Steady-state and time-resolved absorption and emission spectroscopy.

1. Absorption and emission of radiation[1]

Electromagnetic radiation, as the name implies, contains both an electric and a magnetic field component, which are best illustrated by considering linearly polarized light (where the magnetic and electric field components are oriented within one single plane). The electric component takes the form of an oscillating electric field and the magnetic component oscillates with the same frequency but perpendicular to the electric field. If the electromagnetic radiation is travelling along the x-axis, the electric and magnetic field can be oriented along the y- and z-axis respectively according to:

$$E_y = Asin(2\pi\nu t - kx)$$
$$H_z = Asin(2\pi\nu t - kx) \qquad \text{(eq. 1)}$$

With A the amplitude, ν the frequency and $k = 2\pi/\lambda$ with λ the wavelength. For linearly polarized light, there is no phase difference between the magnetic and electric field. The plane of polarization is conventionally taken as the plane containing the direction of E and that of the propagation, so in this case, the light is polarized in the xy plane. An example can be found in Figure 1.

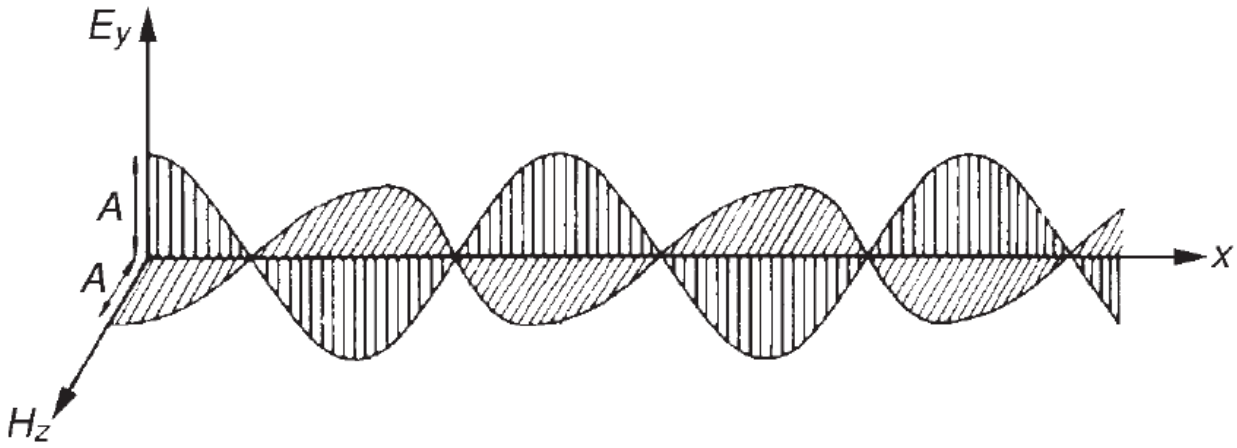


Figure 1: Plane-polarized electromagnetic radiation travelling along the x-axis with the magnetic and electric field in the xy and xz plane respectively.

Now assume that we have an atom, a molecule or a nanoparticle with two different energy states '1' and *'2'* which are stationary states (time-independent energy states of the system). These two states can be electronic states but also vibrational or rotational energy states of the system. If such a 2-level system is subjected to electromagnetic radiation with energy proportional to the energy difference between the two states, *i.e.* $\Delta E = E_2 - E_1 = h\nu$, then three processes can occur, namely (induced/stimulated) absorption, spontaneous emission and induced/stimulated emission. These three processes are highlighted in Figure 2.

[1] This chapter and its figures are directly based on chapter 2 of the book of Michael J. Hollas, Modern Spectroscopy, by John Wiley & Sons (4th edition) but given in a more condensed form, in view of the course 'Microscopy and Spectroscopy of Nanosystems'.

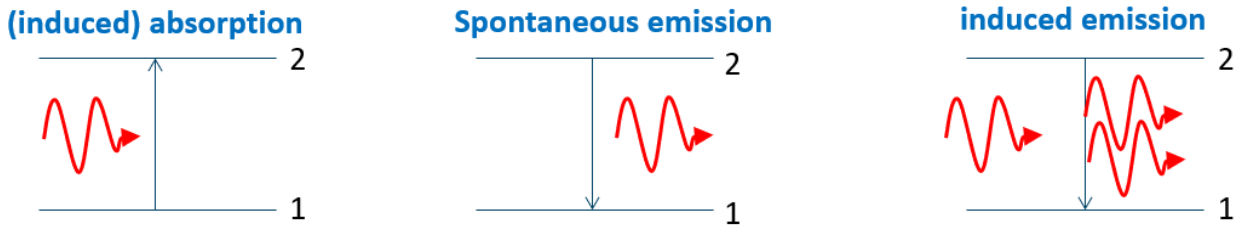


Figure 2: Absorption and emission processes between two stationary states '1' and '2'

1. In induced absorption, the molecule (or atom/system) absorbs a photon and thereby is excited from state '1' to state '2'.
2. After a certain time, the excited system spontaneously emits a photon with the same energy, equal to the energy difference between the two states. The time difference between absorption and emission can be probed in time-resolved emission spectroscopy, which will be discussed further on.
3. The third process is induced emission. In this case an incoming photon induces the emission process (before spontaneous emission occurs). This process lies at the basis of laser physics, since one photon results in two photons, and both photons will have the same energy, same phase and the same travelling direction, such that both electromagnetic waves can be added constructively to create an electromagnetic wave with double the amplitude. The reason why absorption is strictly referred to as induced or stimulated absorption is now also clear, as it requires the presence of radiation with a certain energy.

At a given temperature T, the energy levels will be populated according to the Boltzmann factor, such that those three processes can occur depending on the population of the states:

$$\frac{N_1}{N_2} = \frac{e^{-E_1/kT}}{e^{-E_2/kT}} = e^{-(E_1-E_2)/kT} \qquad \text{(eq. 2)}$$

The **absorption process** can be described by the following process,

$$\frac{dN_2}{dt} = N_1 B_{12} \rho(\tilde{\nu}) \qquad \text{(eq. 3)}$$

Where the rate of change of the population of state '2' depends on the number of molecules that are in state '1', the so-called Einstein coefficient B_{12} describing the efficiency of the absorption process and $\rho(\tilde{\nu})$ the spectral density (the number of photons present to excite the system). This spectral density can be either originating from the system itself (so-called black body radiation) or can be induced by a photon source such as a lamp or a laser.

Likewise, **induced emission** changes the population of the state according to following equation:

$$\frac{dN_2}{dt} = -N_2 B_{21} \rho(\tilde{\nu}) \qquad \text{(eq. 4)}$$

Where the minus sign refers to the decrease of the number of molecules in state '2', and with B_{21} the Einstein coefficient describing the efficiency of the induced emission.

Finally, **spontaneous emission** can be best described by:

$$\frac{dN_2}{dt} = -N_2 A_{21} \qquad \text{(eq. 5)}$$

In this case, no photons are involved, *i.e.* $\rho(\tilde{\nu})$ is absent from the equation, and A_{21} represents another Einstein coefficient.

