
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Double aims of the present talk:

principal messages of the lectures of the school

+ use of lasers in biophysics

1) Basic properties of laser light

**2) The most interesting and hidden
property: the coherence**

**3) Some applications of lasers in
biophysics research**

1) Principles of laser operation and major properties of laser radiation

- Absorption and spontaneous and stimulated emissions.
- Population inversion and the optical gain.
- Laser oscillator
- Continuous and pulsed operation
- Non-linear effects (frequency doubling)
- Modulators in the cavity: Q-switch and Pockels cell
- Tuning the laser
- Laser types: solid state, semiconductor, gas and dye lasers.
- Basic properties of laser light: monochromatism, spatial and time coherence, small divergence and high intensity
- What is unique in laser light? Distinction between thermal and laser light sources.
 - Utilization of high coherence of laser light.
 - Coherence and photon statistics (Poisson and Bose-Einstein statistics). Photon counting processes (blackening of photographic layers and photosynthesis).

Absorption and emission transitions and the Einstein coefficients in a two-level system.

Because of the detailed balance or microscopic reversibility, symmetry exists between the two states:

$$B_{12} = B_{21}$$

In equilibrium with the radiation field $\rho(\nu) \cdot B_{21} \cdot (n_1 - n_2) - A_{21} \cdot n_2 = 0$

Here $\rho(\nu)$ denotes the density of light energy per unit frequency of the radiation field given by the Planck expression at temperature T :

$$\rho(\nu) = \frac{4 h \nu^3}{c^3} \cdot \frac{1}{e^{h\nu/k_B T} - 1}$$

Boltzmann equation related to the populations of the two states: $\frac{n_2}{n_1} = \exp\left(-\frac{h \nu_{12}}{k_B T}\right)$

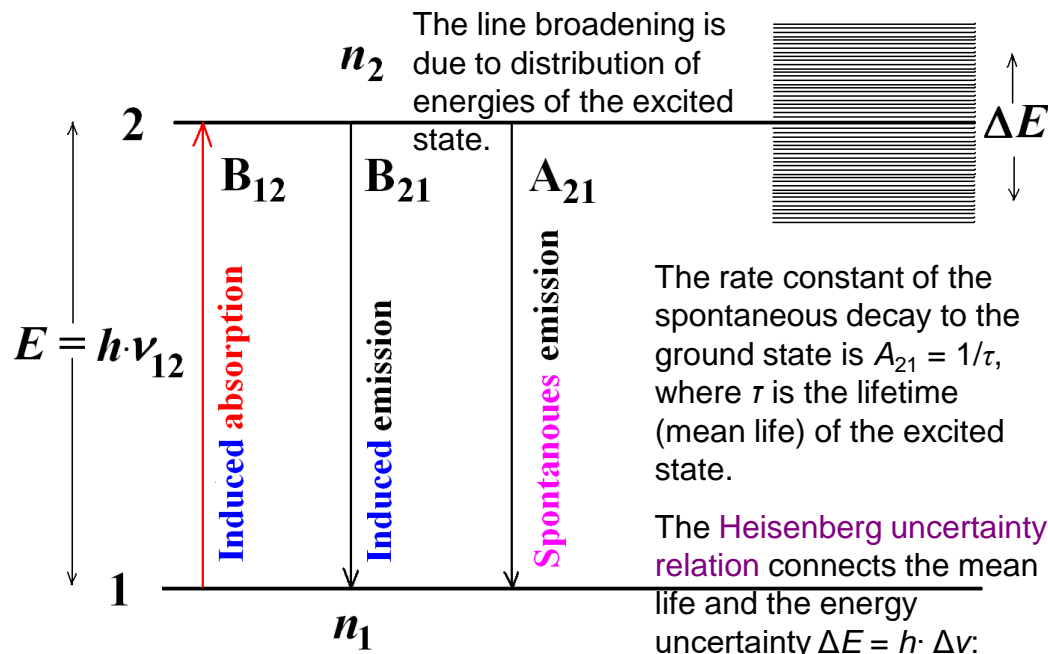
$$A_{21} = \frac{4 \cdot h \nu_{12}^3}{c^3} \cdot B_{12}$$

Differences between induced and spontaneous emissions

1. The spontaneous emission dominates if E is large, and the induced emission is the major form of emission if E is small:

$$\frac{A_{21}}{B_{21}} = \frac{4}{h^2 c^3} \cdot E^3$$

2. Directional and phase dependence. The direction and phase of the induced emission are the same as those of the incident radiation. In the spontaneous emission, however, neither the phase nor the direction are correlated to those of the external radiation; the direction is determined by the orientation of the molecule.



The rate constant of the spontaneous decay to the ground state is $A_{21} = 1/\tau$, where τ is the lifetime (mean life) of the excited state.

The Heisenberg uncertainty relation connects the mean life and the energy uncertainty $\Delta E = h \cdot \Delta \nu$:

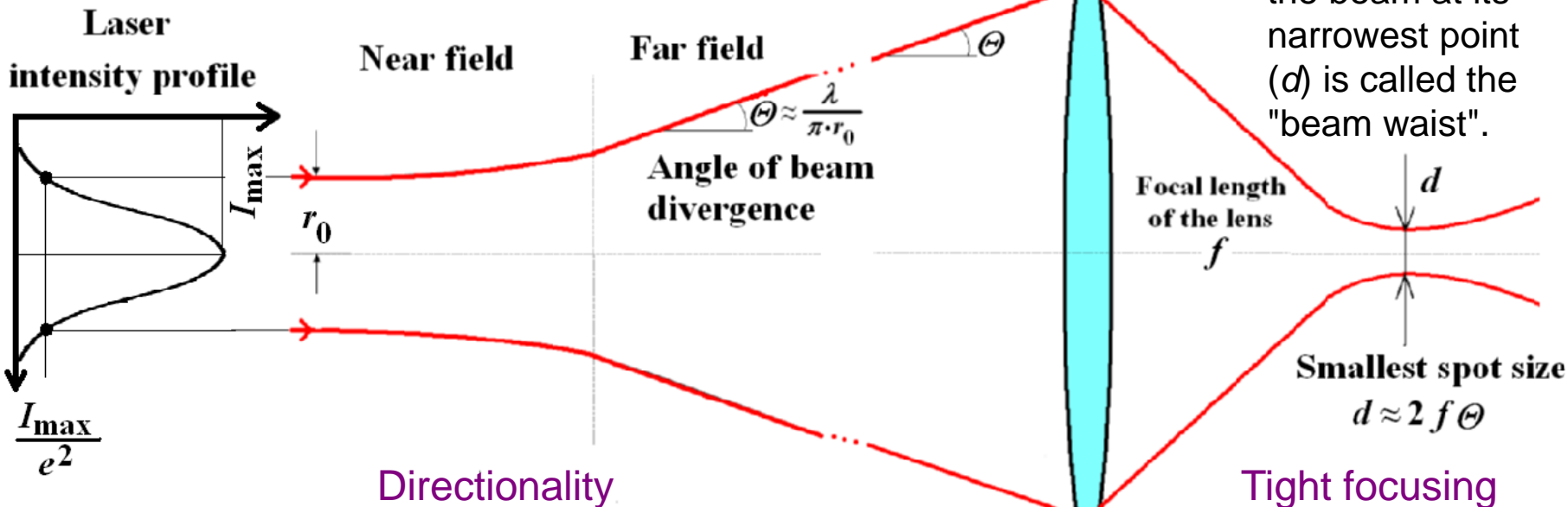
$$\tau \cdot \Delta E = \frac{h}{2\pi}$$

Diffraction limited divergence and convergence of a laser beam due to a converging lens: spatial distribution profile of the laser intensity at I_{\max}/e^2 (note, this is not the path of the ray).

I_{\max}/e^2 (note, this is not the path of the ray).

For a perfect gaussian beam, the diffraction limited divergence:

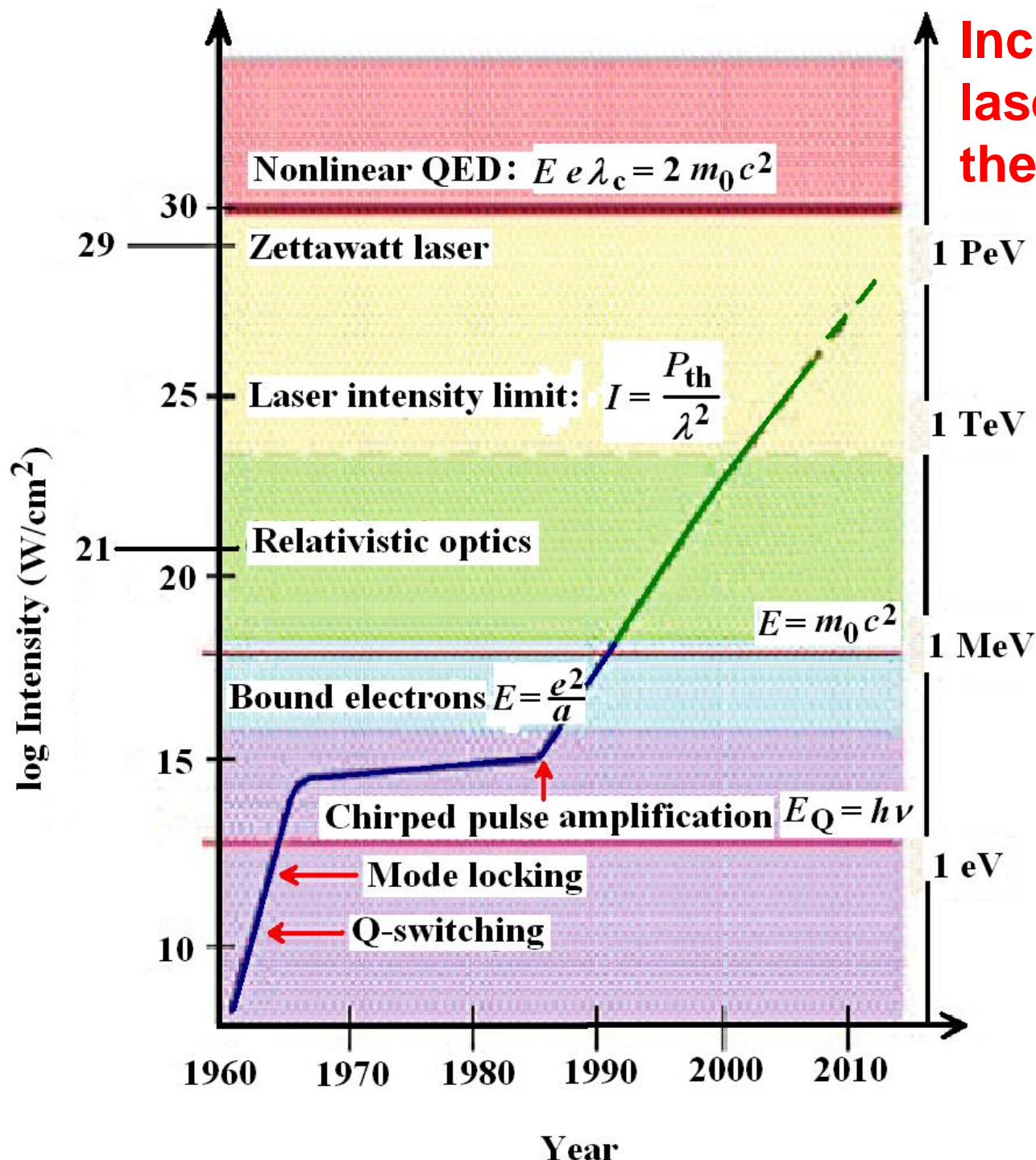
The diameter of the beam at its narrowest point (d) is called the "beam waist".



For a **low gain system**, the use of a Fabry - Perot resonator with parallel feedback mirrors, imposes some directionality on the output of the laser that is equivalent of producing beam with divergence close to the diffraction limit. For a **high gain system** (single pass laser; super radiance), generally larger divergence is produced (many times of the diffraction limit).

Examples. 1) If a laser operating at 500 nm has a beam spot size of 10 cm i.e. $r_0 = 5$ cm, then the diffraction limited beam divergence amounts $\Theta = 3.18 \cdot 10^{-6}$ rad. By illuminating the surface of the Moon at distance $R = 3.84 \cdot 10^5$ km from the Earth, the spread in diameter would be $D = 2 \cdot \theta \cdot R = 2.4$ km. 2) Apollo 11 (1969) placed corner cube reflector on the Moon surface to measure the exact distance between the Earth and the Moon. The measurement was done by time delay with an accuracy of about 1 ns i.e. 30 cm. The round trip of the light took about 2.5 s.

Example. If we take a converging lens of an f -number of 2 (i.e., the focal length is twice the lens diameter), then for a laser wavelength of 600 nm, the radius of the focused spot will be about 1.5 μm . Thus, the area of such a focused spot would be about 7 μm^2 .

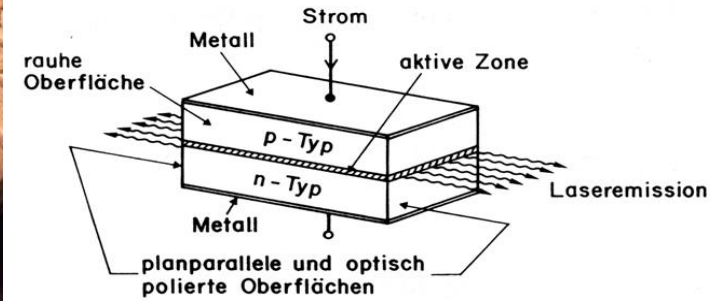


Increase of achievable laser light intensity in the past

The increase has a large slope around 1960 due to the invention of the laser and then again after 1985. (Adapted from Mourou and Yanovsky (2004)).

At intensities of $10^{21} W/m^2$, the electric fields become so high that the electrons accelerate to relativistic velocities (approaching that of light) and at even higher light intensities the photons of the crossing light beams will get into interactions (the light paths are mutually influenced) leading to very interesting and useful **non-linear effects**.

