Fluorescence Lifetime Measurement using Time Correlated Single Photon Counting

Master Thesis

Masters in Photonics

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Popular Science Article

This thesis is about the construction and demonstration of time correlated single photon counting (TSCPC) setup. The technique is essentially digital and is based on detection and counting of single photon, recognizing quantum nature of light. The time is measured between excitation pulse and detected photons and is stored in histogram with x-axis corresponding to time interval. The sample is excited repeatedly and resulting histogram of intensity versus time called as fluorescence decay curve.

Generally speaking, fluorescence or emission spectroscopy is one of the fundamental spectroscopic techniques. This is the study of fluorescence phenomenon which is the emission of photons from singlet excited state. The emission is red shifted relative to absorption maxima due to loss of energy as heat during relaxation process. In the time resolved fluorescence decay experiment, the sample is excited with pulse of laser light, it starts with high intensity and then decays, rapidly. Later on from appropriate fitted functions, type of decay, lifetime and amplitudes are calculated. In case of photon counting, the measured data is in form of discrete time function. But still the fitted functions provide the same information. Then question arises about additional advantage of our TCSPC technique over other life time measurements. The additional benefits come from simplicity of integrated setup i.e. lack of focussing lenses and very low excitation density is required.

The setup has been characterized by measuring the fluorescence decay from organic dye and polymer solar cell material. The data has been fitted with exponential functions and fluorescence life times are calculated with good accuracy. Other than life time measurements, the technique is being successfully used for single molecule detection, TCSPC imaging, and fluorescence correlation spectroscopy in combination with fluorescence microscopy etc.

Abstract

A time correlated single photon counting (TCSPC) setup is built to measure the fluorescence decay of samples, such as solar cell materials. TCSPC is a sensitive technique for measuring fluorescence decays on nanosecond time scale and longer. The principle of TCSPC is based on the precise registration of the arrival time of fluorescence photons from a sample. A fluorescence decay curve is constructed from the TCSPC measurement, this curve is used to extract the fluorescence lifetime. The setup is novel with respect to the conventional method of collecting a fluorescence signal. It is a simplified setup as there is no need of complex geometry of optics to focus the excitation beam or to image the fluorescence on to the detector. Instead, the excitation beam is unfocused and the sample is placed as close to the detector as possible. This approach allows for the usage of low excitation density of photons. The low level of light that this setup could detect made it very sensitive for measuring samples with low emission. The setup is characterised with two different samples: Coumarin-152 (7-N,N-dimethylamino-4-trifluoromethyl-1,2-benzopyrone) and APFO3 (poly[2,7-(9,9-dioctylfluorene)-alt-5,5-(4,7′-di-2-thienyl-2′,1′,3-benzothiadiazole)]). Coumarin-152 is a commercial laser dye and APFO3 is a polymer solar cell material. These measurements reveal that the single and non exponential curves could be obtained using this setup. The verification of the setup is further carried out by classifying different errors that can influence the measurements.

Acknowledgement

The success of this diploma work would have not been possible without the guidance, support, cooperation and the enlightened supervision of my supervisor Arkady Yarstev, Department of Chemical Physics, KemiCentrum, Lund University, Lund Sweden.

In addition to his expertise, rigorous critique and thorough analysis I would like to mention Tomas Österman, who improved not only the quality of this dissertation, but also my overall understanding. I am grateful to their never ending guidance, keen interest, scholarly comments and constructive suggestions throughout the course of my diploma project.

There are no words enough to acknowledge or express my obligations to my affectionate and adoring parents, whose prayers, moral and financial support had led me so far.

I would like to extend my heartiest gratitude to my friends without whom I would not have been able to achieve this. My friends share the struggle and hard work I is able to put in because of them. I would like to say thank you my dear friends.

May Allah Almighty give me the energy and will power to fulfil my entire future endeavour and I thank ALMIGHTY ALLAH for that entire he has blessed me with.