All three processes are continuously going on at the same time, and, when in thermal equilibrium, we have:

$$N_1 B_{12} \rho(\tilde{\nu}) = N_2 A_{21} + N_2 B_{21} \rho(\tilde{\nu}) \qquad \text{(eq. 6)}$$

Or, combining this with equation 2, we find that:

$$\frac{N_1}{N_2} = \frac{A_{21} + B_{21}\rho(\tilde{\nu})}{B_{12}\rho(\tilde{\nu})} = \frac{g_1}{g_2} e^{-(E_1 - E_2)/kT} \qquad \text{(eq. 7)}$$

Or in other words:

$$g_2 A_{21} = \rho(\tilde{\nu})(g_1 B_{12} e^{(E_2 - E_1)/kT} - g_2 B_{21}) \qquad \text{(eq. 8)}$$

2. Absorption spectroscopy

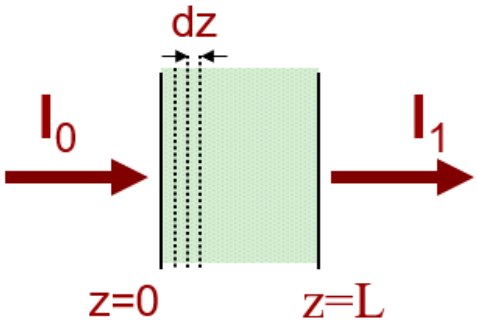


Figure 3: Typical absorption experiment leading to the Lambert-Beer law.

In an absorption experiment, an incident intensity I_0 of (typically) monochromatic light is send through the sample. Depending on the absorption coefficient of the sample, (part of) the light will be absorbed resulting in an intensity $I_1 < I_0$ passing through the sample. Measuring the change in intensity as a function of wavelength of the incoming monochromatic light yields the absorption spectrum. If considering that the absorption coefficient $\alpha(\lambda)$ defines the efficiency of the absorption for a given wavelength, then one can write for the light passing through an infinitesimal small slab with pathlength dz that:

$$dI = -\alpha(\lambda) I dz \qquad \text{(eq. 23)}$$

To then find the absorption of a sample with pathlength L one needs to integrate over this equation to get:

$$\int_{I_0}^{I_1} \frac{dI}{I} = -\alpha(\lambda) \int_0^L dz \qquad \text{(eq. 24)}$$

Giving as a result the law of Lambert-Beer:

$$-\mathrm{A} = \log\left(\frac{I_1}{I_0}\right) = -\alpha(\lambda) L \qquad \text{(eq. 25)}$$

With A the absorbance or optical density, $\alpha(\lambda)$ the absorption coefficient and L the optical path length. In a solution, the absorption coefficient is proportional to the concentration (number of molecules), making that the extinction coefficient ε, with $\varepsilon c = \alpha$ with c the concentration is a better measure. When knowing the extinction coefficient, absorption spectroscopy can thus provide quantitative information on the concentration of a particular sample. Similarly, as the absorption coefficient, the extinction coefficient also depends strongly on the wavelength of the radiation.

A typical absorption spectrometer will consist of four different parts:

(1) The light source, consisting typically out of 2 or more lamps to cover the full excitation wavelength range required. For example, a deuterium lamp to cover the ultraviolet spectrum and a halogen lamp to cover the visible and IR part of the optical spectrum
(2) A dispersive element, to extract a single wavelength from the lamp spectrum. This dispersive element is typically a diffraction grating monochromator, but can also be a combination of two of such grating spectrometers to provide a higher spectral resolution. Typical spectral resolutions of high-end absorption spectrometers are 1-2nm, but can be down to 0.1nm, though at the cost of light intensity.
(3) A sample chamber.
(4) Multiple detectors to cover the full sensitivity range of typical absorption spectrometers. For example, a PbS detector for the infrared part, and a photomultiplier for the UV and visible part of the spectrum.

Note that typical absorption spectrometers can measure optical densities up to $A = 4$, with some exceptions even up to $A = 8$, which means according to the above equation that the light has been reduced in intensity by a factor of $10^4 - 10^8$. In such cases, the remaining signal is typically buried in the noise and a special detection scheme is required to distinguish the remaining intensity from the noise. Such a detection scheme is called LOCK-IN or PHASE-SENSITIVE detection and has been found a very important technique to detect small signals in many different experimental techniques.

3. Phase-sensitive detection or lock-in detection.

In case a weak signal is buried in the noise we can still detect it quite well if we apply so-called phase-sensitive detection. The main idea behind the technique is that we will create a repetitive signal, with a known and fixed frequency (*e.g.* by modulating the light intensity of the incoming light beam), such that the signal is alternating with a known frequency. The superimposed noise is however not modulated by this frequency, and thus we can extract only the signal by phase-sensitive detection. The detection scheme is given in Figure 4.

A signal generator creates a modulation of the light beam (*e.g.* through a chopper wheel or a rotating attenuator) such that the signal is modulated with the same frequency. If we assume for simplicity that the modulation is given by a sine function with a certain modulation amplitude V_{ref} and frequency ω_{ref} we get:

$$V_{ref} \sin(\omega_{ref} t) \qquad \text{(eq. 26)}$$

After passing the sample, we will detect a sum of the real signal (which is modulated with the same frequency) and the noise (random frequencies). Both can be shifted in time (if there is e.g. a delayed response time), which is given by a certain phase ϕ.

$$\sum_{sig} V_{sig} \sin(\omega_{sig} t + \phi_{sig}) \qquad \text{(eq. 27)}$$

If we now multiply our reference with the obtained signal, and we add a certain phase to it, we will get the following:

$$V_{ref} \sin(\omega_{ref} t + \phi_{ref}) \sum_{sig} V_{sig} \sin(\omega_{sig} t + \phi_{sig}) \qquad \text{(eq. 28)}$$

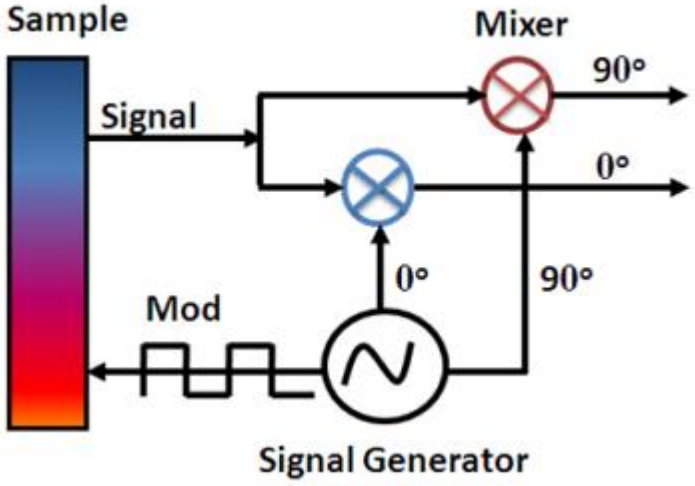


Figure 4: Principle of phase-sensitive detection.

According to the multiplication formula of sinus functions we get:

$$\frac{1}{2}V_{ref}\sum_{sig}V_{sig}\cos\big((\omega_{sig}-\omega_{ref})t+(\phi_{sig}-\phi_{ref})\big) - V_{sig}\cos\big((\omega_{sig}+\omega_{ref})t+(\phi_{sig}+\phi_{ref})\big) \qquad \text{(eq. 29)}$$

For our 'real' signal that we want to extract, we know that $\omega_{sig}=\omega_{ref}$, or in other words, that the first cosine for that particular frequency becomes a constant (no time-component anymore) and thus gives a DC signal. All other components with different frequencies will give AC components. When applying a selective filter that only passes the DC component and removes all the AC components, only that signal that has the same frequency as the signal generator reference signal will be obtained. As such one can e.g. detect the absorption with high accuracy, even if the remaining light intensity is much weaker than the other noise components. This DC component then amounts to:

$$DC=\frac{1}{2}V_{ref}V_{sig}\cos\big(\phi_{sig}-\phi_{ref}\big) \qquad \text{(eq. 30)}$$

And thus becomes maximum if $\phi_{sig}=\phi_{ref}$. Note that the intensity of the signal, which is proportional to V_{sig}, can only be determined if the phase of the signal is known. As initially the phase of the signal (delay of the response) is unknown, one solves this by doing the above for two different phases $\phi_{ref}=0°$ and $\phi_{ref}=90°$. As such one can easily obtain the phase of the signal from the difference between the two DC signals, and thus also the intensity.