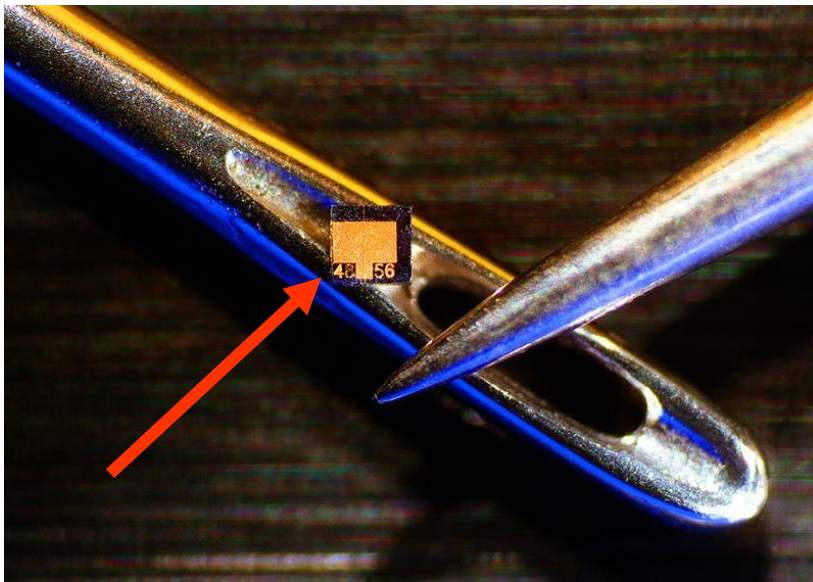
Extreme differences between lasers



National Ignition Facility:

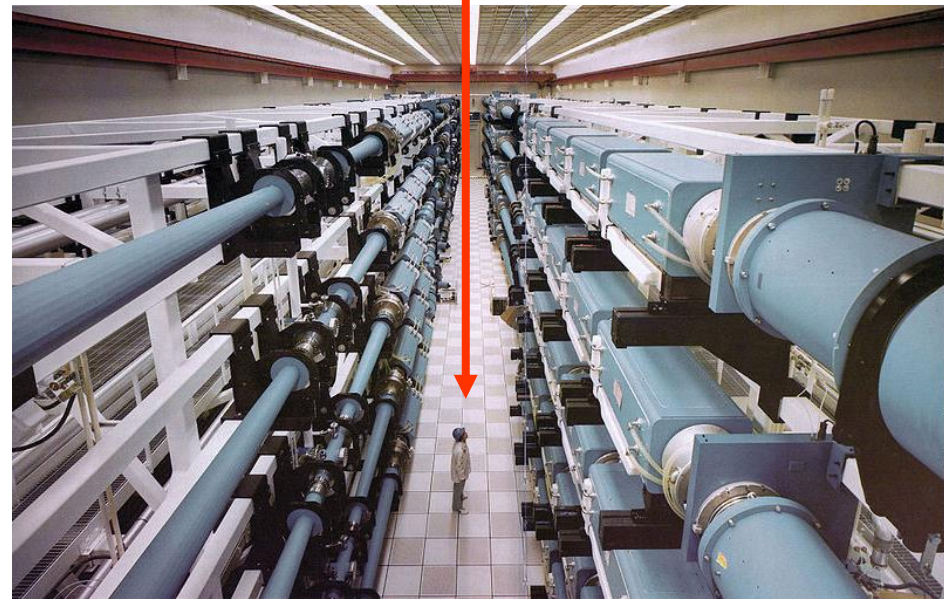
beams of 192 lasers are focussed on 1 mm³ capsule filled with either D-T gas or D-T ice.

man



tunable laserdiode

„Laser of poor scientists”



NOVA Laser in Lawrence Livermore

National Laboratory

High energy, power, intensity and brightness

In a laser system, large energy can be stored and released in a very short time resulting in enormous output power. The extremely high power can also be focused to very small areas generating extremely high-intensity values.

Continuous wave lasers having power levels $\sim 10^5$ W and pulsed lasers having a total energy ~ 50 kJ have applications in welding, cutting, laser fusion, etc.

Examples. 1) Compare the **brightness of the Sun** with that of a typical He-Ne gas laser. The Sun has a surface temperature of $\sim 6,000$ K and the power density of its radiation is $\rho = \sigma T^4 = 5.6696 \cdot 10^{-8} \cdot (6,000)^4 \text{ W} \cdot \text{m}^{-2} = 7.4 \cdot 10^7 \text{ W} \cdot \text{m}^{-2}$ derived from the Stefan-Boltzmann's law. Thus, the brightness of the sun (power density in unit of solid angle) amounts $\rho / 4\pi = 5.9 \cdot 10^6 \text{ W/m}^2/\text{sr}$.

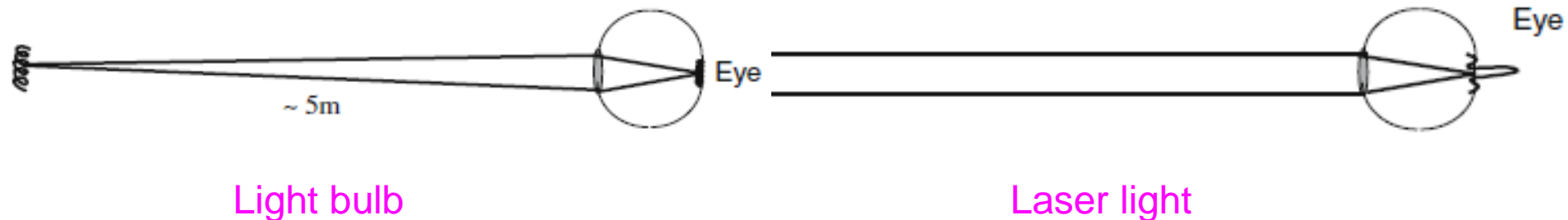
A typical He-Ne laser has a spot size $2 \cdot r_0 = 1$ mm, power $P = 1$ mW and divergence $\Theta = 0.5$ mrad. The power density of the laser amounts $\rho = P / (r_0^2 2\pi) = 1273 \text{ W/m}^2$. The solid angle is $\Omega = A / d^2 = \pi \cdot \Theta^2 = 7.85 \cdot 10^{-7} \text{ sr}$, after replacing the area $A = r^2 \pi = (d \cdot \Theta)^2 \pi$ at distance d from the laser.

Thus, the brightness of the He – Ne laser is $\rho / \Omega = 1.62 \cdot 10^9 \text{ W/m}^2/\text{sr}$ which is about 300 times larger than that of the Sun.

High energy, power, intensity and brightness

Examples. 2) Looking at a 20 W bulb at a distance of about 5 m, the eye produces an image of the bulb on the retina of an **intensity** of only about 10 W/m^2 .

On the other hand, a low-power ($\approx 2 \text{ mW}$) diffraction-limited laser beam incident on the eye gets focused to a very small spot and can produce an intensity of about 10^6 W/m^2 on the retina which could damage the retina. Thus, whereas it is quite safe to look at a 20 W bulb, it is very dangerous to look directly into a 2 mW laser beam. (Similarly dangerous looking directly at the sun not only because of the high power density of the image formed in the retina ($\sim 3 \cdot 10^4 \text{ W/m}^2$) but also because of the large ultraviolet content of the sunlight). As the laser beam can be focused to very narrow areas, it has found important applications in areas like eye surgery and laser cutting.



2) NON-CONVENTIONAL PROPERTY OF LASER LIGHT:

COHERENCE

BASICS

and

APPLICATIONS

Thermal light sources



Lasers

Properties

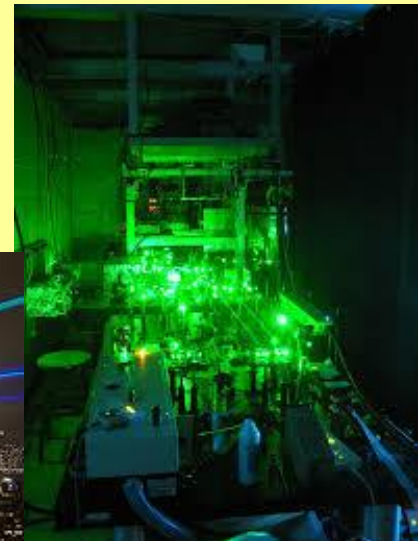
Directionality?
Small spot size
(tight focusing)?

High energy,
power, intensity
and brightness?

Monochromaticity
(spectral purity)?

Coherence?

Photon distribution?



The incident light can come either from
Frederick William Herschel (~1800)



thermal light source

or from



laser

(Audi Quattro headlight).

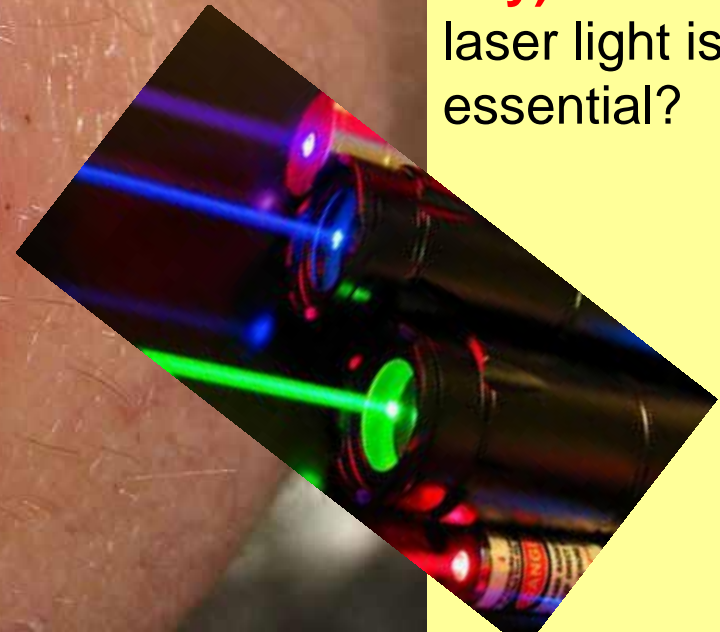
How to decide from inside?

This is an interesting and theoretical question of not much practical use. However, there are more practical (medical) consequences of the principal question.

Wound healing by (laser) light therapy



Which property (**if any**) of the laser light is essential?



LIGHT SOURCES : THERMAL AND COHERENT LIGHT

Thermal Light



- Various Frequencies
- Out of Phase
- Bose-Einstein Distribution
- Blackbody

Coherent Light

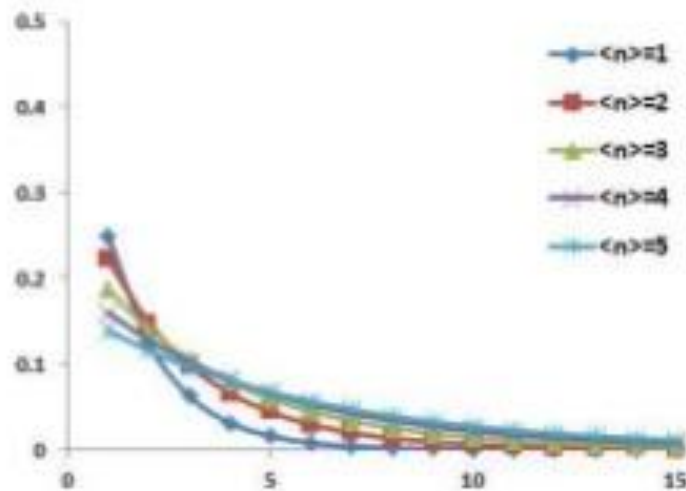


- Single Frequency
- In Phase
- Poisson Distribution
- Laser

LIGHT SOURCES : THERMAL AND COHERENT LIGHT

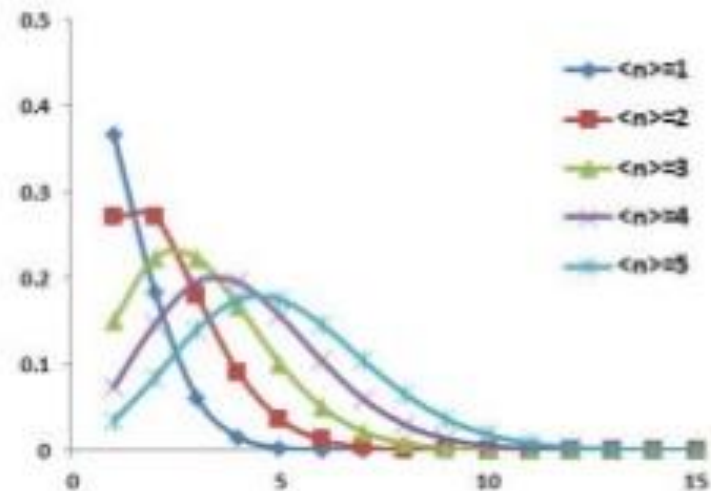
Thermal Light :
Bose Einstein Distribution

$$p_n = \frac{e^{-E_n/k_B T}}{\sum_n e^{-E_n/k_B T}} = \frac{\bar{n}^n}{(1 + \bar{n})^{1+n}}$$

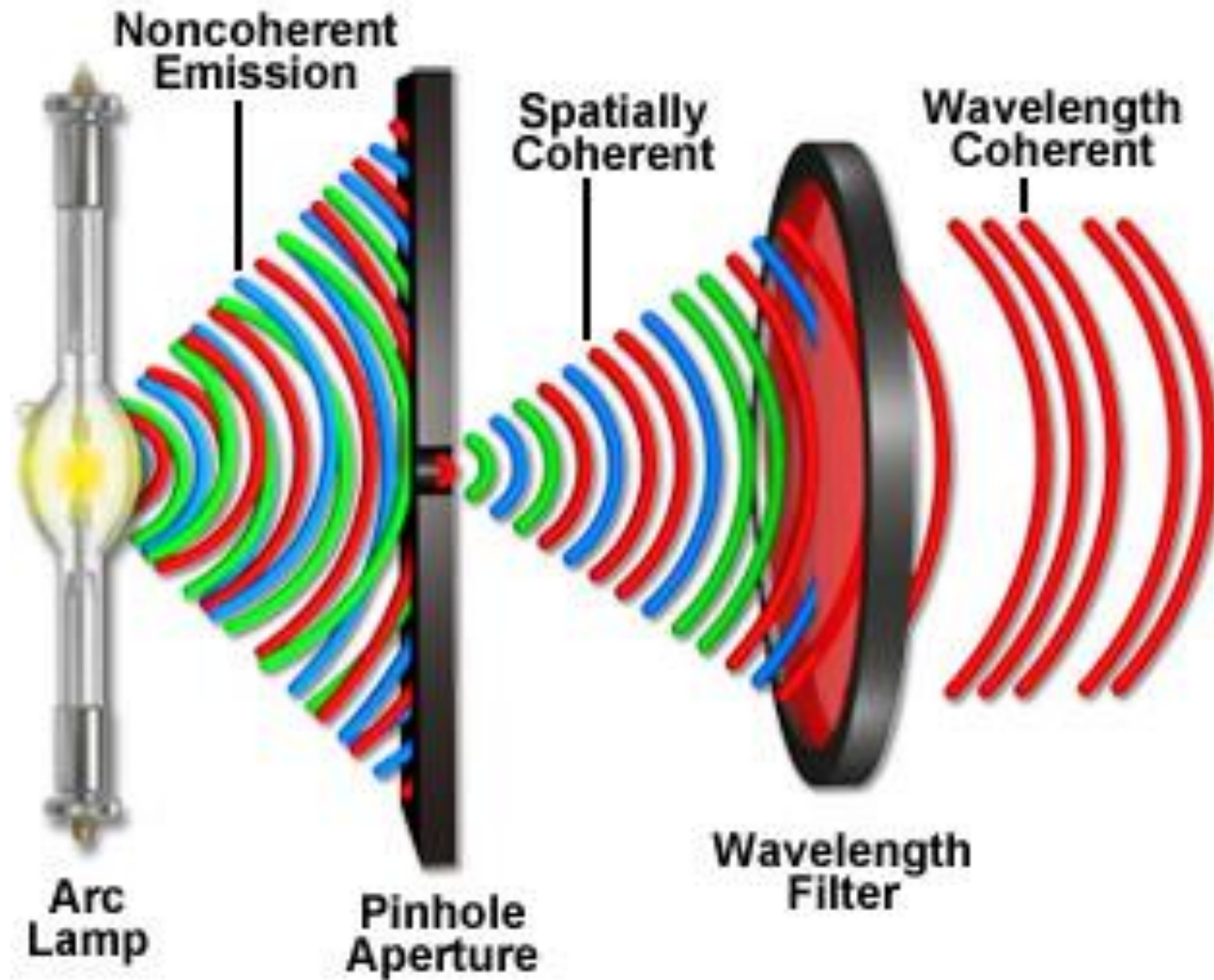


Coherent Light :
Poisson Distribution

$$p_n = \langle n | \alpha \rangle^2 = e^{-|\alpha|^2} \frac{|\alpha|^{2n}}{n!} = e^{-\bar{n}} \frac{\bar{n}^n}{n!}$$