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1. Introduction

The development of new instruments and techniques broadens the understanding of matter. Numerous spectroscopic techniques are used to study different properties of a sample, for example their chemical composition, and their lifetime etc. Any technique is selected based on the information to be extracted from a sample. In spectroscopy a common tool is laser. The laser light interaction with matter is used in photo chemistry, absorption spectroscopy, fluorescence spectroscopy, time resolved spectroscopy, environment monitoring, material processing, medical applications etc . In the field of spectroscopy, laser is used to characterise light induced processes in a sample. The processes a laser can probe depend on the absorption and emission of the sample . Absorption of light is followed by emission which has a distinct lifetime and can be used to characterise a process in a sample. One way to measure this lifetime is to use the Time Correlated Single Photon Counting (TCSPC) technique. It is a sensitive technique which can detect very low emission from a sample and measure the emission lifetime. TCSPC is based on light behaving as quanta (photon). TCSPC has many applications other than fluorescence lifetime measurement, such as Ultrafast recording of optical waveforms, Detection and Identification of Single Molecules, DNA sequencing, Optical Tomography, and Fluorescence lifetime imaging .

1.1 Motivation

The aim of my diploma project is to design and demonstrate the highly light sensitive, time resolved fluorescence setup, based on single photon counting. The key advantage of TCSPC over steady state fluorescence measurement is that all the detected photons are accumulated in histogram without added instrumental noise, thus providing high signal to noise ratio and high sensitivity. This instrument allows reproducible measurements of rates and mechanism of light induced dynamics, with fast time resolution of ns. It is also the objective to avoid the artifacts arising from emissive optical components. This is circumvented by using a series of interference filters. These approaches are manifested in development of new instruments for study of fluorescence samples (Coumarin-152, APFO3), investigation of decay kinetics and therefore model assessments. The later is the subject of complementary diploma project.

2. Theory and Background

The study of light matter interaction, as a function of wavelength is called the spectroscopy. The most widely used spectroscopic techniques are UV/Visible absorption (electronic) and fluorescence spectroscopy. Absorption of UV/Visible radiations by a molecule excites it from electronic ground state to any vibrational level of electronically excited state S_1. Fluorescence occurs when this excited molecule returns back to the ground state with an emission of photon. Fluorescence measurements are classified into two main types: 1) steady state measurements, where the sample is excited with continuous beam of light and emission intensity is measured 2) time resolved measurements of fluorescence intensity decay when sample is excited by a pulsed laser. Time resolved measurements are used extensively in fluorescence spectroscopy, particularly investigating dynamic conformational changes in biological macromolecules, mechanism of energy transfer and quenching etc. Further, most of the time resolved measurements are being carried out by single photon counting . Single photon counting is a digital technique based on detection and counts of individual photons from illuminated sample. Since, this technique is insensitive to instrumental noise it enables to analyze the low intensity fluorescence decays. The decay curves are data collected in form of photon counts versus time. In the following theory part, we discuss in detail about above mentioned spectroscopy techniques.

2.1. Absorption spectroscopy

Absorption spectroscopy is a technique that measures the absorption of radiation, as a function of frequency or wavelength, due to its interaction with a sample. It is a tool for investigating molecular structures, atomic physics, astronomical spectroscopy and remote sensing etc. . Absorption of radiation by a sample is measured by the ratio of the transmitted intensity and incident intensity of light. A common way is to illuminate the sample from one side and detect the transmitted intensity of the light on the other side. Absorbance of a sample is given by the Beer Lambert law in Eq.1.

$$I = I_0 e^{-\sigma lN} \quad (1)$$

Incident intensity of light I_0, has to be measured. The intensity of the transmitted light, I, is determined by the absorption cross section, σ, the path length l, and the number of absorbing molecules, N.

2.2. Fluorescence Spectroscopy

The quantum mechanical picture states that excitation to a higher energy state is absorption of a photon and radiatively relaxing down to ground state with emission of a photon is fluorescence. Both absorption and fluorescence are very fast processes but the time between them is typically on nanosecond timescale depending on the sample . The law of nature states that every system is stable at a lower energy state and the radiative relaxation process is spontaneous.

Fluorescence emission takes place from the lowest vibrational state of the excited state to the ground state of the molecule. The emission is red shifted compared to the absorption. The process of excitation-emission is illustrated by the Jablonski diagram, Figure 1.

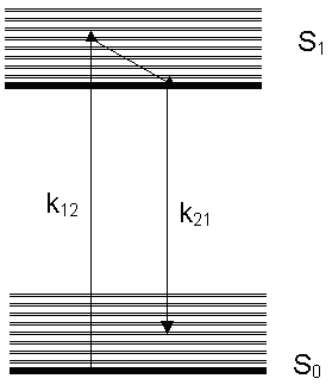

Figure 1. Illustration of the absorption and emission.