This phase-sensitive detection is important in all spectroscopic measurements that try to detect weak signals that are buried in the noise. Typically, the signal generator that also includes the signal mixing with the two different phases is obtained through **a so-called lock-in amplifier**.

4. Emission spectroscopy[2]

In emission spectroscopy, the emitted light coming from a sample is detected as a function of emission wavelength, after exciting the sample with a monochromatic light beam with a given wavelength.

A typical emission spectrometer consists of very similar set of components as an absorption spectrometer:

(1) A light source and corresponding dispersive element: can be a lamp + monochromator, or a laser
(2) A sample chamber
(3) A detector to collect the emitted light.

Important is that the emission is typically incoherent and non-directional (*i.e.* photons are emitted at random times, and with random phases and orientation). Therefore, in a typical experiment one cannot collect the full emission from a sample, but typically only a fraction of it (specific light captured e.g. by a lens). An integration sphere can provide a solution for this, but not all instruments are equipped with it.

In most cases, an emission spectrum will be measured for only one specific excitation wavelength. Nevertheless, a fluorescence-excitation map can also be obtained, where emission is measured as a function of excitation wavelength. In the case of nanomaterials and in particular 0D and 1D nanomaterials, this is a very powerful technique, which we will discuss by a few examples in the class.

Nowadays, highly sensitive detectors exist, than can even detect down to single photons. When detecting an emission spectrum, the efficiency of the detector as a function of emission wavelength is very important to take into account. Typical detectors consist of a particular semiconductor, that can collect photons with an energy larger than the bandgap. For visible light, a silicon detector is mostly used (band gap ~1eV), while for infrared light other semiconductors with smaller band gap are required (for example InGaAs). To detect a spectrum at once, a detector having an array of pixels is used, such that all wavelengths can be detected at once (in contrast to absorption spectroscopy where absorption is measured as a function of wavelength, thus one wavelength after the other).

A typical, well-known detector for detecting visible light is a SiCCD, or Silicon-based charge coupled device. One pixel of such a SiCCD consists of a MOS device (metal-oxide-semiconductor) with a structure as in figure 5, left panel. The substrate consists of p-doped Silicon, *i.e.* in which part of the Si atoms are replaced by B atoms and therefore an excess of holes is present (as denoted schematically by the green circles in Figure 5). On top of this substrate, a dielectric layer is placed, typically SiO_2, and on top of that a metallic gate, typically polysilicon. By putting a positive voltage on the gate, the holes of the p-doped Si get repelled from the area close to the gate and a depletion layer starts to form (indicated by the light-blue shaded area in Figure 5). When a photon with sufficient energy falls on the detector, an electron and hole pair is formed. During light collection, the positive voltage on the gate is maintained such that when a photon hits the Si in the depletion layer, the electron is directly attracted to the gate (due to the positive voltage), while the hole is diffused to the substrate.

The more photons are falling in the pixel, the more electrons are collected at the gate. After the collection time, the number of electrons in the pixel needs to be converted to a digital number corresponding to the intensity of incident photons in that specific pixel. In a CCD, the p-type substrate has a series of pixels built in, as schematically presented in the right panel in Figure 5. Each pixel, will have a different number of electrons, which are collected that are afterwards read out, one-by-one by each time shifting the electrons to the next pixel and then reading it out in the last pixel of the row. To do so, one can play with the voltages on each of the different gates. For example, if V_3 is put much higher than V_2, the depletion layer will extend itself and connect pixels 2 and 3 such that the electrons of pixel 2 can move to pixel 3. In a CCD, by changing these voltages one by one, each pixel is read out one after the other and then converted into a digital number using an analog-to-digital convertor.

[2] Partly based on the course: Physics of the Daily Live – the digital camera (1st bachelor of physics – S. Cambré).

A CCD is different from a CMOS in the fact that in a CMOS each pixel has its won analog-to-digital convertor, allowing to read-out the detector much faster, yet at the cost of a higher noise level.

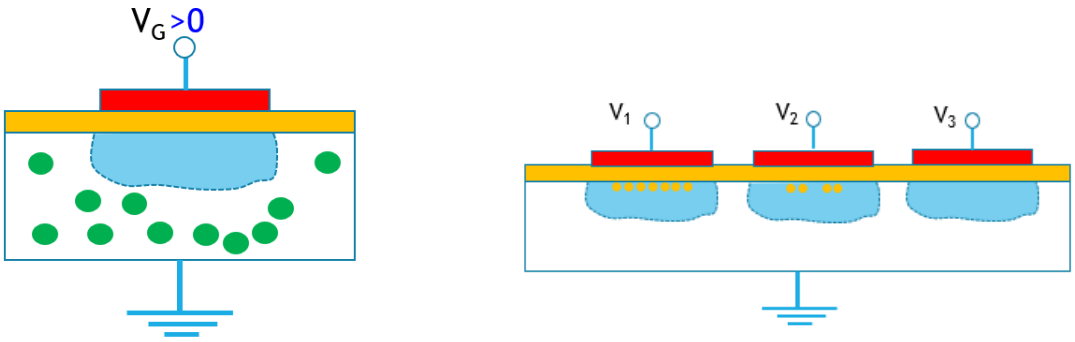


Figure 5: (left) Schematics of one pixel in a CCD device comprising a metallic gate (in red), SiO_2 as dielectric material and p-type Si as the body. The green circles represent the 'holes' of the p-type doping. When putting a positive voltage on the gate, a depletion layer consists in which the electrons (after photon capture) can be stored until they are read out. **(right)** series of pixels on the same body where the read-out can be controlled by the different voltages on the gate.

There are many aspects that need to be taken into account when selecting a particular detector for a given experiment: the efficiency, full-well capacity, dark current, etc…

The **charge collection efficiency**, i.*e.* the chance that a photon is absorbed and creates a photon should be as high as possible. This will depend on the energy of the photon (and its relation to the bandgap of the semiconductor), but also on other factors, such as the available space for the photon to penetrate to the depletion layer (as the metallic gate is typically not transparent). For the best SiCCDs this efficiency can be very high, up to >90% in particular wavelength ranges.

To increase the efficiency, sometimes the illumination is performed from the back of the MOS, instead of from the top, by thinning the Si substrate such that the incoming light does not need to pass by the gate. This is important to detect high energetic photons (UV-region). Such detectors are called: back-illuminated detectors.

Each pixel will have a maximum number of electrons that it can keep within the pixel, depending on the size of the pixel and the specific voltage applied. This is typically referred to as the '**full well capacity**' and expressed in number of electrons per pixel (typical values are of the order of 25.000-100.000 electrons per pixel). When over illuminating a CCD, blooming and streaking artefacts can occur, in which adjacent pixels get unwillingly a part of the collected electrons, and thus unwanted signals arise (see examples in class).

Aside from the real measured intensities, the typical temperature at which experiments are performed can also sometimes create an electron that is wrongfully identified as a photon that entered the system. This is called the **dark current** of the device. Typical emission detectors are therefore cooled down to liquid nitrogen temperature to reduce this dark-current. Dark-currents play an important role for detectors in the infrared, where the band gaps are lower and thus the chance of creating an random electron in the conduction bands are very high. Even when cooling down to liquid nitrogen temperature, integration of the detector for a few seconds can lead to a significant dark current.