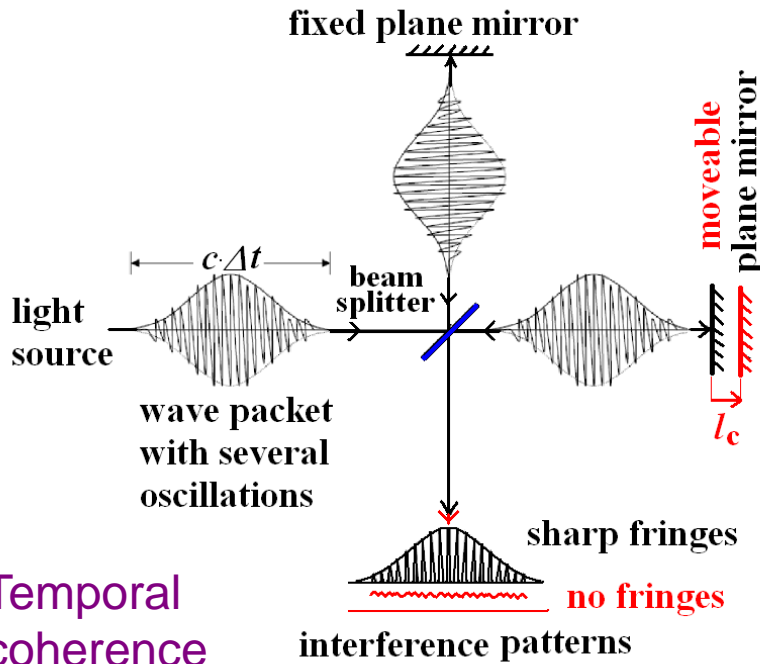


Light wave coherence

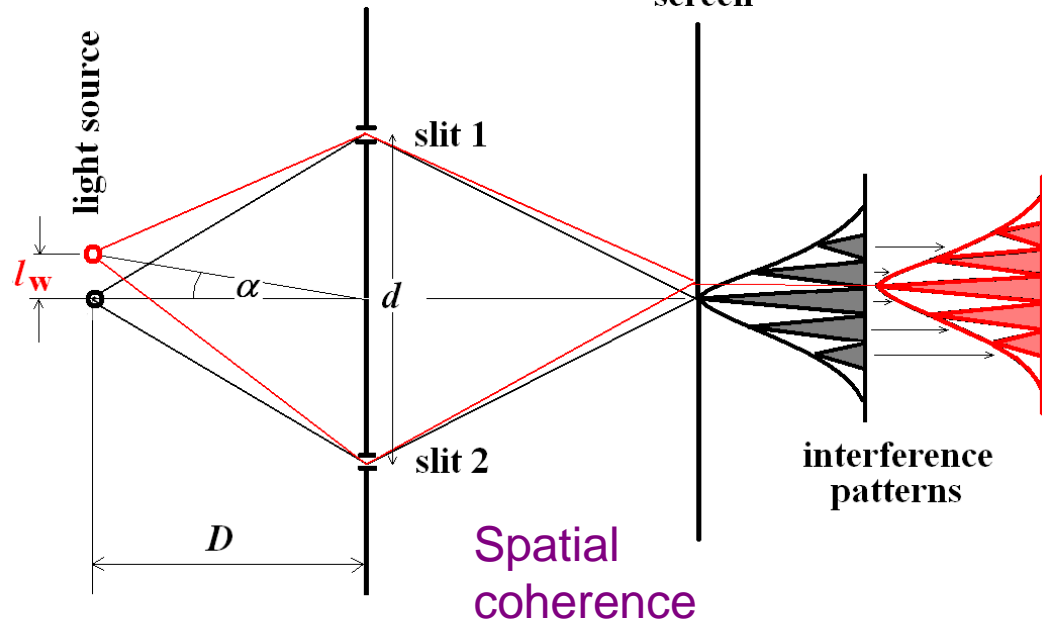


Michelson and Young's double slit interferometers to measure the temporal ($\ell_c = c\Delta t = c \cdot \tau_c$) and spatial (ℓ_w) coherence of light sources, respectively.

Michelson interferometer



Young's double slit interferometer



The electromagnetic wave has perfect spatial coherence when the phase difference remains the same for any two points anywhere on the wave front. The spatial coherence is related to the directionality and to the uniphase wave fronts. A plane wave is highly directional, the phase difference between two points is constant (if the two points are on the wave front, then the difference is 0) and remains so at all time. This is characteristic for a coherent laser beam. At spontaneous emission, however, the wave front is distorted, the phase difference between two points will change and therefore the spatial coherence is highly limited. As the spatial coherence is high for sphere waves and plane waves, **light from distant stars**, though far from monochromatic, **has extremely high spatial coherence**.

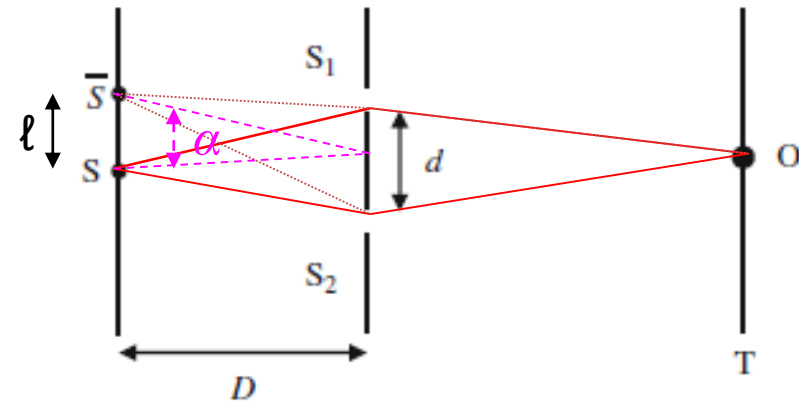
As the emission of a photon occurs within a finite (life) time Δt , we can visualize the photon as a wave packet of a finite spread in time with several oscillations. Because the life time Δt is related to the linewidth $\Delta \nu$ as $\Delta t = 1/\Delta \nu$, we can introduce the time of coherence as $\tau_c \approx \Delta t$ or more precisely

$$\tau_c = \frac{1}{\Delta \nu}$$

We can similarly visualize the extension of the wave packet in space and define the (longitudinal) length of coherence as

$$l_c = c \cdot \tau_c$$

Spatial coherence measured by Young's double-hole experiment



$$\bar{S}S_2 = \left[D^2 + \left(\frac{d}{2} + l \right)^2 \right]^{1/2} \approx D + \frac{1}{2D} \left(\frac{d}{2} + l \right)^2$$

$$\bar{S}S_1 = \left[D^2 + \left(\frac{d}{2} - l \right)^2 \right]^{1/2} \approx D + \frac{1}{2D} \left(\frac{d}{2} - l \right)^2$$

For disappearance of fringes $\bar{S}S_2 - \bar{S}S_1 = \frac{\lambda}{2} \approx \frac{ld}{D}$ or $l \approx \frac{\lambda D}{2d}$

For an extended source made up of independent point sources, one may say that good interference fringes will be observed as long as

$$l \ll \frac{\lambda D}{d}$$

Equivalently for a given source of width l , interference fringes of good contrast will be formed by interference of light from two point sources S_1 and S_2 separated by a distance d as long as

$$d \ll \frac{\lambda D}{l}$$

Since l/D is the angle (say α) subtended by the source at the slits the above equation can also be written as

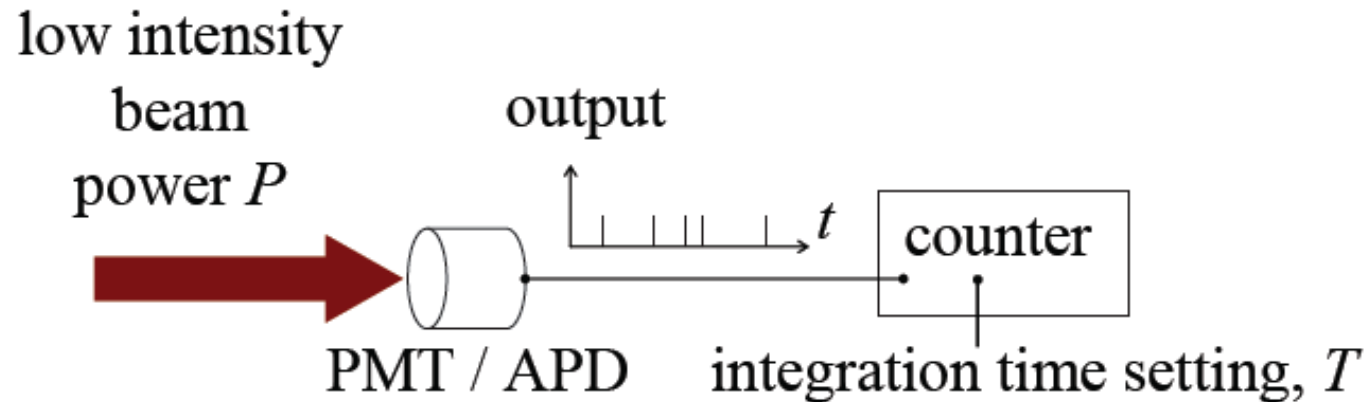
$$d \ll \frac{\lambda}{\alpha}$$

The distance $l_w (= \lambda/\alpha)$ is referred to as the **lateral coherence width**. It can be seen that l_w depends inversely on α .

$$l_w = \frac{\lambda}{\alpha}$$

Example. The angle subtended by sun on the earth is 32 s of arc which is approximately 0.01 radians. Thus assuming a wavelength of 500 nm, the lateral coherence width of the sun would be 50 μm . Thus if we have a pair of pinholes separated by a distance much less than 50 μm , and illuminated by the sun, interference pattern of good contrast will be obtained on the screen.