In Figure 1, k_{12} is the rate of absorption of light (photon) from ground state S_0, to the excited state S_1. The rate of emission (fluorescence) is k_{21}, and it is from the lowest

vibrational state of the excited state, S_1, to the ground state, S_0. Eq. 2 and Eq.3 describe the transitions between ground state and excited state .

$$S_0 + h\nu \rightarrow S_1 \quad (2)$$

$$S_1 \rightarrow S_0 + h\nu_1 \quad (3)$$

The $h\nu$ in Eq. 2 is the absorbed photon to excite the electron from S_0 to S_1 state. The $h\nu_1$ in Eq. 3 corresponds to the emitted photon when the electron relaxes to the ground state. Eq. 2 and Eq. 3 represent fundamental excited and ground state interaction with a photon.

Lifetime of an excited molecule is the time it takes for an ensemble of such molecules to decay to 1/e of their initial excited state population. The fluorescence lifetime thus refers to the average time a molecule stays in its excited state before emitting a photon. The decay of fluorescence is usually represented by a first order rate equation Eq. 4.

$$A(t) = A_0 e^{-\frac{t}{\tau}} \quad (4)$$

$$[S_1] = [S_1]_0 e^{-kt} \quad (5)$$

In Eq. 4 the initial intensity of fluorescence, A_0, decays with time, t. τ is the fluorescence lifetime. Eq. 6 states that the initial population, $[S_1]_0$, of the excited state decays with a decay rate k . The decay rate, k, and the decay lifetime, τ, are related by Eq. 6.

$$k = \frac{1}{\tau} \quad (6)$$

2.3. Steady state fluorescence spectroscopy

Steady state fluorescence spectroscopy is performed by constantly illuminating the sample and observing the total fluorescence intensity over a broad electromagnetic spectrum. The spectral shape and the peak of emission are the parameters that can be obtained to identify a sample or a process in a sample.

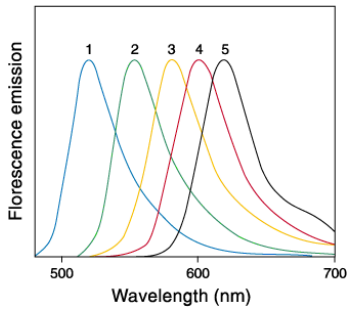

Figure 2. Steady state normalized fluorescence spectra .

In Figure 2, the steady state fluorescence spectrum has five curves. These curves represent the five different samples.

2.4. Time Resolved Fluorescence Spectroscopy

Time resolved spectroscopy is the study of dynamic processes in materials or chemical compounds by means of spectroscopic techniques. Most often, the processes studied occur after illumination of a sample. Using pulsed lasers, it is possible to study processes that occur on time scales as short as femtoseconds .

The fluorescence of a sample is monitored as a function of time after excitation by a laser pulse is called time resolved fluorescence spectroscopy. The time resolution can be obtained in a number of ways, depending on the required sensitivity and the sample. Time resolved fluorescence is used to extract the lifetimes of different samples. The fluorescence from different samples can be spectrally overlapping. Due to the possibility of spectral overlap the steady state fluorescence technique is inadequate. These samples have most probably a distinct fluorescence lifetime which can be extracted by doing time resolved fluorescence spectroscopy .

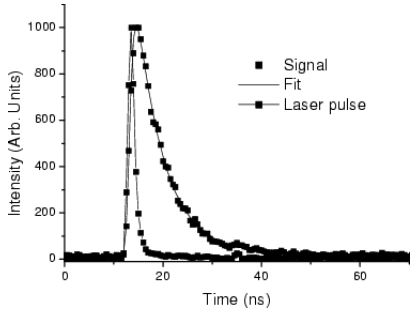

Figure 3. Time-resolved fluorescence spectrum .

In Figure 3, there are two curves for recorded time resolved fluorescence signal and laser excitation pulse.