5. Time-resolved spectroscopy[3]

Time-resolved spectroscopy focuses on the time evolution of emission and absorption processes to obtain information about the dynamics of chemical, physical or biological systems. From an historical perspective, it started on the millisecond time scale in the stopped-flow method. In this method, used to study chemical reactions, two liquid samples are contained in two syringes, and mixed together while measuring optical absorption (or emission) spectra. Afterwards flash photolysis was capable of reaching the microsecond and approaching the nanosecond time scale. However, with he development of ultrafast pulsed lasers the time scales have significantly decreased in the latest years, reaching easily the time scales of atomic motions in the pico- and femtosecond time scale.

Time-resolved laser spectroscopy methods provide information about the dynamics of various processes, such as:

1. Dynamics of reaction coordinates (e.g. cis/trans isomerization, excited state energy or charge transfer, conformational changes, ..)
2. Reorientational relaxation
3. Vibrational dephasing/relaxation in the ground and excited electronic state
4. Lifetimes of excited states
5. ….

Most of these processes can be described in a so-called Jablonski Diagram, of which an example is shown in figure 6.

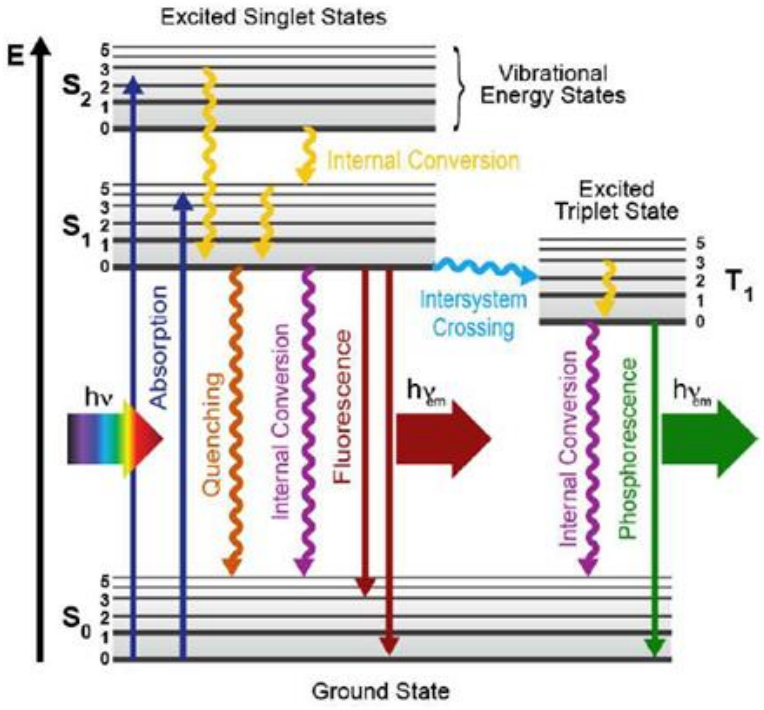


Figure 6: Example Jablonski diagram showing absorption, emission (fluorescence and phosphorescence) and vibrational relaxation processes as well as intersystem crossing from singlet to triplet states.[4]

For example, processes in liquids and solids occur in time scales down to femtoseconds, electron relaxation in the conduction band occurs below 1 picosecond, tunneling and transport processes were previously observed in the 50ps-10ns regime and vibrations of molecules typically happen in the less than 1ps up to 10ps regime,

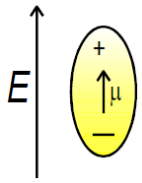
[3] Based on chapter 8 of the book "Introduction to Laser Spectroscopy" by Halina Abramczyk (pages 175-189)

[4] This figure was obtained from: https://www.simtrum.com

while vibrational relaxation times are in the ps to ns range. It is therefore important to gain access on both the shorter (fs) as longer (ns, ms) timescales in the optical spectroscopic techniques like absorption and emission.

Time-resolved spectroscopy may be classified using a variety of criteria, but the simplest classification depends on the temporal resolution determined by the laser pulse duration: nanosecond (10^{-9}s), picosecond (10^{-12}s) and femtosecond (10^{-15}s) spectroscopy. Time-resolved spectroscopy is typically performed in resonant conditions (like absorption, emission) but can also be done to monitor vibrational timescales.

When using pulsed lasers, both linear and nonlinear optical phenomena can occur. When looking at how molecules interact with an electric field, $\vec{E}$, we typically think of the following 'linear' equations:



$$\vec{\mu} = \overrightarrow{\mu_0} + \alpha\vec{E} \qquad \text{(eq. 31)}$$

$$\vec{P} = \overrightarrow{P_0} + \chi^{(1)}\vec{E} \qquad \text{(eq. 32)}$$

With $\vec{\mu}$ the dipole moment of the molecule, $\overrightarrow{\mu_0}$ the intrinsic dipole moment in the absence of an electric field and α the linear polarizability of the material. The intrinsic dipole moment can be either zero, but can be quite large for molecules with a built in dipole, e.g. a molecule having a conjugated backbone in which electrons can move quickly, and a donor and acceptor group at opposite ends of this backbone. The linear polarizability α describes the volumetric measure of the electron cloud displacement in the path of the incident light, relative to the nuclear framework. This polarizability is closely related to the structure and bonding properties of the molecules and is always non-zero. Likewise, equation (32) discusses the interaction of the electric field with a macroscopic material, with $\vec{P}$ the polarization of a material and $\chi^{(1)}$ the linear susceptibility. In the general courses of physics it has been shown that this linear susceptibility relates to the refractive index, and can thus describe absorption of a material.

What is important to realize, is that the above equations only hold for small electric field and that they are actually abbreviations of a Taylor expansion. When working with pulsed lasers, quite strong electric fields can occur and therefore also the higher terms in the Taylor expansion become increasingly important, leading to nonlinear optical effects. Assuming that the electric field component of the laser can be written as $E_j \cos(2\pi\nu t)$, the Taylor expansion becomes:

$$\mu_i = \mu_0 + \alpha_{ij}E_j\cos(2\pi\nu t) + \beta_{ijk}E_j\,E_k\cos^2(2\pi\nu t) + \gamma_{ijkl}E_j\,E_k E_l\cos^3(2\pi\nu t) + \ldots \qquad \text{(eq. 33)}$$

$$P_i = P_0 + \chi^{(1)}_{ij}E_j\cos(2\pi\nu t) + \chi^{(2)}_{ijk}E_j\,E_k\cos^2(2\pi\nu t) + \chi^{(3)}_{ijkl}E_j\,E_k E_l\cos^3(2\pi\nu t) + \ldots \qquad \text{(eq. 34)}$$

In this case, α_{ij}, β_{ijk}, γ_{ijkl}, represent the linear, and 2nd and 3rd order polarizability tensors, and the subscripts $i, j, k, l, \ldots$ refer to the components of the tensor expressed in the molecular frame. Accordingly, $\chi^{(1)}$, $\chi^{(2)}$, … represent the first, second and higher order macroscopic susceptibilities of a material.

From equations 33 and 34, one can immediately see that when sending a laser with frequency $\omega = 2\pi\nu$, the molecule/material will respond nonlinearly and will oscillate also with frequency 2ω, 3ω, ... as schematically presented in the figure below.

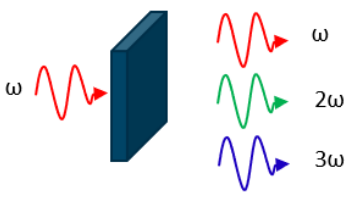


Figure 7: schematic representation of the nonlinear polarizability resulting in frequency doubling or tripling within a material.