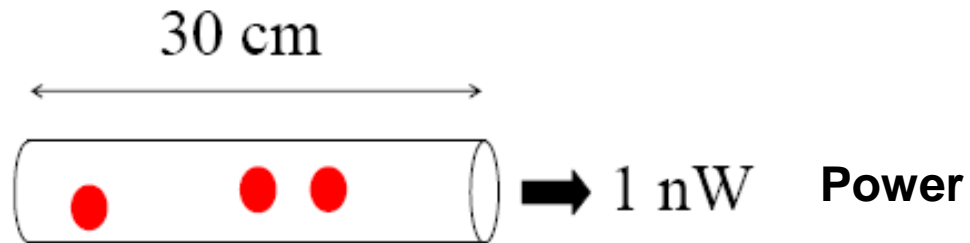
Photon streams and counting



He-Ne Gas Laser

wavelength

$$\lambda = 633 \text{ nm}$$



$$\Phi = 3.1 \times 10^9 \text{ photons / s} \quad \text{Photon flux}$$

\Rightarrow average of 3 photons in 30 cm of beam

\Rightarrow timing random on very short time scales

\Rightarrow Poissonian statistics

Poisson statistics

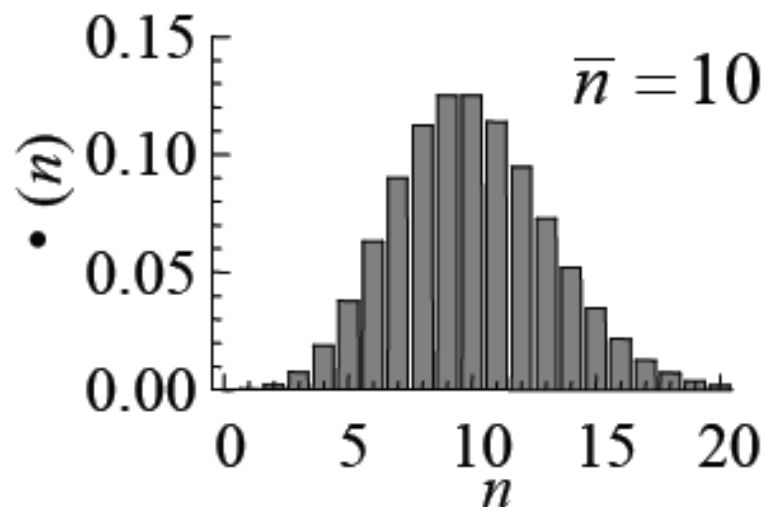
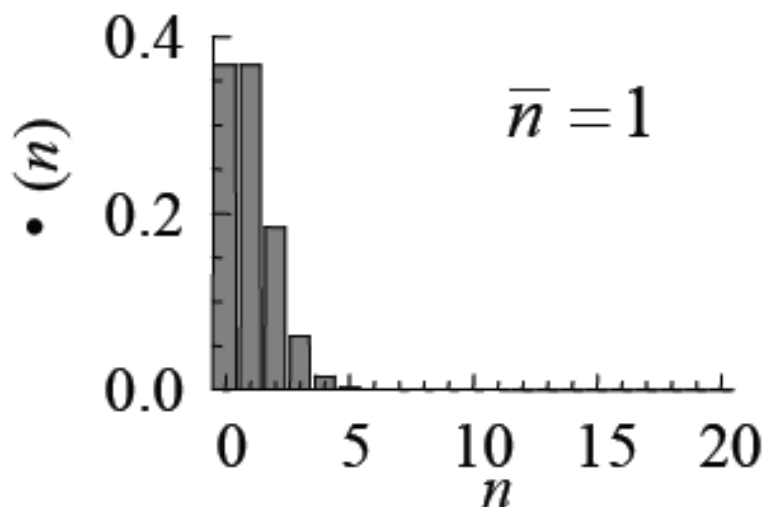
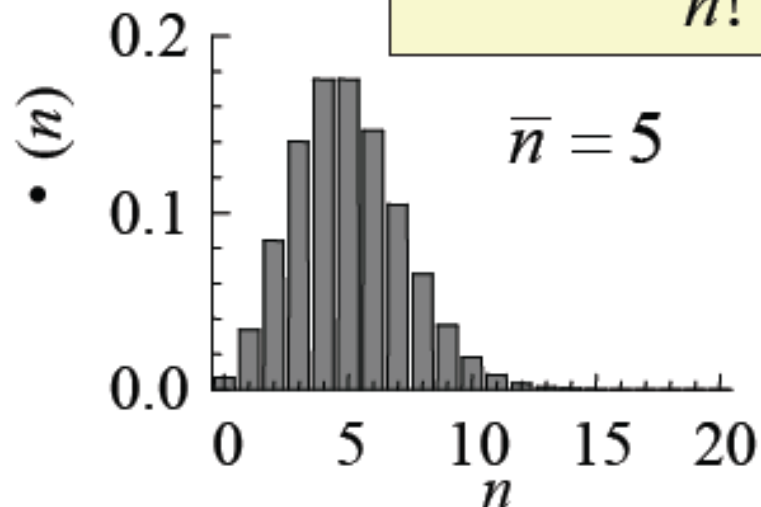
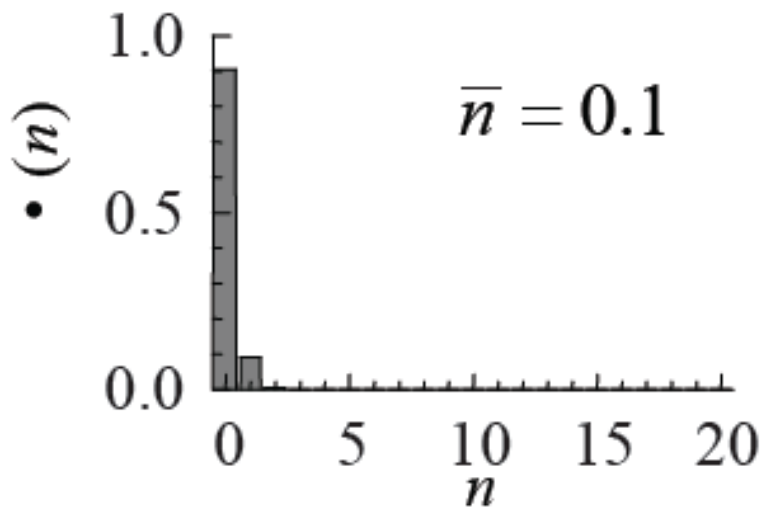
$$P(n) = \frac{\bar{n}^n}{n!} \exp(-\bar{n}) \quad \bar{n} = \text{mean}$$
$$\Delta n = \sqrt{\bar{n}} \quad \Delta n = \text{standard deviation}$$

- Random events with *discrete* outcomes. [cf normal (Gaussian) distribution for *continuous* variables.]
- Average well-defined, but individual events random
- Examples:
 - number of rain drops falling in time T
 - number of radioactive decays in time T
 - number of photons from starlight detected in time T

The photons from a **coherent light** source follow **Poisson statistics**: the individual events (appearance of photons) are **rare** and **random** and the outcomes are **discrete**.

Poisson distributions

$$P(n) = \frac{\bar{n}^n}{n!} \exp(-\bar{n})$$



Thermal light (black body radiation)

Bose-Einstein distribution

For a chaotic (incoherent) light source, like a light bulb, the numbers of detected photons are distributed according to a Bose-Einstein distribution:

$$P(n) = \frac{1}{\bar{n} + 1} \left(\frac{\bar{n}}{\bar{n} + 1} \right)^n$$

where the mean (expected) number of photons depends on the temperature T :

$$\bar{n} = \frac{1}{\exp(f(\hbar\omega)/k_B T) - 1}$$

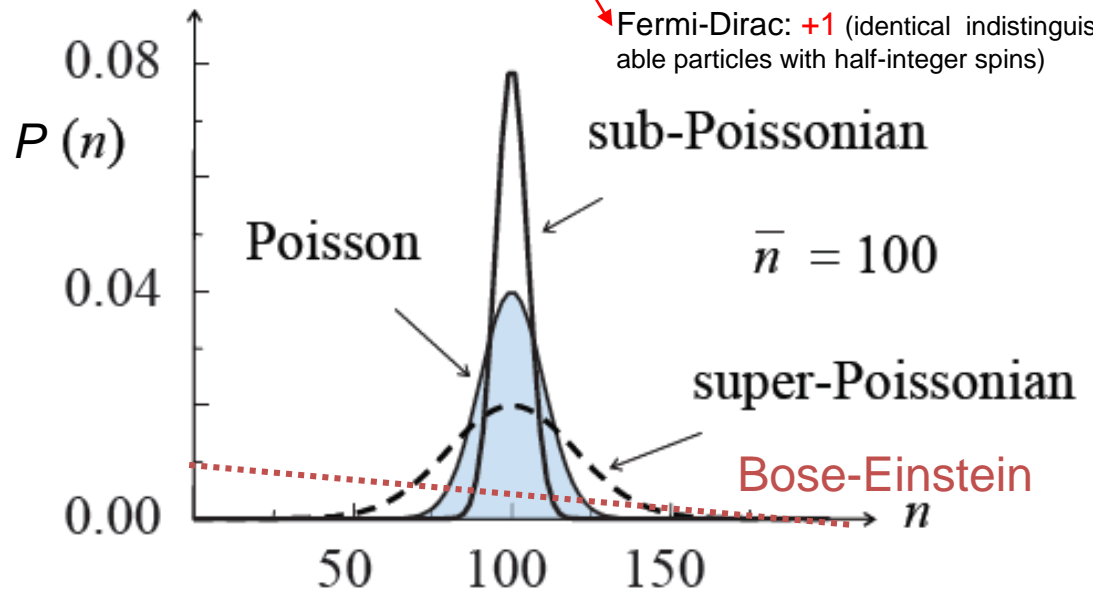
Maxwell-Boltzmann: **0** (classical, identical but distinguishable particles)

Bose-Einstein: **-1** (identical indistinguishable particles with integer spins)

Fermi-Dirac: **+1** (identical indistinguishable particles with half-integer spins)

The standard deviation of the Bose-Einstein distribution is larger than that of the Poisson distribution (super Poisson distribution):

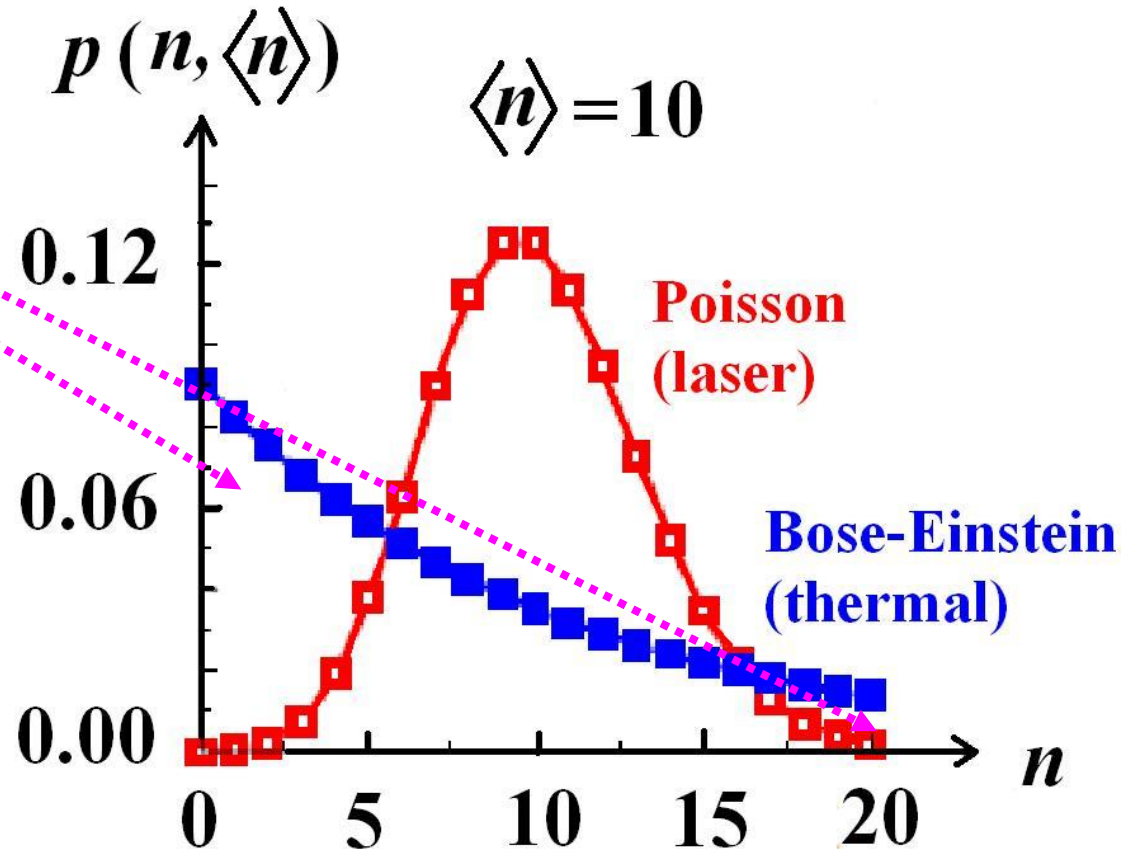
$$\Delta n = \sqrt{\bar{n} + \bar{n}^2}$$



Poisson and Bose-Einstein photon distribution

for identical mean photon number $\langle n \rangle = 10$

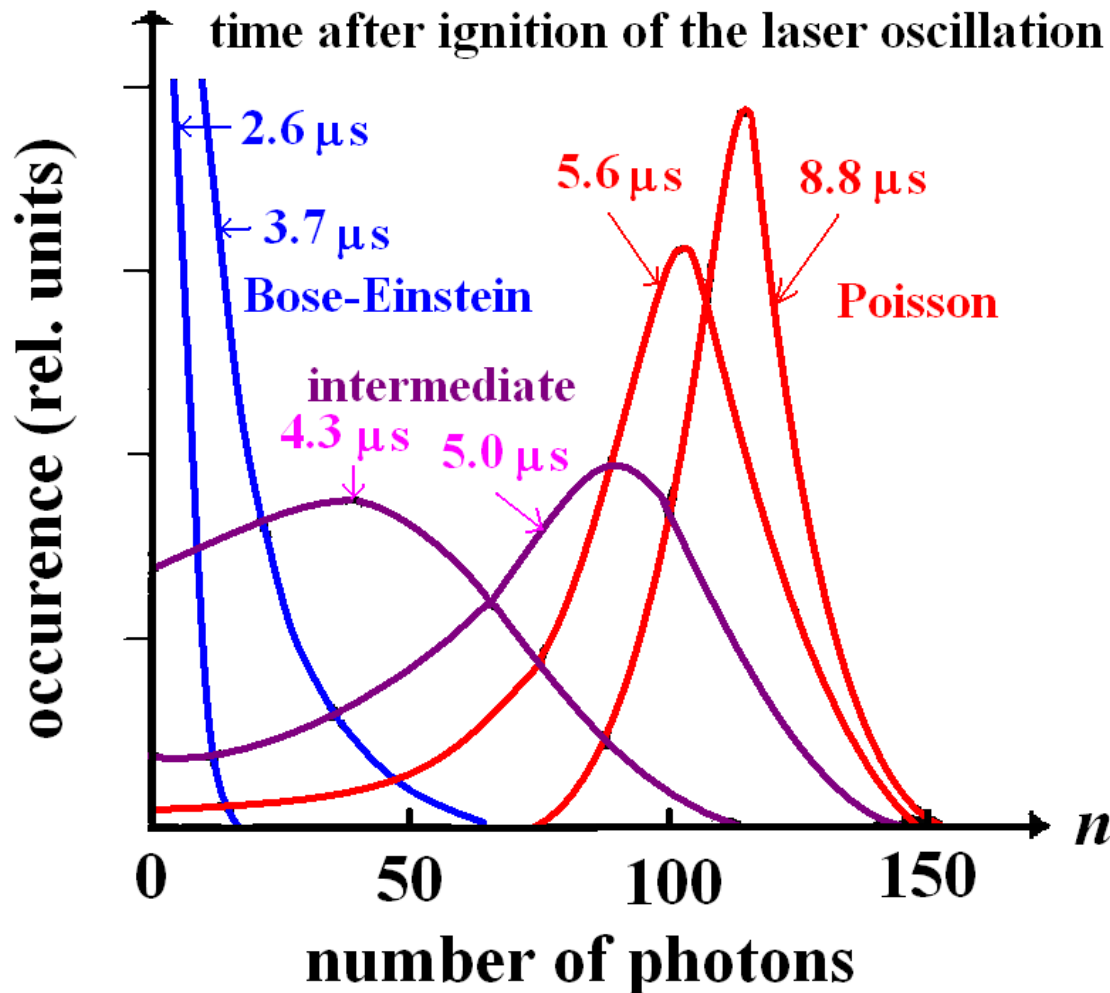
Significant **clumping** of photons (increase of p) occurs in the **Bose-Einstein** statistics relative to **Poisson** statistics at low photon numbers.



Both functions have the same expectation value (mean). For the Poisson distribution, the expectation value and variance of the photon number coincide; on the other hand, the variance of the thermal photon number distribution goes beyond its expectation value.

Statistical distributions of photons

detected at different times following the startup of the laser oscillation.