2.5. Time Correlated Single Photon Counting

Time correlated single photon counting (TCSPC) is a technique to record low level light signal. TCSPC is based on the detection of single photons of a periodical light (pulsed laser) signal, the measurement of the detection times of the individual photons, and the construction of the decay curves from the individual time measurement . The measurement requirement is that the probability of detecting a single photon per pulse should be less than one. For higher counting rate the histogram is biased to shorter times. This is because with TCSPC only the first photon is observed. Therefore, the detection of several photons can be neglected. In fact, the detection rate is typically 1 photon per 10000 excitation pulses. The time is measured between the excitation pulse and the observed photon, see Figure 4. This measured time is stored in a histogram .

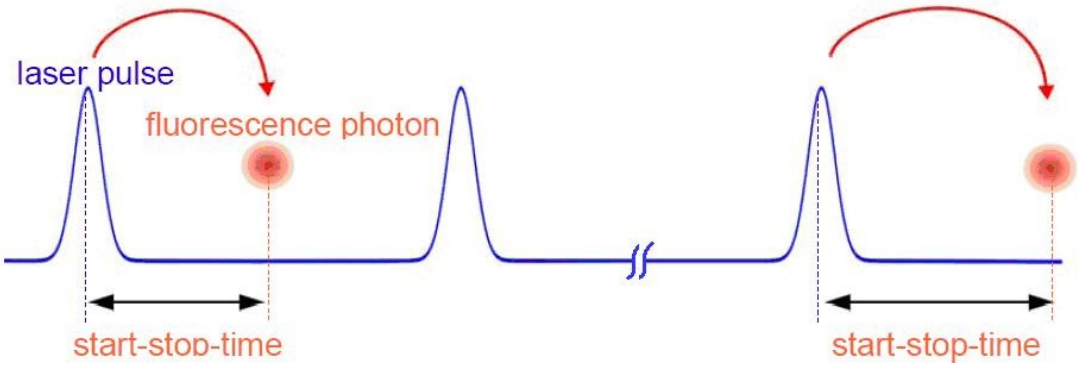

Figure 4. The time measurement between excitation and emission of the fluorescence photon .

A start and stop signal is needed to initiate and halt a measurement cycle. These two signals control the triggering, counting and timing of the measurement cycle. The start signal is a pulse generated from the laser driver which is detected and its time of arrival is stored by the fast electronics. Secondly, the stop signal is another pulse generated when the fluorescence photon arrives at the detector. The arrival time of this stop pulse is also stored. The time difference between the start and stop pulse

arrival is the time of detecting a single photon. This whole cycle is repeated over and over to get a good decay curve.

The sample is excited many times i.e. the excited state of a molecule is populated continuously. The decay of the population from the molecule's excited state is observed. For each photon detected its time after the last excitation pulse is measured. After repeating this cycle over a certain time, the accumulated photons and their arrival times are used to construct the fluorescence decay curve, see Figure 5.

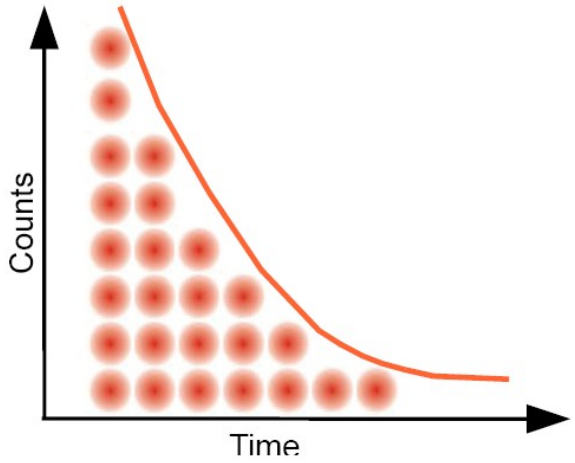

Figure 5. The histogramming procedure to illustrate the decay obtained .

TCSPC differs from methods with analog signal processing in that time resolution is not limited by the width of the detector impulse response. For TCSPC only the timing accuracy in the detection channel is essential. This accuracy is determined by the transit time spread of the single photon pulses in the detector and the trigger accuracy in the electronics. The timing accuracy can be up to 10 times better than the half width of the detector impulse response . For a given number of photons N the signal to noise ratio is given by Eq. 7.

$$SNR = \sqrt{N} \tag{7}$$

If the emission intensity is low enough to avoid pile up, all detected photons contribute to the decay curve . In TCSPC noise due to leakage currents, gain instabilities and stochastic gain mechanism of the detector do not appear in the decay curve. This yields a very good signal to noise ratio.