Fluorescence (emission) and absorption are linear phenomena that depend on the first order susceptibility of a material, while pump-probe methods and transient gating use the third order nonlinear phenomena (see further). There is a great variety in time-resolved spectroscopy methods. In this chapter we will briefly survey two of them, namely fluorescence decay and pump-probe methods.

Depending on the different time-scales, these techniques will use different detection schemes to provide for the ultrafast detection of photons. One can classify these detection schemes in two different manners: (i) electrical detection and (ii) pump-probe techniques. While electrical detection can access time-scales down to about 10ps using either a streak-camera or ultrafast photodetectors (see further), pump-probe techniques can measure down to a time resolution of a few fs. The main idea in these pump-probe techniques is that an exciting laser pulse (the pump) changes the state of the sample. After a given delay in time a probe laser pulse interrogates the changes. The delays can be as short as fs and can be introduced by a longer path-length of the probe pulse versus the pump in a variable delay line.

5.1 Fluorescence Decay

Conceptually, the simplest time-resolved method is fluorescence decay or time-resolved fluorescence. A single laser pulse excites the system to a higher energy level, and the spontaneously emitted light is detected as a function of time. This emitted light is proportional to the incident light intensity and this can thus be considered a linear process. In the above Jablonski diagram presented in Figure 6, one can see the different processes the excited state can undergo before emission takes place. The incident laser pulse will induce absorption from the singlet ground state, S_0, promoting the molecule to the excited electronic state S_1, when the energy of the photon is larger or equal to the energy difference between these two electronic states. The molecule will exist for a short time in this excited state, after which it returns to the ground state in a radiative or non-radiative manner. Generally, the molecule gives back the excess energy as a result of the following processes:

- Radiative channel of energy dissipation, called fluorescence, where a photon is emitted with energy equal to the energy difference between the two electronic states. The molecule will end up in its ground state again.
- Nonradiative channel of energy dissipation to the surrounding molecules (e.g. conversion to heat) followed by the molecule's return to the single ground state, typically called 'internal conversion'
- Nonradiative transfer of energy from the excited singlet state S_1 to the triplet state T_1 via so-called intersystem crossing. This is the transition between the electronic states of different spin multiplicity (the singlet state has a total spin of 0 and the triplet state a total spin of 1). In theory, this change in spin multiplicity is forbidden by the selection rules within the dipole approximation but can become allowed, e.g. in case of heavy atoms in the vicinity with a high spin-orbit coupling.
- Radiative transfer of the energy from the excited triplet state to the singlet ground state, called phosphorescence. The lifetimes of the excited triplet states are much longer (because in theory the phosphorescence is forbidden, similarly as intersystem crossing). Triplet states typically also have a lower energy than the singlet states, such that the phosphorescence channel emits photons with a lower energy as the fluorescence channel.
- Higher-order processes such as triplet-triplet annihilation, in which two triplets recombine into an excited singlet state and a singlet ground state. The excited singlet state can then emit again, but given the much longer lifetime of the triplet excitons, this process is typically referred to as delayed fluorescence.

Let us now only consider the first channel above, fluorescence. As the fluorescence lifetime, τ, of the molecule in the excited state depends on molecular motions in its environment, it is important to monitor the time evolution of the emission spectrum. The lifetime measurements provide important information about the dynamics and structure, type of interactions and molecular motions of the surrounding molecules. In contrast to the fluorescence spectrum in the wavelength dimension in steady-state spectroscopy, which represents a picture in which all the objects are static, the measurement in the time domain can be compared to a movie in which movement in time provides additional information about the system.

In the time-resolved measurements, fluorescence spectrometers use light from a pulsed laser source. The fluorescence is focused onto the monochromator slit, which induces spatial separation of various fluorescence

spectral components. In order to measure the fluorescence lifetime, τ, different detection methods may be used, such as: streak camera detection, time-correlated single photon counting, averaging by the boxcar integrator, light-gating techniques, and phase-modulation methods. The streak camera, time-correlated single photon counting and averaging by the boxcar integrator use electronic devices to obtain the time-resolved emission of the sample versus time. The light-gating technique explores the correlation between the excitqtion pulse, the gate-opening pulse, and the observed emission. In the simplest cases, when measuring the fluorescence decay as a function of time, one typically obtains an exponential decay that can be fitted from which the lifetime τ can be deduced. This can be directly seen from equation 5. Indeed, at time $t=0$, i.e. just after the initial laser pulse, the energy state '2' is highly populated. Afterwards, we observe the decay of state '2' to the ground state '1' according to equation 5. For more complicated systems, multi-exponential decays can be found, e.g. a combination of direct emission and delayed fluorescence or because other non-radiative processes play a role which were neglected when composing equation 5. Modern software allows to determine also those lifetimes, treating them as parameters in standard fitting procedures.

Let us now discuss the different methods that have already been posted above, streak-camera detection, time-correlated single-photon counting, box-car integration and light-gating techniques.

5.2 Streak-camera detection

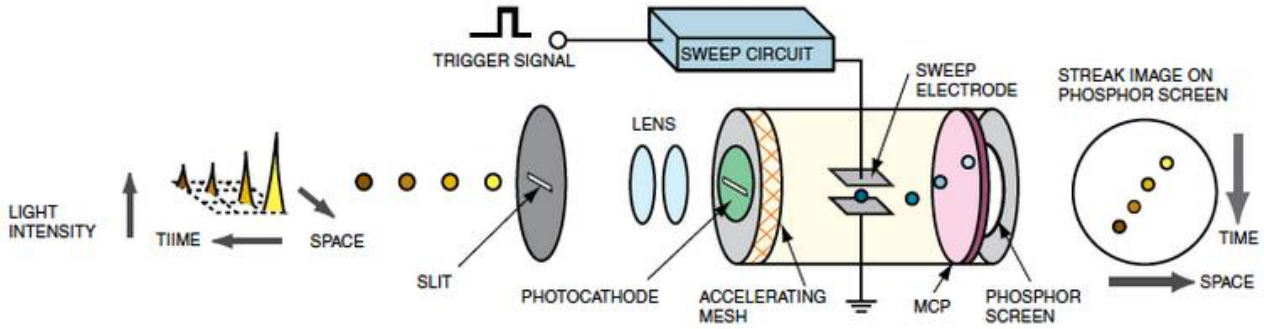


Figure 8: Schematic representation of a streak-camera, able to deliver 2D information, *i.e.* fluorescence as a function of time and wavelength. [5]

Streak cameras are high speed light detectors that enable the observation of fluorescence both as a function of time and wavelength. A schematic representation is presented in Figure 8. To enable measurements as a function of emission wavelength, the streak-camera is combined with a monochromator (not shown in Figure 8) which separates the different wavelengths along one axis, the space-axis in Figure 8. Single photons will arrive to the first slit of the streak-camera as a function of time and space, the latter thus originating from the spatial separation due to the different wavelengths. After passing through the slit, the photons will be focused by lenses on the photocathode, where the photons are converted into a number of electrons proportional to the intensity of the incident light. The electrons are then accelerated and conducted towards the microchannel plate (MCP) after which they are bombarded against the phosphor screen of the streak-tube and converted to an optical image. To obtain the time axis, the electrons run through a sweep electrode, where as a function of time a voltage is changed such that the electrons deflect in the opposite direction as the space axis.
In a typical experiment, the light intensity is strongly reduced to be able to measure in single-photon regime, and a long integration is performed until sufficient statistics is obtained. A typical experiment is shown in Figure 9, where each 'dot' in the spectrum represents a single photon that was detected as a function of emission wavelength and time.