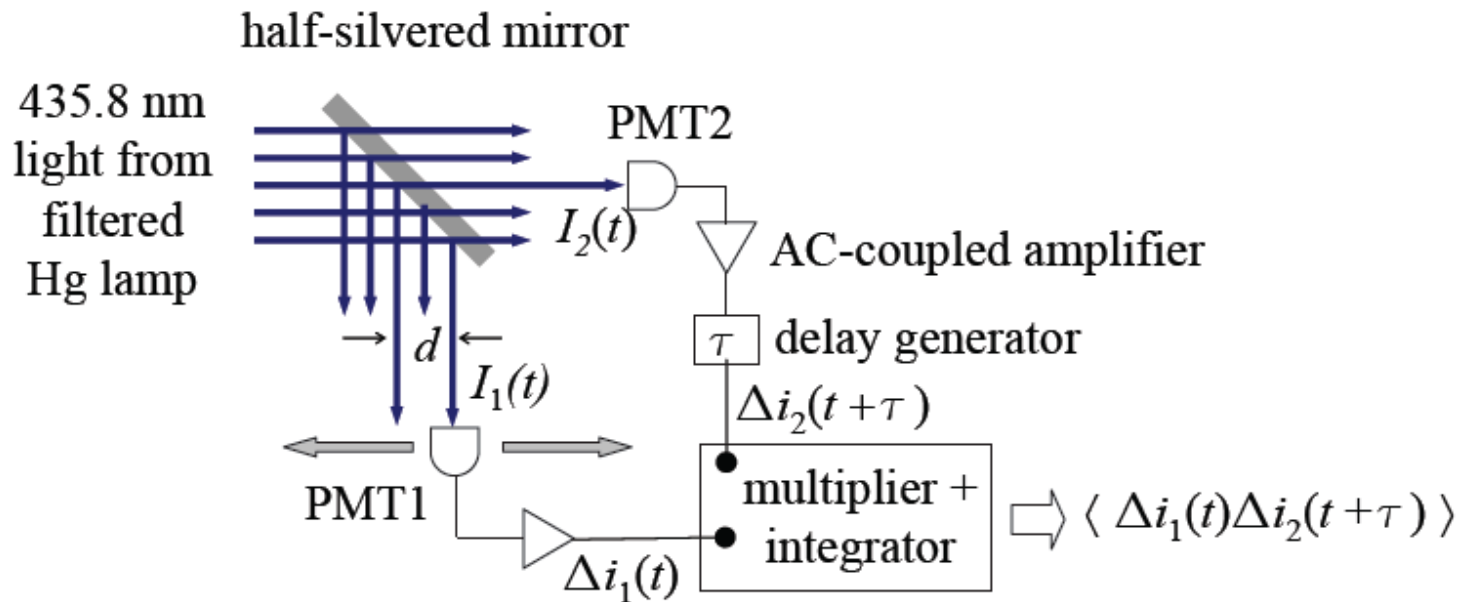
At short times ($< 3 \mu\text{s}$), the source is chaotic and the distribution is of **Bose-Einstein** type.

At longer times ($> 8 \mu\text{s}$), the source is a laser and the distribution becomes **Poisson**.

In the intermediate time, the distribution is a **mixture** of the two main statistics.

Second order intensity correlation functions: Hanbury Brown-Twiss experiments (1956) and photon bunching

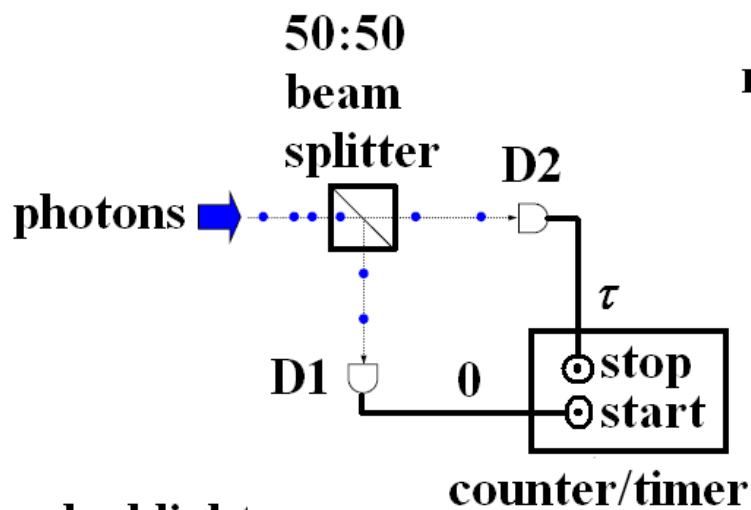
The best way to decide whether a light source is a laser or a thermal source with a very narrow band width is to measure the second order correlation function. The correlation function is obtained from the probabilities that photons arrive in coincidence at two photon detectors as a function of the arrival time difference τ :



Measures second-order correlation function

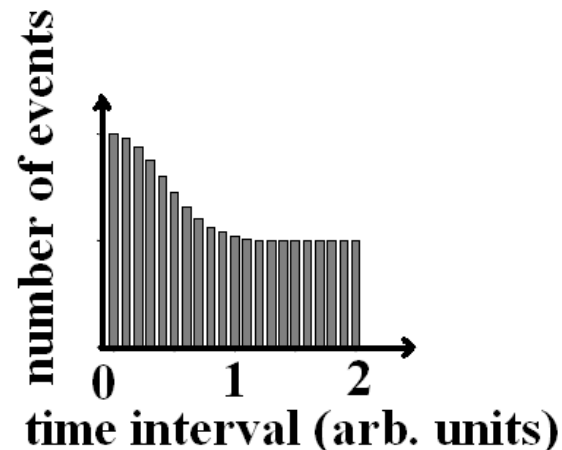
$$g^{(2)}(\tau) = \frac{\langle I(t)I(t + \tau) \rangle}{\langle I(t) \rangle \langle I(t + \tau) \rangle}$$

To determine the second order intensity correlation function $g^2(\tau)$ of the optical field after Hanbury Brown and Twiss (1956)



measures

$$g^{(2)}(\tau)$$



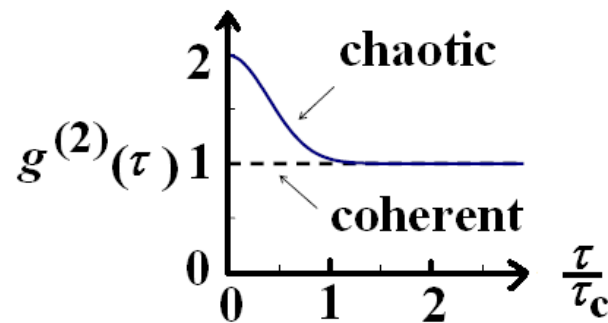
bunched light

... ..

coherent light

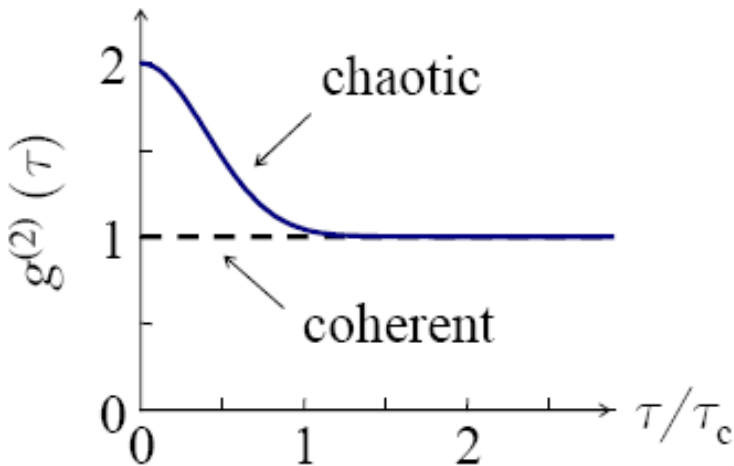
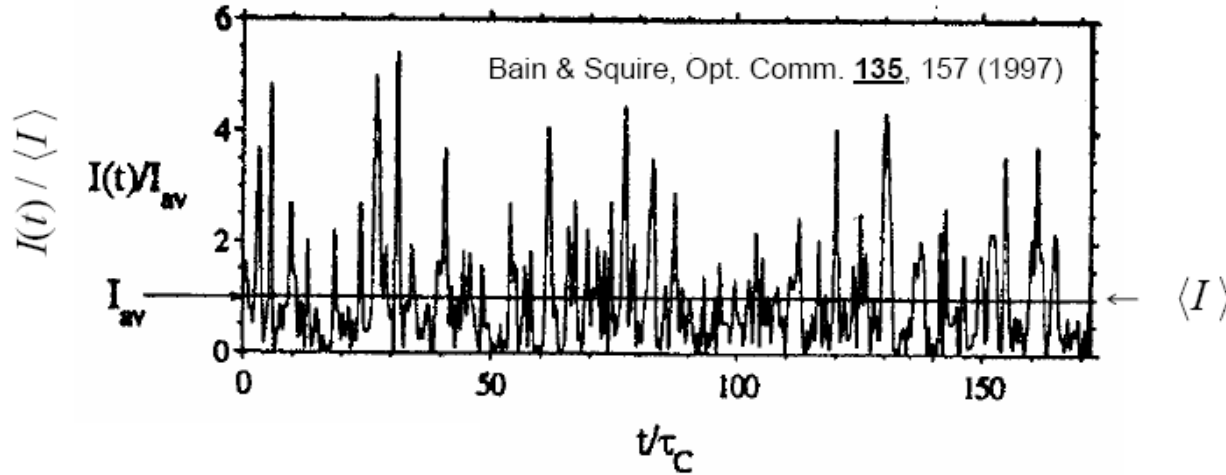
.....

no intensity fluctuations
 random time intervals: all times equally likely
 (Poisson statistics)



The photons in the splitted beams are detected by D1 and D2 photodetectors which initiate and terminate the counter after an arbitrary time interval τ , respectively. From the histogram, the second order correlation function can be derived that indicates the bunching of photons delivered by chaotic light source in less time than the time of coherence.

The intensity of **chaotic source** fluctuates wildly on time scales much more than that of the coherence time τ_c



Coherent light:

$$g^{(2)}(\tau) = 1 \text{ for all } \tau$$

Gaussian chaotic light:

$$g^{(2)}(\tau) = 1 + \exp[-\pi(\tau/\tau_c)^2]$$

All classical light:

$$g^{(2)}(0) \geq 1$$

$$g^{(2)}(0) \geq g^{(2)}(\tau) \text{ for all } \tau$$

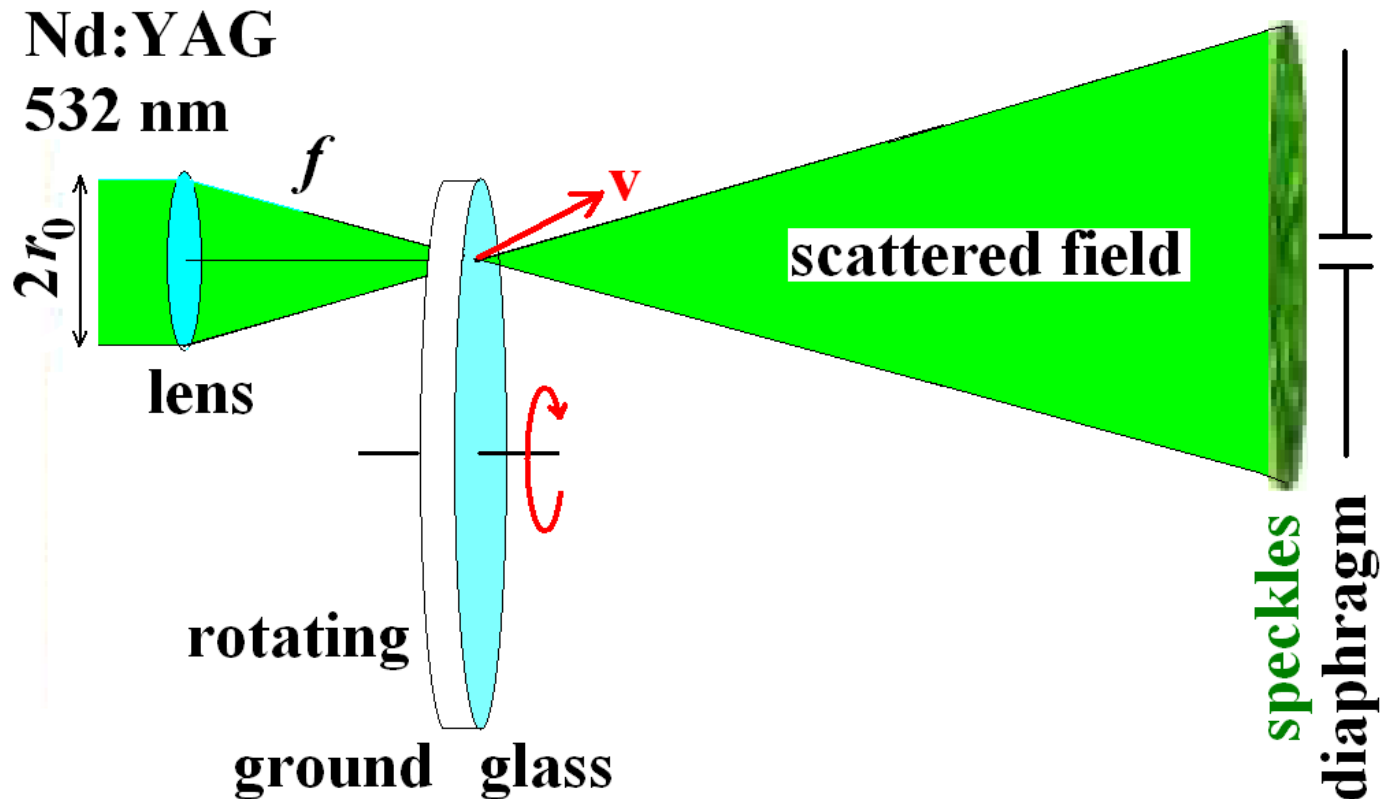
Photons emitted by a **chaotic source** have the tendency to arrive in packets (bunches) to the detector whereas the photon emission by a **laser** is always regular.

Clumping of photons can be observed within an observation time smaller than the time of coherence ($\tau < \tau_c$). At longer time of exposure ($\tau > \tau_c$), the bunching of photons becomes negligible and the photons arrive regularly. It is important to note that the statistics is a property of the source and not of the photons.

Many events near $\tau = 0$ if photons come **in bunches**.

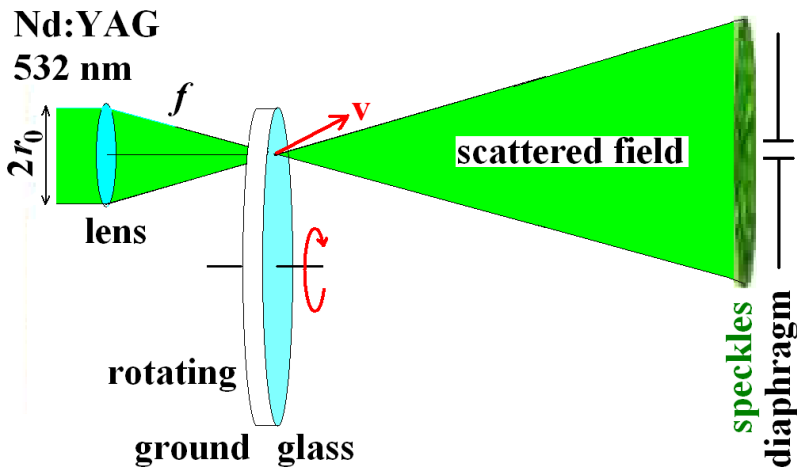
No events at $\tau = 0$ if photons come **one by one**.