3. Test Samples

The constructed TCSPC setup is verified after implementation so that other samples can be measured accurately. For the verification of the technique, two test samples are used. The purpose of these test samples is to characterize the setup by showing reproducibility and credibility of the measured data. In Figure 6, the chemical structure of Coumarin-152 (7-N,N-dimethylamino-4-trifluoromethyl-1,2-benzopyrone) is shown . It is dissolved in ethanol and placed in a cuvette of 1mm thickness. Coumarin-152 is used because it is expected to have a single exponent decay which will be shown in the Results and Analysis chapter.

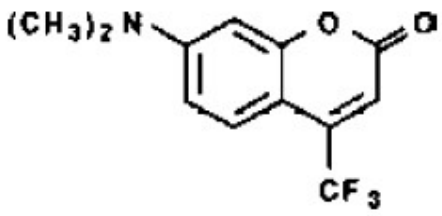

Figure 6. Coumarin-152 .

The other sample is a conjugated polymer. Figure 7 shows the chemical structure of the polymer, APFO3 (poly[2,7-(9,9-dioctylfluorene)-alt-5,5-(4,7′-di-2-thienyl-2′,1′,3-benzothiadiazole)]) . APFO3 is spin coated on a glass substrate. The thickness of the APFO3 after spin coating is approximately 100nm. It is sealed by another glass substrate on top.

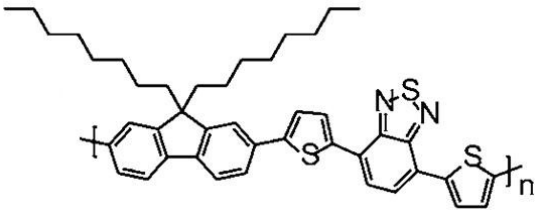

Figure 7. APFO3 polymer .

This polymer is selected as it is expected to be inhomogeneous. Due to the inhomogeneity the sample has non-exponential fluorescence decay. To check whether our TCSPC setup could record non exponential decay this sample is used.

4. Experimental Setup

4.1 Time Correlated Single Photon Counting Setup Overview

The block diagram in Figure 8 is the optical layout of TCSPC setup. The setup has number of components. Each of these components will be discussed in detail in this chapter. The setup has a laser driver with three laser heads, the detector APD, the photon counter PicoHarp, the sample holder with interference filters, the computer for controlling all the instruments, flipping mirrors and neutral density filters. The purpose of the figure is to highlight the setup construction and the instruments used. The operation and function of these components are described individually in following sections.