[5] Figure and further reading: https://hofstragroup.com/product/hamamatsu-c4334-02s-streak-camera-system-for-time-resolved-spectroscopy/

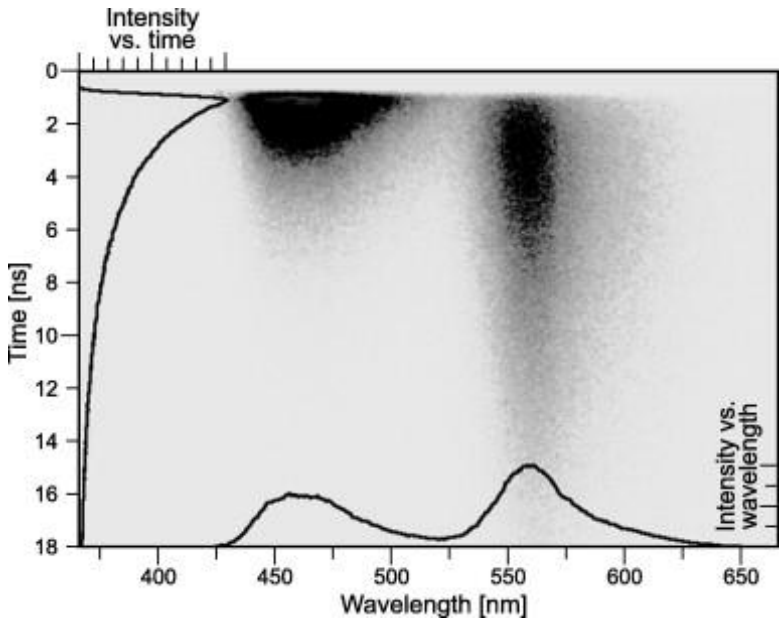


Figure 9: Typical streak-camera experiment for two different molecules in solution with different lifetimes and emission wavelengths. Integration on the time-axis yields the expected exponential intensity decay, while integration on the wavelength axis yields the emission spectrum.

Key to this streak-camera detection is the ability to detect single photons. In the example above a so-called **MCP, or microchannel plate** is used. Such an MCP has several channels that work independently as an electron multiplier. A single electron enters one of the channels and this creates an avalanche of electrons which then create a measurable signal on that particular 'pixel'. The first electron enters the channel and will be accelerated by the voltage across the channel. When hitting the wall of the channel, it will emit a second electron. Secondary electrons are accelerated by an electric field developed by the voltage across the MCP. They will travel in parabolic trajectories until they strike the channel surface, producing more secondary electrons. This process is repeated multiple times along the channel, as a result, this avalanche yields a measurable signal (typically a few thousand of electrons). The process is the same is an in a conventional photomultiplier, however providing at the same time spatial resolution.
The MCPs have high gain (avalanche), high spatial resolution (determined by the number of channels in the plate) and a high temporal resolution and are therefore ideally suited to be used in the streak-camera. The gain of an MCP is typically determined by the length-to-diameter ratio of the channel, the gain factor G of the channel (chance of producing secondary electrons) which depends on the wall characteristics and the applied electric field. Generally, the length-to-diameter ratio is designed around 40, which produces a gain of 10^4 with an applied voltage of 1kV. The transit time of MCPs is very small, in the ps range. The dark current is typically also very low (a few events per second per active area), such that each incoming electron can easily be recognized as a real signal. Spatial resolution can be down to 10µm.

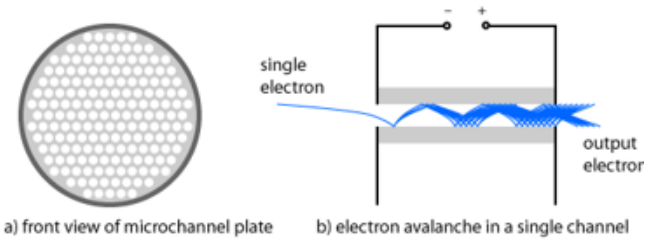


Figure 10: Schematic representation of a microchannel plate.[6]

[6] Figure and further reading: https://www.rp-photonics.com/microchannel_plates.html

5.3 Time-correlated single-photon counting

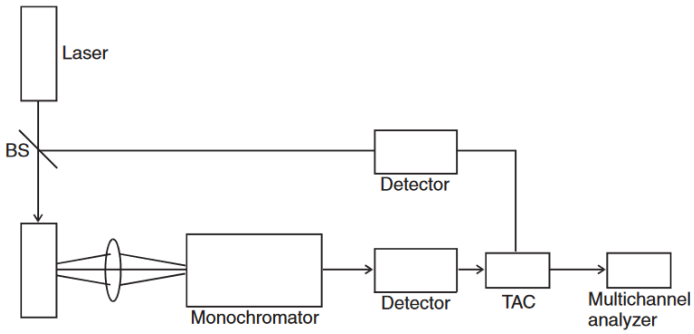


Figure 11: Schematic setup for time-correlated single-photon counting (BS= beam splitter, TAC = time-amplitude convertor

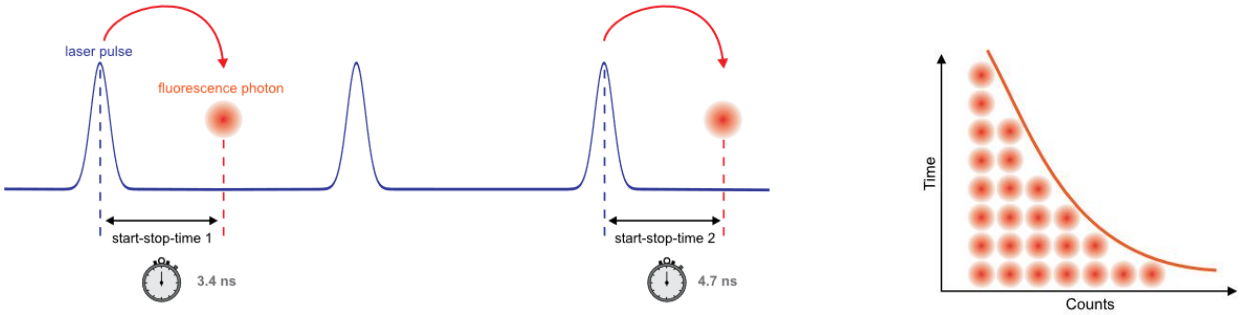


Figure 12: Principle of time-correlated single photon counting.[7]

Figure 11 presents a typical experimental setup and Figure 12 presents the principle of time-correlated single photon counting (TCSPC) measurements. The method is based on the repetitive, precisely timed registration of single photons. A laser pulse of duration Δt, which is short in comparison with the measured signal decay times, excites a sample at $t = t_0$, inducing fluorescence. This laser pulse will pass through a beam splitter such that part of this laser pulse will directly go to a detector and starts the 'clock', i.e. sets $t = t_0$. The other fraction of the laser pulse excites the sample, after which the emitted photons pass a monochromator (to select the emission wavelength) and fall on a similar detector. As soon as this second detector detects one photon, the clock will stop and the time between the start and the stop will be registered in a histogram. This process is repeated several times to get sufficient statistics which then builds up the fluorescence decay curve. When no photon is detected between two laser pulses, the clock will be reset by the second laser pulse so that each time a new measurement will start. The **TAC, time-amplitude convertor** is the system that measures the time between 'start' and 'stop'. Similarly as in the streak-camera detection, one thus requires a detector that can detect single photons, which can be the above mentioned microchannel plate, but can in this case also be a photomultiplier tube (PMT) or a single-photon avalanche diode (SAPD). Each of these detectors will be described below, but let's first discuss the principle of the TAC which determines the precision of the time-registration between laser pulse and photon detection.
The TAC is in principle a highly linear ramp generator that is started by one signal and stopped by the other signal (of the two photodiodes). The result is a voltage, proportional to the time difference between the two signals (see Figure 13). This voltage is then kept constant and send to an analog to digital convertor (ADC) which provides the digital timing value used to address the histogram, after which the TAC is reset. This ADC must thus also be very fast in order to keep the dead time of the system short. The dead time can be