Transition between laser and thermal light: Pseudo-thermal light source ("Martinsen-Spiller lamp", 1964)



The laser light is focused by a lens of focal length f on a rotating ground glass disc and a small section from the speckles in the scattered field will be selected by a small diaphragm. By changing the velocity v of the scattering centers, the coherence (and the statistics) of the laser light can be controlled.

Pseudo-thermal light source



The monochromatic laser beam undergoes a Gaussian spectral broadening ($\Delta\nu$) due to the scattering on the randomly distributed grains of the ground glass. This spectral profile of the speckle is characteristic of the thermal light. The coherence time of the scattered field may be deduced from $\Delta\nu$:

$$\tau_c = \left(\frac{1}{\Delta\nu} \right) = \frac{r_0}{v} \cdot \frac{4\pi}{\sqrt{2 \cdot \ln 2 \left(1 + \frac{4k^2 r_0^4}{f^2} \right)}}$$

where r_0 is the radius of the incident beam, v is the velocity of the illuminated area on the rotating disc, k is the wave number and f is the focal length of the focusing lens. The coherence time of this special light source may be varied in a rather simple way: using an arrangement with constant k , r_0 and f , the coherence time τ_c can be varied by altering the angular speed of the rotating disc.

Generalized photon distributions of the pseudo-thermal light

How the photon distribution depends on the ratio of τ/τ_c ? The generalization of the Bose-Einstein distribution can be done by introduction of the degree of freedom M (Mandel 1967). The fluctuation of the number of bosons (photons) within one cell of the phase space, which is equivalent of $\tau \ll \tau_c$, is described by the Bose-Einstein distribution. The probability of finding n photons over a number of phase cells $M (\geq 1)$ is

$$p(n, \langle n \rangle, M) = \frac{\Gamma(n+M)}{n! \Gamma(M)} \left(1 + \frac{M}{\langle n \rangle}\right)^{-n} \left(1 + \frac{\langle n \rangle}{M}\right)^{-M}$$

This should hold for light of arbitrary spectral density. It gives Bose-Einstein distribution for $M = 1$ and Poisson distribution for $M \rightarrow \infty$. The degree of freedom M can be expressed by

$$M = \frac{\tau^2}{2 \int_0^\tau (\tau - \tau') |\gamma(\tau')|^2 d\tau'}$$

where $\gamma(\tau')$ is the normalized autocorrelation function of the amplitude of the optical field and can be approximated by

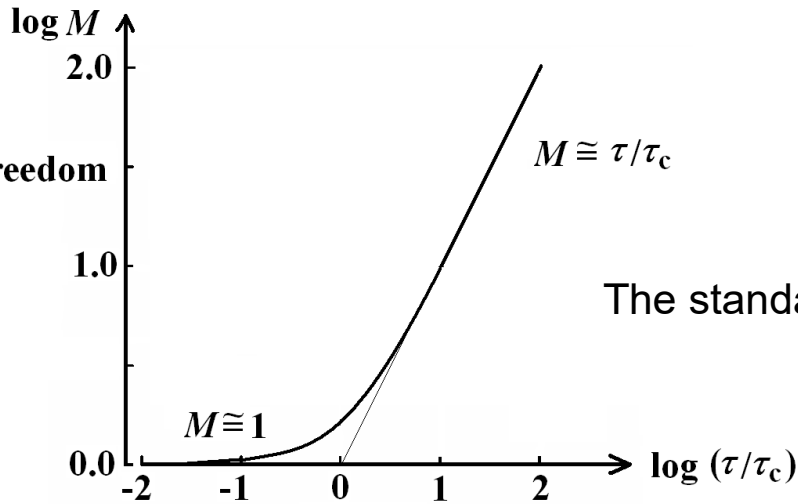
$$\gamma(\tau) = \exp\left(-\frac{2\tau}{\tau_c}\right) \quad \text{After substitution:}$$

$$M = \frac{1}{2} \frac{\left(\frac{2\tau}{\tau_c}\right)^2}{\left(\frac{2\tau}{\tau_c}\right) - 1 + \exp\left(-\frac{2\tau}{\tau_c}\right)}$$

M performs sharp turn around $\tau \approx \tau_c$: $M \approx 1$ (the distribution is Bose-Einstein) if $\tau/\tau_c < 1/10$, and may be approximated by $M \approx \tau/\tau_c$ (approaching the Poisson distribution) if $\tau/\tau_c > 10$.

Advantages of the pseudo-thermal light source

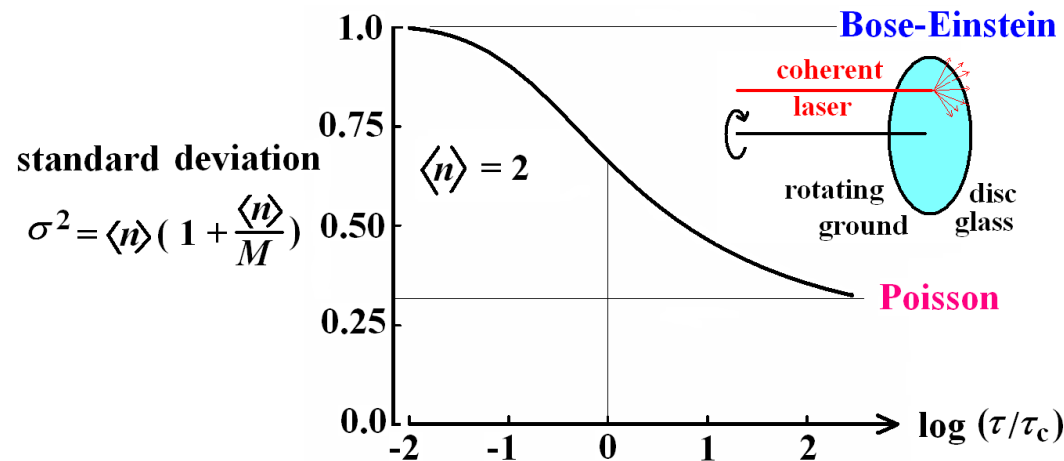
Correlation between the degrees of freedom (M) and the ratio of the exposure time (τ) to the time of coherence (τ_c)



$$M = \frac{1}{2} \frac{\left(\frac{2\tau}{\tau_c}\right)^2}{\left(\frac{2\tau}{\tau_c}\right) - 1 + \exp\left(-\frac{2\tau}{\tau_c}\right)}$$

The standard deviation of the Mandel's distribution:

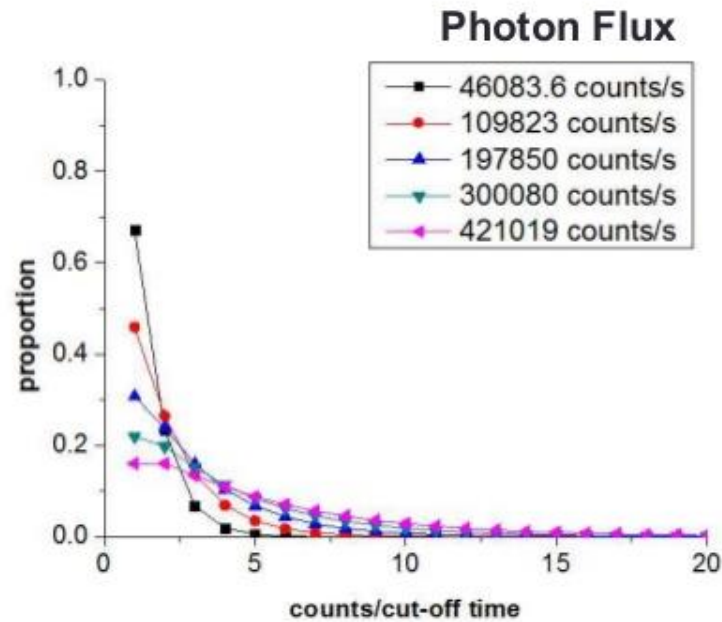
$$\sigma^2 = \langle n \rangle \cdot \left(1 + \frac{\langle n \rangle}{M}\right)$$



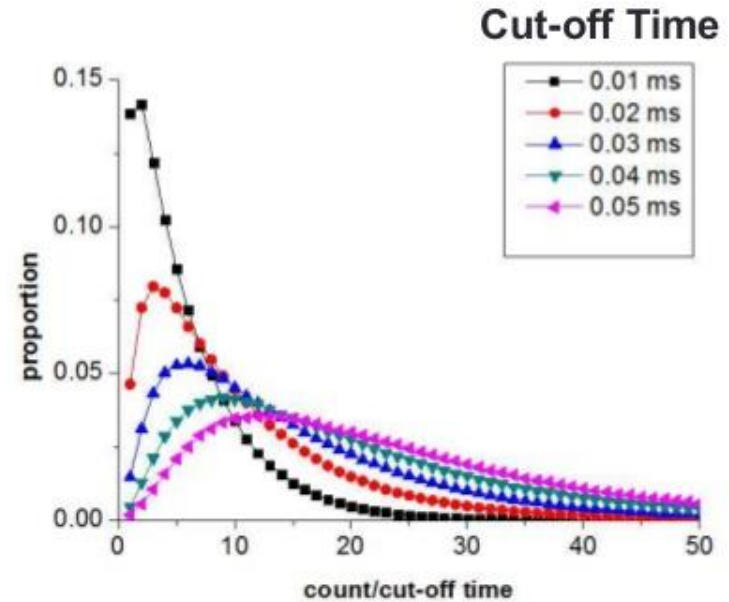
Starting ($M = 1$) from a broad distribution characteristics of the Bose-Einstein statistics, the intermediate distributions become gradually narrower and reach the sharpest (Poisson) distribution finally ($M \rightarrow \infty$). The transition from one limiting distribution to the other occurs within one or two orders of magnitude of τ/τ_c around 1.

The square of the standard deviation (σ^2) of the photon distribution as a function of τ/τ_c at mean photon number of 2.

Pseudo-Thermal Light



Cut-off Time = $10 \mu s$



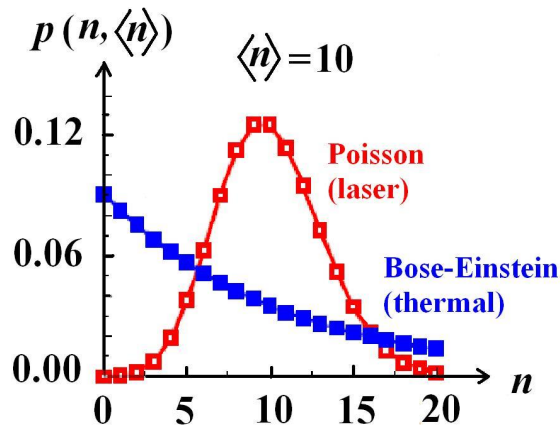
Photon Flux = $\sim 500,000$ counts/s

Coherence time : $10 \sim 20 \mu s$

- Laser follows a Poisson statistics, so laser is a coherent light.
- Rotating glass plate generates a pseudo-thermal light.
- Pseudo-thermal light follows Bose-Einstein statistics when the cut-off time is smaller than $20 \mu\text{s}$.
- The coherence time of the pseudo-thermal light is about $10\sim 20 \mu\text{s}$ in this experiment.
- As the cut-off time increases, statistics of pseudo-thermal light approaches Poisson distribution
- One can say that pseudo-thermal light is approximately a thermal light when time resolution is larger than the coherence time.

Coincidence photon counter models

Reactants in chemical reactions interact with each other and will result in a product. If one of the reactants is a photon, then a photochemical reaction would take place. Special attention should be paid if more than 1 photon is required to the reaction. In that case, the product will come out only if the proper (stoichiometric) number of photons will be available within a definite period of time (“time of coincidence”). The photons should arrive in narrow time gaps: not too early (after the establishment of the relevant precursors) and not too late (before the relaxation of the previous transient states) to drive the reaction properly. The excess photons will not be utilized: the reaction serves as a photon counting process: (chemical) product will be issued as soon as the required number of photons has been accumulated (the threshold is reached) within the time of coincidence and the photons above the threshold value will be lost.

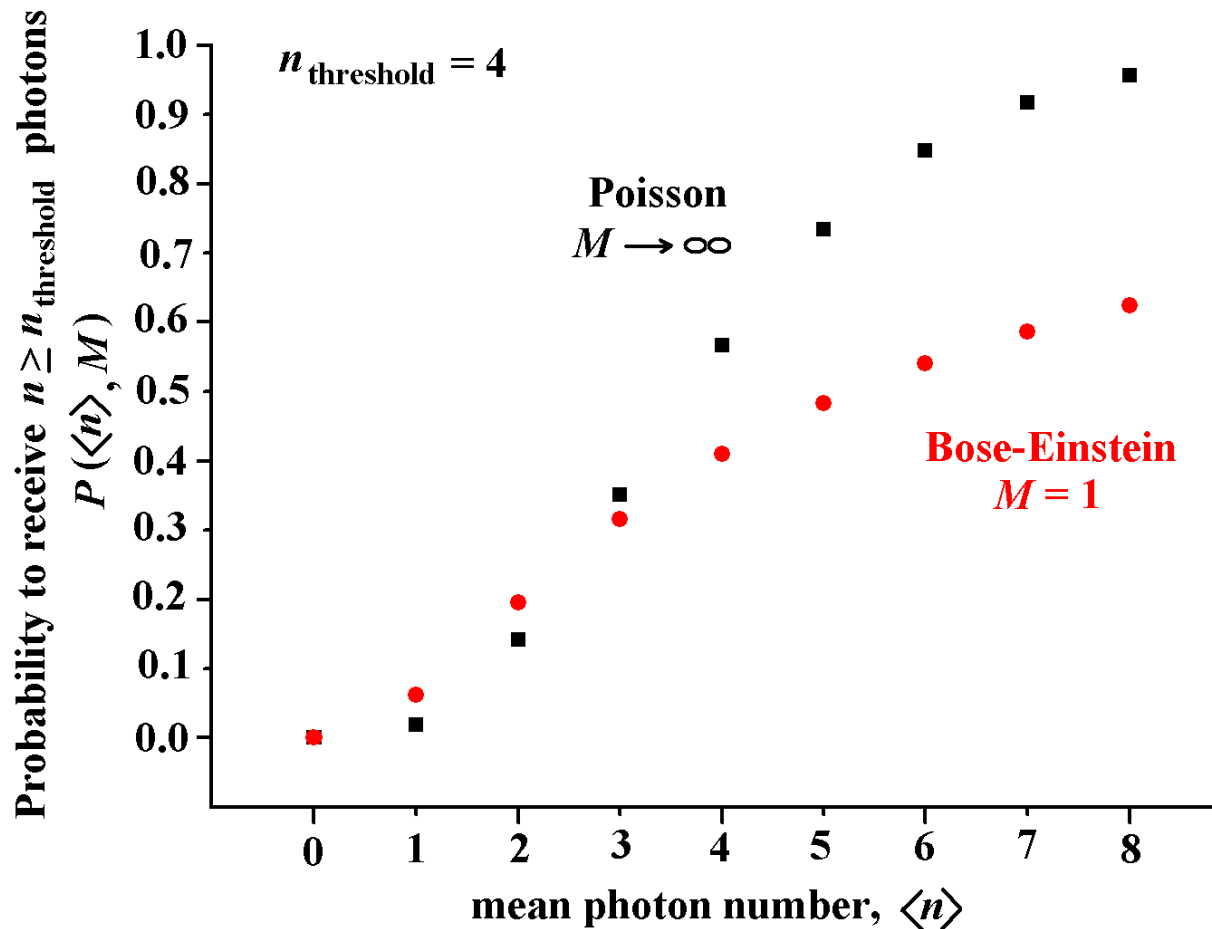


The rate of production of the photon counting mechanism (reaction) should be sensitive to the photon statistics of the illumination of identical mean values. This is the idea behind the application of coherent and (variable degree of) incoherent radiation (pseudo-thermal light source) to photon counting processes.