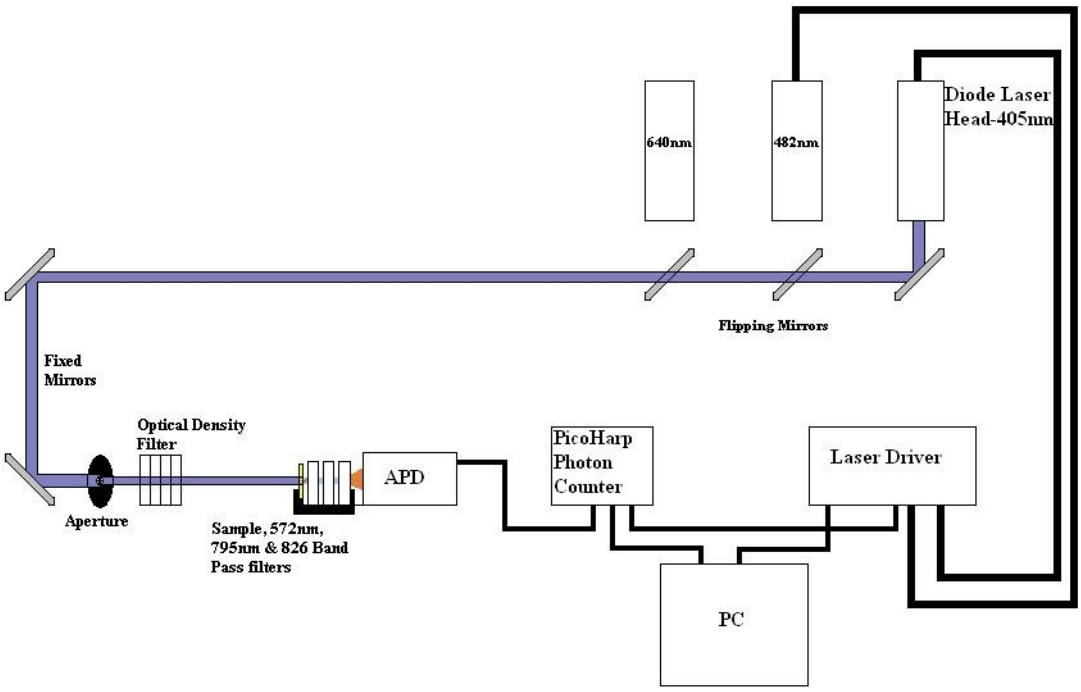

Figure 8. Experimental arrangement of the TCSPC setup built in this work.

4.1.1 Laser Driver and Diode

The laser system used in this setup is *SEPIAII*; it has a set of diode lasers which are driven by the same laser driver . It allows a user to have wavelength, repetition rate and intensity control. Triggering control is completely configured via the computer through USB. The system has three wavelengths 405nm, 480nm and 640nm.

4.1.2 Avalanche Photodiode (APD)

Avalanche photo diodes are semiconductor based detectors. The APD which is used in these experiments is a Micro Photon Device manufactured PDM model . The sensitivity of an APD is equivalent to a Photo Multiplier Tube (PMT). In comparison to PMT the APD has a much smaller chip size and it has lower dark counts. The typical optical power detected by the APD is 1pW. The quantum efficiency at the efficiency maxima is 45%, see Figure 9. The APD is silicon based and therefore the sensitivity is lesser in the longer wavelength region. The two output modes of the APD are the TTL OUT and the NIM OUT .

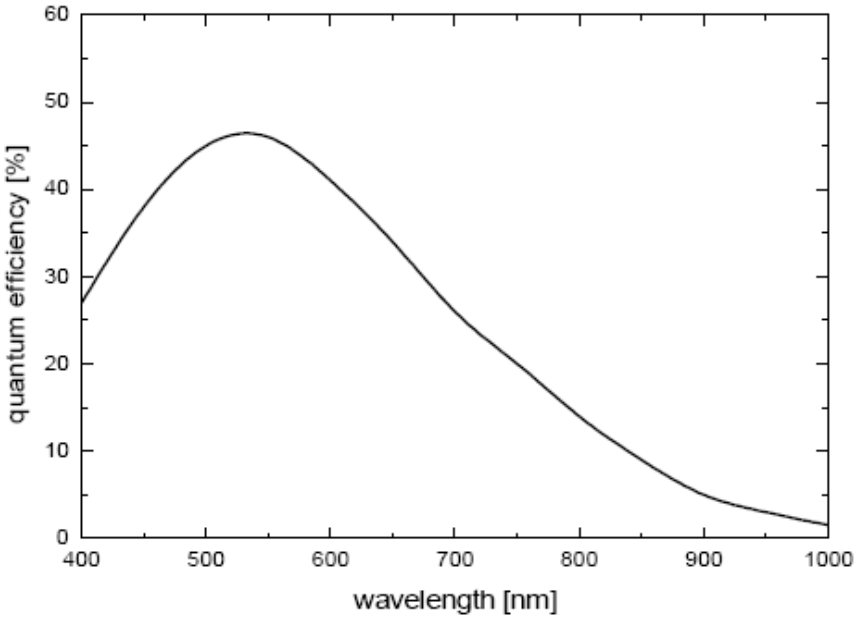

Figure 9. Data sheet specifications of the APD .

TTL OUT has a wider FWHM and does not have a sharp rise of the output stop pulse. TTL OUT of the APD is a standard BNC output connector.

NIM OUT has a shorter FWHM of the output stop pulse. The major advantage of NIM OUT over TTL OUT is the sharp rise of the stop pulse. The sharp rise of NIM OUT gives better timing resolution than TTL OUT.

4.1.3 Photon Counter

PicoHarp 300 is the photon counter that is used in this TCSPC setup. PicoHarp is available from PicoQuant. PicoHarp has high counting rate capability of 10 million counts/sec and timing resolution approximately of 10ps can be achieved.