[7] Figure and further reading: https://www.picoquant.com/images/uploads/page/files/7253/technote_tcspc.pdf

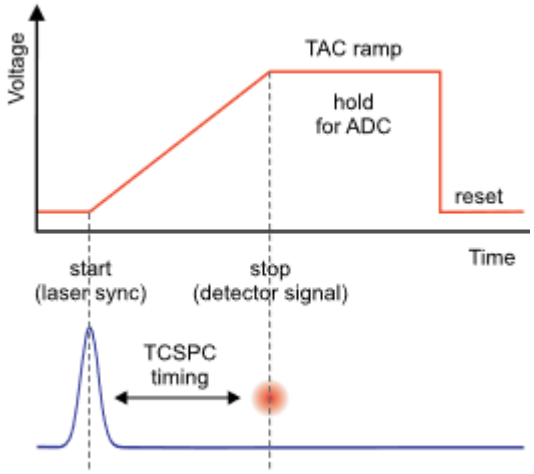


Figure 13: Principle of the TAC device.

considered as the time needed to reset the TAC and thus within that time-frame no photons or laser pulses can be detected.

In order to guarantee single photon statistics, as a rule of thumb, on average only one in 20 to 100 excitation pulses should generate a count at the detector. In other words, the average count rate at the detector should be at most 1 to 5% of the excitation rate. This leads to another issue, the maximum count rate that the system can handle, setting limits to the possible detectors that can be used. With modern integrated TCSPC designs, count rates up to 40 million counts per seconds can be achieved.

It is also worth nothing that in principle, due to quantum mechanics, the actual photon arrival times are random, so that there can be bursts of high count rate and periods of low count rates. Bursts of photons may well exceed the average rate. It should be kept in mind when performing an experiment. Even if the instrumentation in principle can accommodate the average rate photons are emitted, it may drop photons in bursts (only the first photon will stop the TAC, the rest is neglected due to the dead time). Therefore, to obtain correct statistics, collection times should be sufficiently long to thrust the resulting histograms.

In view of the different detectors that can be used, we first discuss the **photomultiplier tube.** A PMT works similarly as a single channel of a MCP that was discussed above. It consists of a light-sensitive photocathode that generates electrons when exposed to light. These electrons are directed onto a charged electrode called a dynode. The collision of the electrons with the dynode produces secondary electrons. Since each electron that strikes the dynode causes several electrons to be emitted, there is a multiplication effect. After further amplification by multiple dynodes, the electrons are collected at the anode of the PMT and output as a current. The current is directly proportional to the intensity of light striking the photocathode. The PMT is thus a photoelectron amplifier with high sensitivity and remarkable low noise, similar as the single channels of an MCP. They moreover have a wide dynamic range, i.e. they can measure single photons up to relatively high levels of light. They are moreover also very fast, thus ideally suited for the TCSPC experiments. In single photon counting experiments, each photon will generate a short output pulse containing millions of electrons. PMTs are usually most sensitive in the blue and red regions of the visible spectrum, with greater quantum efficiency in the blue-green region, depending on the exact materials of the photocathode. Typical peak quantum efficiencies are about 25%. In the near infrared however, the sensitivity drops off rapidly.

The semiconductor equivalent of PMTs are called **avalanche photodiodes** (APDs). Generally, APDs may be used for ultra-low light detection, with single-photon quantum efficiencies of 50% and even maximum efficiencies for some wavelengths at about 80% have been reported. Recent APDs can reach timing accuracies down to 30ps but typically 400ps is more common. A single photon may trigger an avalanche of about 10^8 carriers, but one is not really interested in the amplitude of the output current, because it will carry no information, other than 'there was a photon'. The working principle of an APD is based on a PIN photodiode. It consists of two heavily doped p^+ and n^- regions (in e.g. Si) at opposite sides of the APD and in between two lightly doped regions, typically one is not doped, called the intrinsic (I) region, and one is slightly p-doped. Thus the system consists of the following sequence $p^+ – I – p – n^-$ or in words: a heavily doped p-region, an intrinsic region, a weakly-doped p-region and a heavily doped n region. The heavily doped p-region acts like the anode and the heavily doped n-region acts like the cathode. The APD is operating in reverse bias, meaning that the p-region is connected to the negative terminal and the n-region is connected to the positive terminal of the power source. The reverse bias increases the electric field across the depletion layer between p and n regions. Incident light enters from the p+-region and gets absorbed in the intrinsic (or weakly doped p-region), creating an electron hole pair. The comparatively weaker electric field in this region, causes separation of these pairs such that the electrons drift with their saturation velocity towards the pn region

where a high electric field exists. Like that they are strongly accelerated, and start to collide with other atoms, generating new electron-hole pairs. A large number of electron-hole pairs (avalanche) results then in a measurable current. To obtain this avalanche effect, the reverse bias is set very high (typically 100-200V in silicon), such that the kinetic energy of the charge carriers overcomes the ionization energy. APDs can be designed for different wavelength ranges. For example, Silicon covers the visible spectrum (up to 1050 nm), while InGaAs APDs are sensitive in the near infrared (up to 1600 nm), gallium-nitride diodes are used in the ultraviolet range.

5.4 Box-car integration

All the above methods rely on the detection of single photons to then perform sufficient statistics to obtain the fluorescence decay signals. However, the signal may be controlled with other detection methods, such as synchronized sampling by the boxcar integration. The configuration applied in this method is similar as the TCSPC, but in this case, the TAC is replaced by the so-called box-car integrator. In this electronic device, at a precisely determined moment correlated with the trigger pulse, an electronic entrance gate is opened for a certain short time and the voltage corresponding to the fluorescence is measured. Figure 14 shows the schematics of the box-car integrator which forms the heart of this method. This circuit is simply an RC low-pass filter gated by switch S_1. When the gate opens (switch S_1 closes) the output voltage v_0 starts to rise exponentially as given in Figure 14. The gate time constant (given by the RC) is adjusted so that the output voltage is typically within a few percent of the input voltage v_i by the end of the selected gate width (the time the gate is open). The integral of the gate sample is the output voltage at the end of the gate width that can then be read out to create a signal.

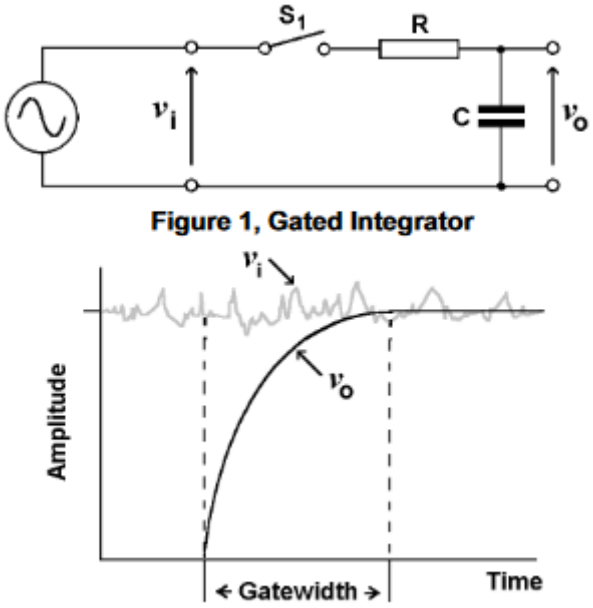


Figure 14: Principle of box-car operation.[8]

In a first type of experiments, referred to as 'static gate boxcar averaging', the length of the trigger delay between laser pulse and gate opening is fixed and the intention is usually to determine the amplitude of some narrow feature of a waveform that is typically much narrower than the repetition period set by the overall trigger rate (see Figure 15). The trigger delay is set to open the gate just before the feature and the gate width is set to as broad as the feature. Each sample results in an integral intensity, representing the area under the curve, and these samples are then averaged in the output average.
In a second type of experiments, the so-called 'waveform recovery mode', the boxcar operates rather like a sampling oscilloscope, with the trigger delay being swept over a range of values while the output is recorded, as a result the signal waveform (the fluorescence decay in this case) is directly recorded.