The probability that a photon counter with n_{th} **threshold value** receives $n \geq n_{\text{th}}$ photons

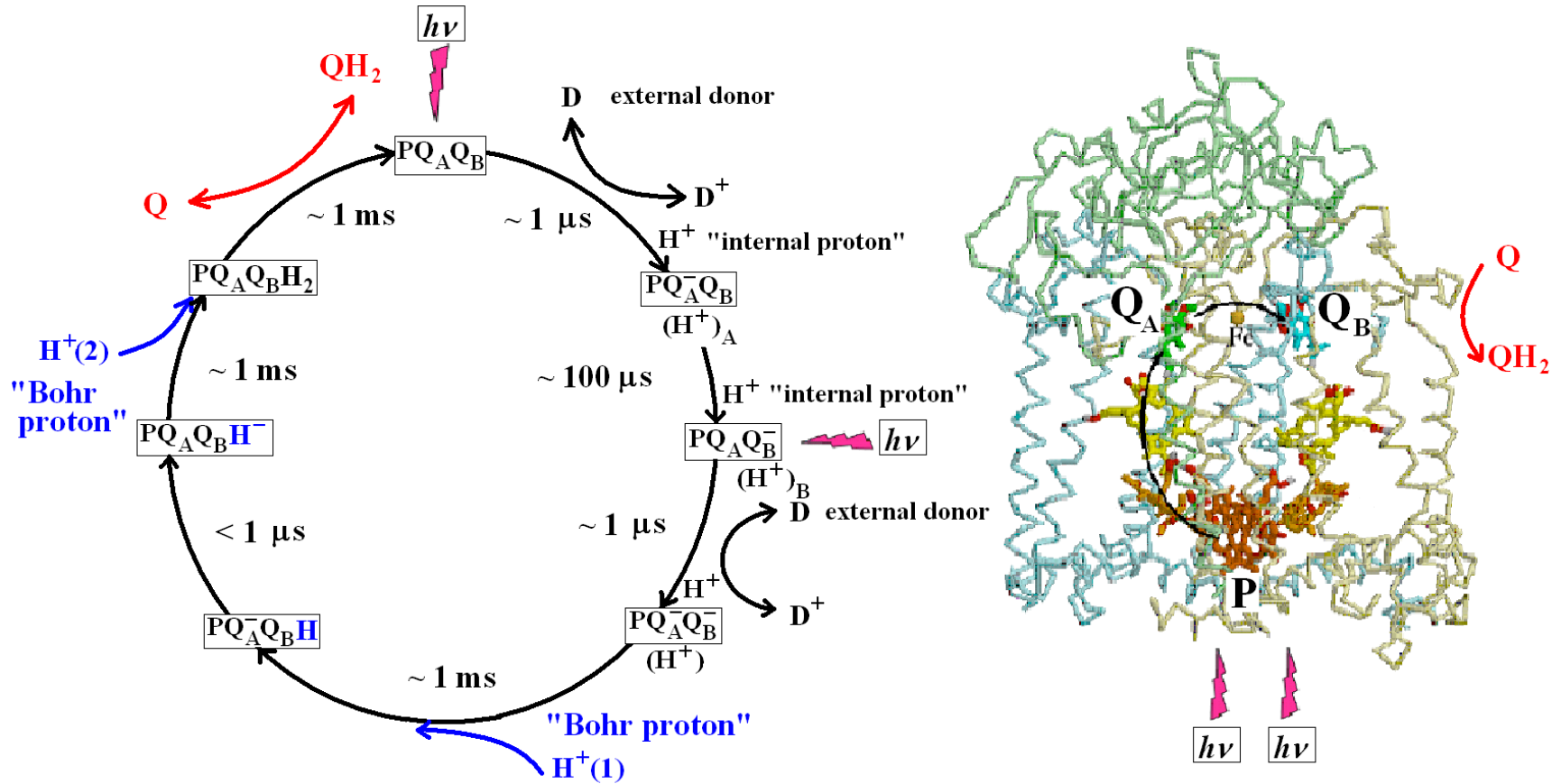
$$P(\langle n \rangle, M) = 1 - \sum_{n=0}^{n_{\text{th}}-1} p(n, \langle n \rangle, M)$$

Difference in outputs of a photon counter of a threshold value of 4 upon illumination with light source of Poisson and Bose-Einstein statistics at different light intensities (average photon numbers)



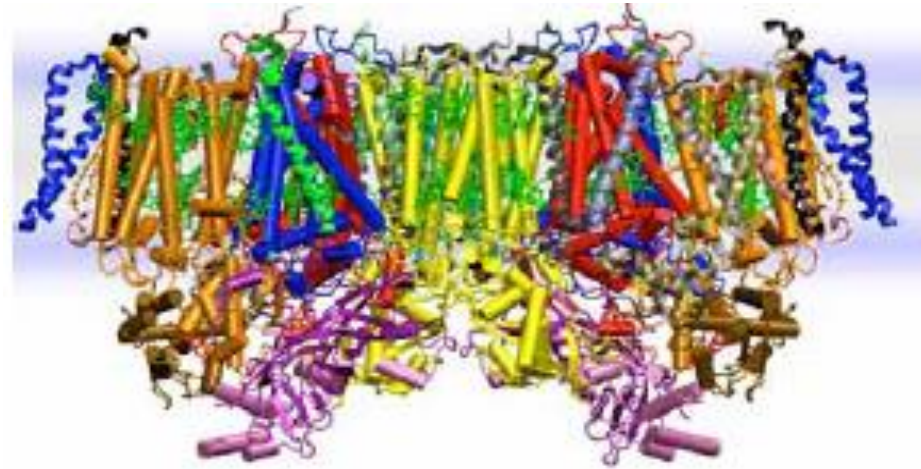
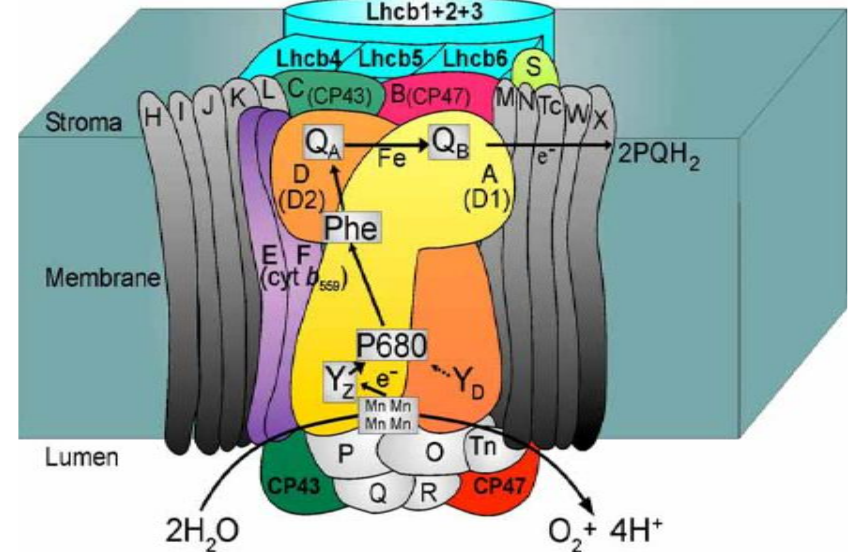
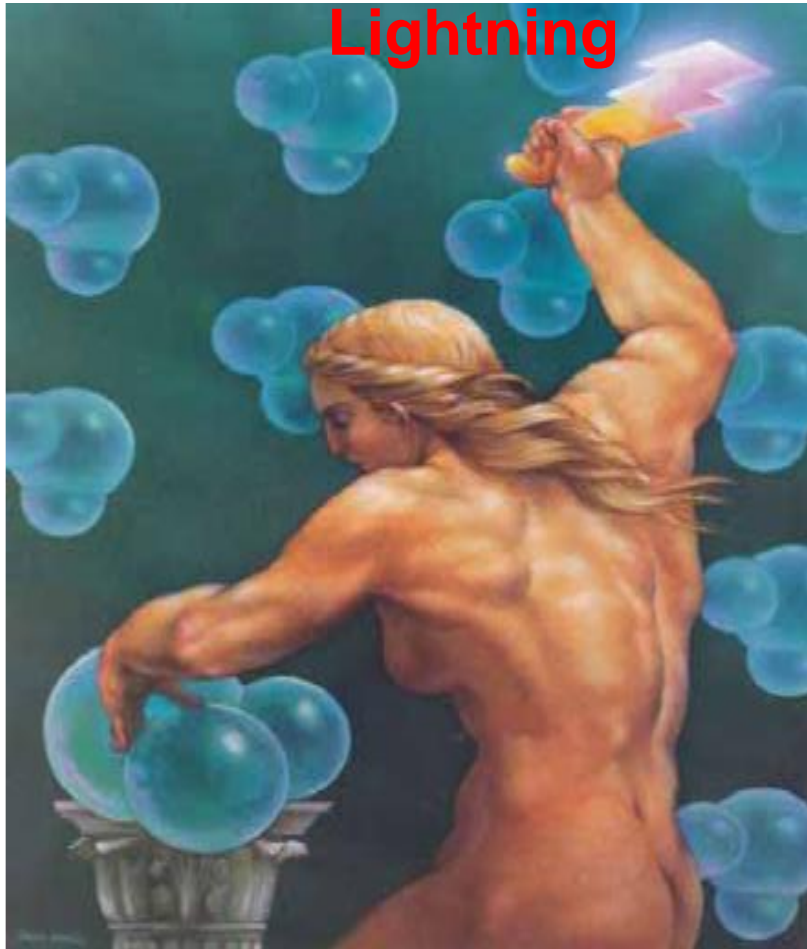
The number of counts of the photoncounter is slightly higher upon **Bose-Einstein** distribution than upon Poisson distribution at low light intensity ($\langle n \rangle < 3$), but it turns to opposite tendency at high light intensities.

Quinone reduction cycle (left) in reaction center protein of photosynthetic bacteria (right) as coincidence photon counter



Two photons at appropriate kinetic sequence are required to reduce the quinone (Q) to quinol (QH_2). The gap (coincidence time) between the first and second photons is determined by the reaction rates of the cycle. The bacteriochlorophyll dimer (P) on the periplasmic side of the membrane can be oxidized by light and subsequently reduced by an external electron donor (D). The two quinones on the cytoplasmic side (Q_A and Q_B) make one- and two-electron chemistry, respectively. The reduction of the different species results in either internal proton rearrangement in the protein ("internal protons") and/or uptake of protons from the aqueous environment ("Bohr protons").