PicoHarp 300 has a built in counter to count the number of photons and their arrival time. If the counting rate exceeds the maximum value then pile up occurs due to the dead time of the detector. The pile up is discussed in the errors and uncertainties chapter. In Figure 10, the schematic of the PicoHarp shows the built in electronic component called Constant Fraction Discriminator (CFD) which controls the start and stop of a photon arrival event. To minimize false readings the signal is restricted to a threshold voltage by the CFD, which allows detection of signal pulses in above a certain voltage level.

The delay time is calculated by passing the arrival time of both excitation pulse and emission pulse to a time-to-amplitude converter (TAC), which generates a voltage ramp that is a voltage that increases linearly with time on nanosecond or faster timescale. Start signal of the voltage ramp comes from the excitation pulse and the stop signal comes from the detected photon (emission pulse). After this the TAC contains a voltage proportional to the time delay between the excitation and emission signals. If the signal is not within this range the event is not registered. The voltage is converted to a digital value that is stored as a single event with the measured time delay, Δt. A histogram of the decay is constructed by repeating this process many times with a pulsed laser .

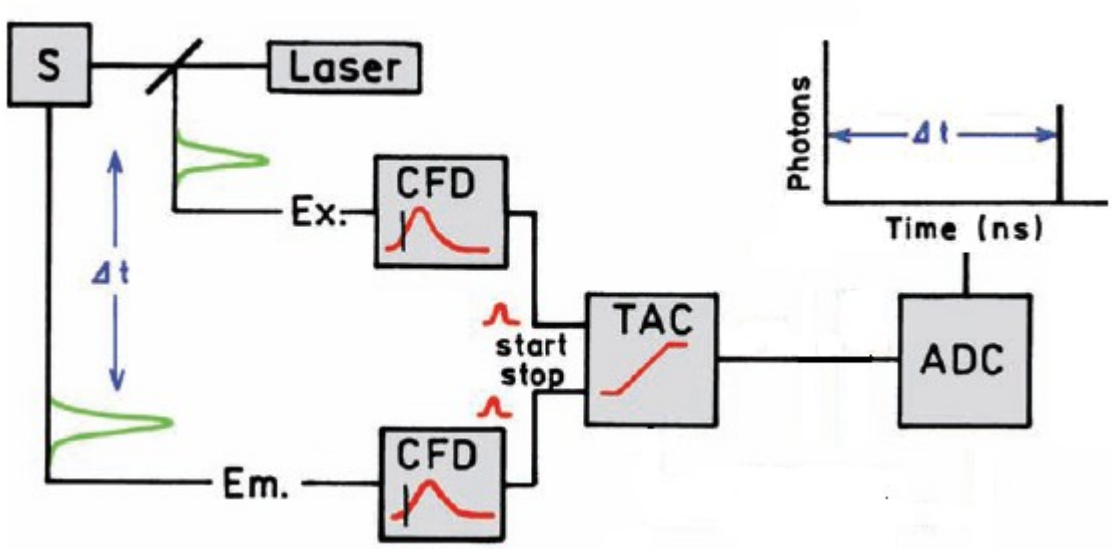

Figure 10. Electronic Schematic of PicoHarp 300.

4.1.4 Interference Filters

The geometry of the setup shows the significance of the interference filters. There are two types of interference filters used; cut-off or long pass filters. The major use of the filters in our geometry is to block out the excitation light. It is important to detect only the fluorescence signal from the sample. These cut-offs are absorbing filters and generally any material that absorbs has some emission. It is therefore expected to have emission from the cut off filters, which contaminates the fluorescence signal and needs to be filtered out along with the excitation wavelength.

The fluorescence decay has two parameters of information about the sample; wavelength and lifetime. The emission at particular wavelength is observed with the use of band pass filters. The cut-off filters used in the setup are from CHROMA Technologies. The cut off filters block all wavelengths below 572nm and 795nm. The band pass filters have a bandwidth of around 5-8nm. The spectral properties further characterise a sample.

4.2 Additional Instruments

The interference filters and the test samples are characterised by measuring their absorption and emission spectra. The instruments used for these measurements are described below.