[8] Figures and further reading: https://123.physics.ucdavis.edu/week_1_files/Boxcar_Averager.pdf

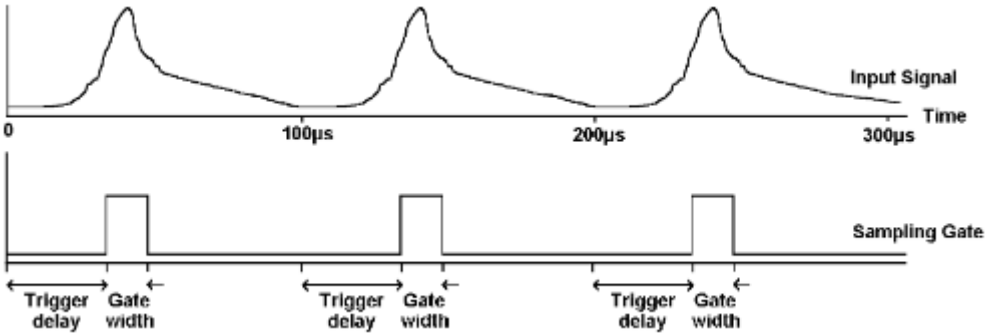


Figure 15: boxcar average in the static gate mode.[9]

5.5 Light-gating techniques

While the previous experiments all relied on electronic types of gating in the detection analysis, it is also possible to use the above-mentioned nonlinear optical effects to provide for light gating. Instead of a continuous fluorescence beam reaching a detector, one can provide discrete portions of fluorescent light to the detector at controlled time intervals, being the basis of the light gating method. A schematic of this method is provided in Figure 16.

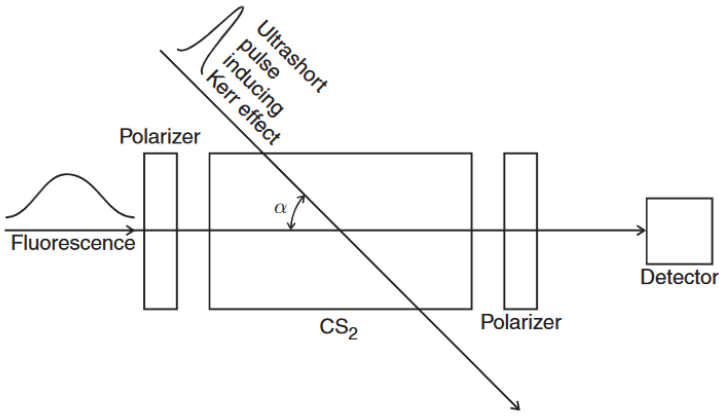


Figure 16: Principle of light-gating techniques.

The light, emitted as a result of fluorescence, illuminates carbon disulphide (CS_2) placed between two crossed polarizers. In this case the light does not reach the detector, because the crossed polarizers to not transmit the light to the detector. CS_2 is an isotropic liquid and does not affect the polarization of the incident light. However, if this material is placed in a strong electric field, induced e.g. through a short laser pulse, the molecules begin to align in the direction of the electric field, and this ordering makes the liquid anisotropic and birefringent. This birefringence generated in liquids by an electric field is known as the Kerr effect. As a result of birefringence, the incident light beam splits into two rays: the ordinary and the extraordinary rays, with mutually perpendicular polarisations, and the intensity of each depends on the angle between the polarization of the incident ray and the optical axis of the Kerr material. Thus the Kerr effect changes the polarization of the beam that travels through the medium, allowing the fluorescence to pass to the detector. If we apply an ultrashort laser pulse, its electric field will induce an ultrashort Kerr effect, and thus the liquid placed between the two polarisers will act as an ultrafast shutter, which transmits the emission while the CS_2 is birefringent, which for CS_2 is about 3.5ps.

[9] Figures and further reading: https://123.physics.ucdavis.edu/week_1_files/Boxcar_Averager.pdf

A second way to use light-gating is based on the nonlinear optics. When a short laser pulse and the fluorescence beam are incident simultaneously on a nonlinear crystal at an angle allowing phase matching to be fulfilled, the interaction of the beams produces a signal of a frequency equal to the sum frequency, called the up-conversion signal. The up-conversion signal is generated only when the beams overlap, that is only when the laser pulse illuminates the crystal. When the fluorescence intensity changes with time, the up-conversion signal changes also, as its intensity linearly depends on the intensity of each of the superimposed beams.

5.6 Pump-probe methods

All the above methods can only reach a time resolution of the order of several tens of picoseconds at the most. In many cases this is an insufficient resolution to study dynamics of excited states that take place at the pico- or femtosecond time scale. One way to extend the time resolution to lower timescales is to use pump-probe methods. The principle is provided in Figure 17. The sample is first excited by a pump beam, and later interrogated by a probe beam. The delay between pump and probe can be managed by changing the path length difference between probe and pump beam, through the simple formula: $\Delta t = \Delta x/c$ with c the speed of light and Δx the pathlength difference between pump and probe. Recording the signal for different delay times (by changing the path length difference) allows to monitor the dynamics of the studied process. In old experiments, the pump and probe beam had the same wavelength, however, with tunable pulsed lasers becoming increasingly represented, and even white-continuum sources emitting light pulses with a broad spectral range, any kind of combinations of pump and probe beams can be available.

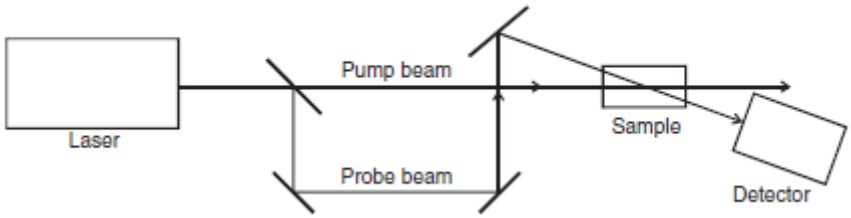


Figure 17: Principle of pump-probe spectroscopy.

Pump-probe methods are mostly measuring the change in absorption of the sample. Assume a simple 2-level system. After an initial pulse by the pump beam, the system is no longer in thermal equilibrium, with the population of state '2' now higher than the population of state '1'. When interrogating the sample with the probe beam, the absorption spectrum will have decreased, as the number of molecules in state '1' is much lower, called 'photobleaching'. When the time delay increases, the thermal equilibrium is restored (e.g. through fluorescence), and the original absorption spectrum starts to reform. Aside from intensity changes, also spectral shifts can be observed, for which then a white-light continuum probe beam is more ideal. Important to observe any changes, the pump probe must have an intensity close to saturating the transition such that the energy level population is changed to the most.
Aside form monitoring changes in absorption, superimposed one can also find changes in emission or changes in vibrational light scattering that can give information on the vibrational lifetimes of the system.