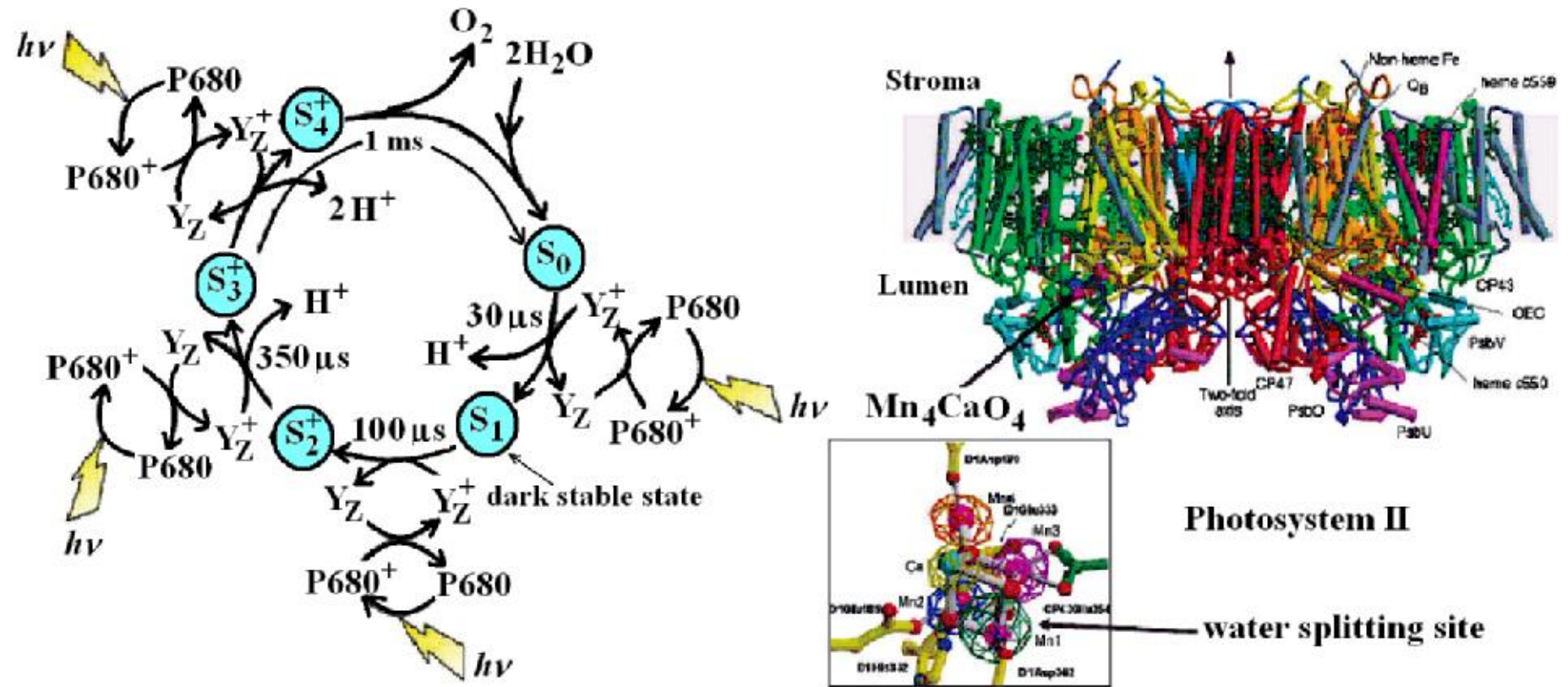
Water splitting in green plants



The artistic visualisation

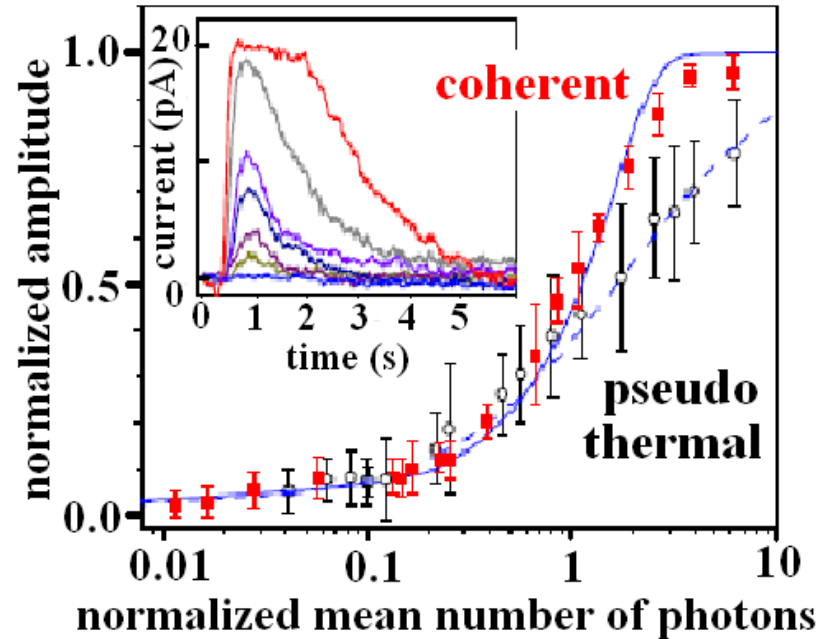
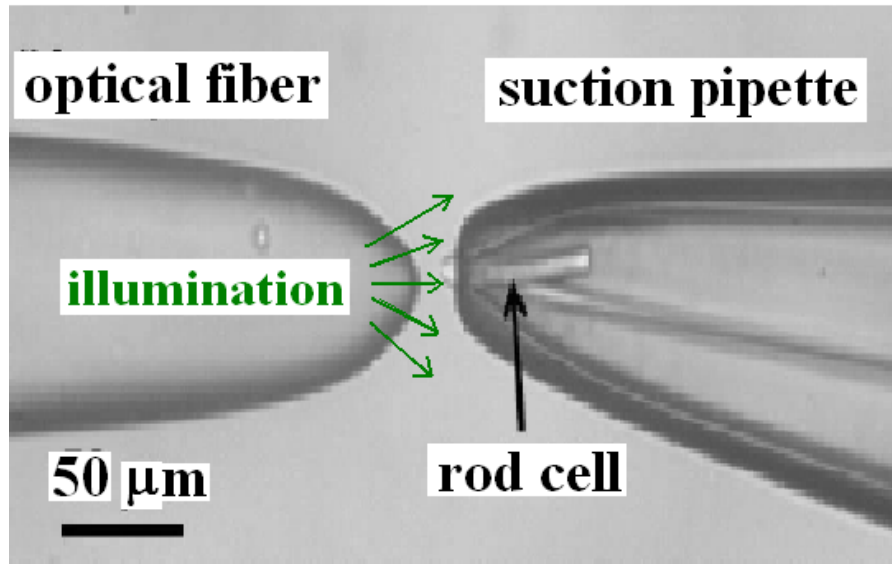
The fascinating molecular realisation

Water splitting reaction cycle in molecular complexes of Photosystem II of green plants



The accumulation of 4 oxidizing equivalents on S states is required to evolve 1 oxygen molecule. The photons drive the reaction forward step by step. The coincidence time among photons is determined by the forward and backward (relaxation) reaction rates.

Microscope image (top view) of the optical fiber with light from pseudo-thermal light source that stimulates the visual rod cell constrained in a suction micropipette (left)



Dependence of the normalized average amplitude of the rod photocurrent on the average number of impinging photons for coherent (red squares, solid line) and pseudo-thermal (black circles, dashed line) sources (right). Each data point is an average response to 80-100 light pulses. Amplitudes are normalized by the saturation value and the numbers of photons are normalized by values (in the range of 550-2,500 photons/pulse) which initiated a response of a half saturation amplitude. Inset shows raw waveforms of the rod photocurrent in response to coherent pulses of different average intensities (adapted from Sim et al. 2012).

3) Applications of laser light in biophysics research

overview

Laser spectrometers and fluorometers

- Laser aided UV and visible spectroscopy
 - Absorption, fluorescence and anisotropy spectra of biomolecules.
 - Spectral hole burning
 - Infrared and laser-Raman spectroscopy
 - Fourier transform infrared (**FTIR**) spectrometers
 - Optical coherence tomography (**OCT**): utilizes echos of infrared light waves backscattered off the internal microstructures within biological objects to obtain images on a μm scale.
 - Attenuated total reflection (**ATR**) infrared spectroscopy: the coefficient of internal total reflection of an IR beam in a waveguide is changed by a sample deposited on the surface of the waveguide.
 - Scanning IR microscopy enables the inspection of most strongly scattering samples.
 - Raman and resonance Raman spectroscopy of biomolecules
- Comparison of information obtained from IR and Raman spectroscopy

Time-resolved (kinetic) spectrometers

- (Bacterio)chlorophyll fluorometer.

The photosynthetic capacity of intact cells (bacteria) can be measured by tracking the kinetics of (bacterio)chlorophyll fluorescence excited by pulsed laser diodes.

- Lifetime measurements of fluorescence and delayed fluorescence

Different techniques: analog measurement, phase modulation and photon counting

- Femto- and picosecond biology

- The "big bang" of biophysics: light absorption by pigments, photosynthetic charge separation, and the fastest electron transfer and ligand reactions in proteins.

- Femtosecond coherence spectroscopy, direct measurements of coherent protein oscillations and vibration population decay (cooling).

Lasers to reveal structural dynamics of biophysical systems

- How the dynamics of proteins, DNA, biomembranes etc. does determine the physiological function?
- Laser temperature-jump methods for investigating biomolecular dynamics and protein folding/unfolding.
- Transient grating spectroscopy (**TGS**) and optical heterodyne detection (OHD): large steady-state reference field is mixed coherently on a detector with the weak and time-varying signal field of interest.

Unique feature: both signal amplification and linearization are simultaneously achieved.

Separation of transient grating signals of different origins; TG signals of biological interest (enzymatic reactions, photosynthesis etc.)

- Fluorescence correlation spectroscopy (**FCS**) is a technique in which spontaneous fluorescence intensity fluctuations are measured in a microscopic detection volume of about 10^{-15} L defined by a tightly focused laser beam. Fluorescence intensity fluctuations measured by FCS represent changes in either the number or the fluorescence quantum yield of molecules resident in the detection volume.

Laser-flash photolysis

Photobleaching by lasers

Fluorescence recovery after photobleaching (**FRAP**):

the pigments in a small field of a microscope are bleached by a strong laser flash and the repopulation of this area from the surroundings is tested by the measurement of the fluorescence of the pigments.

Principle of operation, instrumentation

Measuring the diffusion of proteins in the cytoplasm of *E. coli*

Green Fluorescent Protein inside *E. coli*

Kinetic analysis of the recovery trace: slow and fast diffusion, crowding effect

Flow cytometry and cell separation

Quantitative characterization and separation of heterogeneous cell population

Important features of flow cytometers:

the nozzle and the sheath fluid; hydrodynamic focusing

Forward Angle Light Scattering (FALS)

Fluorescence detection

Multi-parameter flow cytometers:

Lasers used in flow cytometry

Optical configurations of flow cytometers

Data processing and storage

Applications

Determination of the DNA content of cells

Immunofluorescence

Laser tweezers

Optical trap: enables a microscopic object (e.g. a single cell) to be lifted without causing any perceivable damage and placed virtually anywhere while performing measurements with other optical, spectroscopic or microfluiddynamic techniques.

Forces in the laser tweezers; light gradients in the excitation beam;
Models, optical light paths, changes of the impulses of the photons
Perturbing effects: absorption (heat), displacement of the particles due to light pressure, different geometries

Combination of tweezers with other photonics methods (e.g. spectroscopy)

Two-photon microfabrication, two-photon polymerization

Manipulating microsized objects: rotation of microscopic propellers, optically driven micropumps

Characterization of cell and molecule interactions: direct measurement of torque of double-strand DNA

Single-molecule spectroscopy and mechanical studies

Essential differences between spectroscopy of the bulk (ensemble average of large (Avogadro's) number of biomolecules) and of a single molecule.

Force (momentum) – elongation (torsion) characteristics on single elastic biomolecules.

Elasticity of cytoskeletal filaments

Motor proteins

Muscle contraction

Mass spectrometry

Important analytical technique for the identification of molecules by way of measuring their mass-to-charge ratios in the ionized state brought about by strong laser irradiation.

Time-of-flight mass spectrometer (TOF)

Matrix-assisted laser desorption ionization (MALDI)

- Principle of operation and type of spectrometers
- Peptide (protein) sequencing by sequential degradation after Edman and identified by mass spectrometry.

Laser-aided microscopy

The principle of scanning: scanning tunneling microscope (**STM**) for imaging individual atoms.

Atomic force microscopy (**AFM**) for providing images of mainly surface structures in their natural environments with atomic or even subatomic resolution.

Confocal laser scanning microscopy (**CLSM**) produces an image of a selected layer at a certain depth by scanning it from point-to-point. It is capable of imaging series of thin, optically clearly separated layers.

Improving the resolution of the confocal microscope

- Two-photon excitation
- Theta-microscopy
- 4-Pi microscopy

Scanning near field optical microscopy (SNOM)

Evanescent fluorescence and the total internal fluorescence microscopy;

super-resolution fluorescence microscopy

Lasers in medicine

Laser-tissue interactions: heat (laserthermia, vaporisation, carbonization), photodissociation, ionization (plasma, mechanical shock waves, cavitation), fluorescence, photochemistry.

Diagnostics

Photodynamic diagnostics

Laser Doppler velocity meter

Optical tomography

Therapy

PUVA (Psoralen + UVA) therapy

Photodynamic therapy

Blue light therapy

Surgical applications of lasers; photoacoustics (**lithotripsy**), reshaping the cornea (PRK, **LASIK**, LASEK)

Remote sensing

Laser spectroscopy is exquisitely suitable for remote sensing of clouds of biological agents.

Estimation of the size and physiological state of corn field (crop) and canopy of plants (forests) by detection of the chlorophyll fluorescence on global scale.

Light detection and ranging (**LIDAR**): the detector senses the backscattered laser light (the return of the echo). Mobile commercial LIDAR systems employ integrated global positioning system (GPS).

Masers (microwave-amplified stimulated emission of radiation);
microwave auditory effects and thought transmission technology

Theoretical concept: background of the concept of thought
transmission technology, description of the technology

Potential biomedical applications

Take home messages

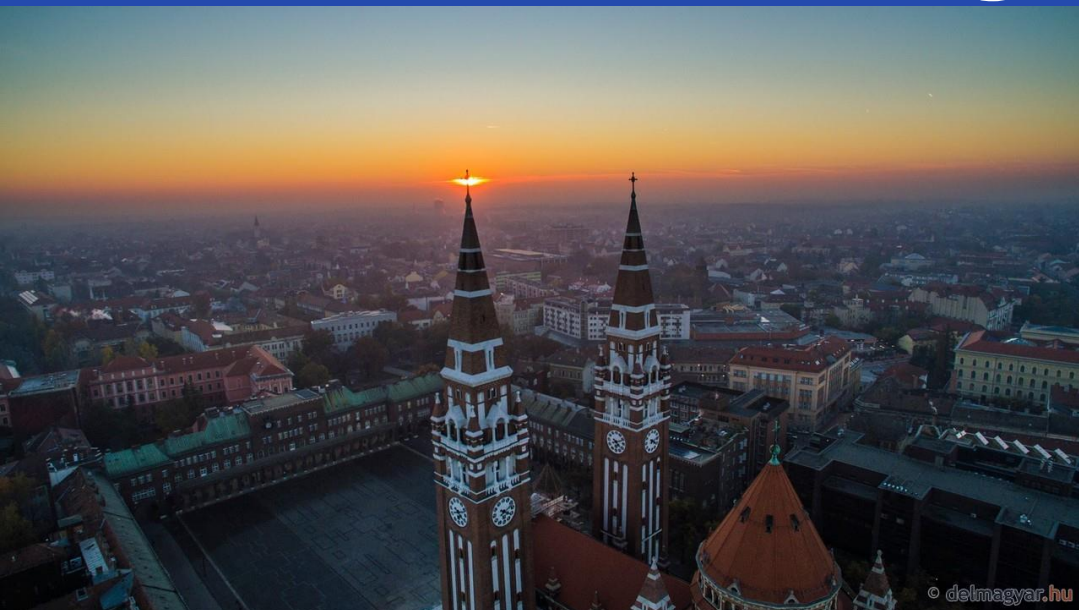
- Induced emission and optical gain in the resonator determine the principal properties of the laser radiation that could be distinct from those of the thermal light sources: **directionality**, **monochromaticity**, **high power** and **coherence**. On one hand, there are lasers of large divergence (laser diodes), small power, low temporal (e.g. short lived laser flashes) and spatial (cw lasers of poor construction) coherence and wide spectral band. On the other hand, light from thermal sources can be made highly parallel (radiation of the sun and other stars), monochromatic (by use of monochromators), intense (by focusing) and spatially coherent (low view angle). Consequently, **neither of these properties are absolutely unique to lasers**.
- The **photon statistics** can be more characteristic to light sources.
- Coherent beams are both directional and monochromatic and show Poisson photon distribution.
- The photons from thermal light sources have super-Poisson (in special case Bose-Einstein) statistics when the illumination time is smaller than the time of coherence.
- Pseudo-thermal light source can convert Poisson statistics to Bose-Einstein in a well controlled way by keeping the mean number of photons and the illumination conditions constant.
- The light sources of different photon statistics can be used to study mechanisms of various coincidence photon counters in biophysics (photosynthesis and electrophysiology).



'ELITEAM'- ESTABLISHMENT OF THE ELI INSTITUTE AT THE
UNIVERSITY OF SZEGED: FOUNDATION OF INTERDISCIPLINARY
RESEARCH IN THE FIELD OF LASERS AND THEIR APPLICATIONS

**THANK YOU FOR YOUR
ATTENTION!**

**I hope you had a pleasant stay in
Szeged.**



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