4.2.1 Absorption Spectrometer-Agilent 8453 UV-Visible

The absorption spectrometer that is used in this set up is as an Agilent 8453 UV-Visible spectrophotometer, see Figure 11. It uses a photodiode array (PDA) which has a range of diodes measuring from the UV to visible. The advantage of PDA is that the measurements are fast as the whole wavelength range is acquired in a single run. The PDA is wavelength calibrated, so the transmitted intensity of light for each wavelength is recorded. Agilent 8453 has a short start up time, as the lamps are stable and warm up quickly. Overall the instrument has low electronic noise.

The output from the spectrometer is the optical density spectrum of the sample over a range of wavelengths (190-1100nm). For measuring a sample is illuminated by a beam of broad band light from the two lamps, deuterium and tungsten. The intensity

of light reaching the detector after passing through the sample is measured by the Photodiode Array (PDA) .

The absorption spectrum of the interference filters and the test samples are measured to check their optical specifications and characteristics. In constructing the setup we needed to find out which interference filters to use for our setup, for blocking the excitation light and filtering out the fluorescence emission. Secondly, we have to find out where our samples have maximum absorption and also to look for a common absorption peak if there is any so that we could use a single excitation wavelength.

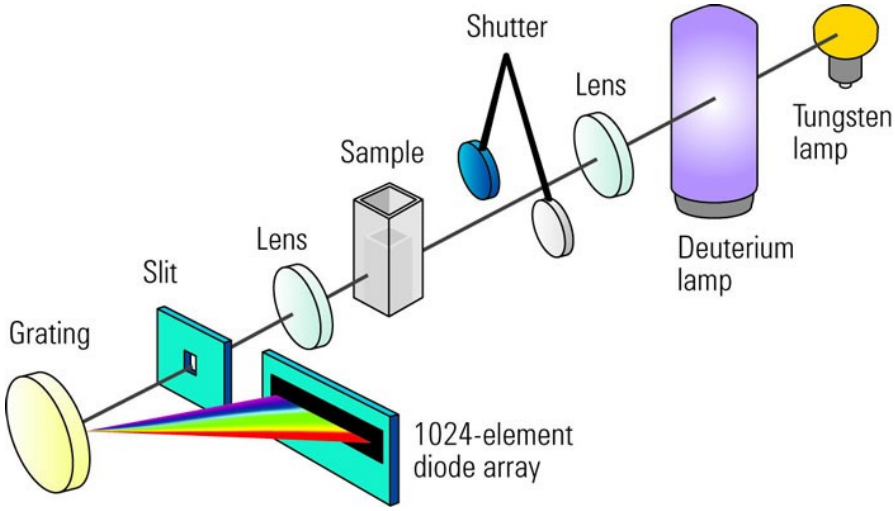

Figure 11. Schematic of the Agilent Absorption Spectrometer .

4.2.2 Steady State Fluorescence Spectrometer

The steady state fluorescence is measured by a SPEX spectrometer, see Figure 12. It gives the emission spectrum of the sample over a broad wavelength range (200-1200nm). The major components of the steady state fluorescence spectrometer are the Xenon lamp, the excitation monochromator, the sample holder, the emission monochromator, and the detector. Each component of the SPEX is calibrated. The calibration of the excitation monochromator is performed by a reference diode and the Xenon lamp. The calibration of the emission monochromator is done by measuring the scattered light of known wavelength.

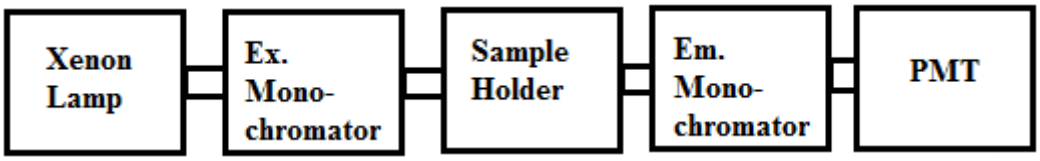

Figure 12. Schematic of SPEX.

The SPEX has two detectors: an IR and an UV/VISIBLE PMT. These PMTs are highly sensitive detectors and need to be cooled down to lower dark current. The incident photon generates electrons which are amplified approximately 10 million times. The steady state emission of the cut off filters and the test samples are measured using SPEX.

5. Results and Data Analysis

For the credibility and reproducibility of the data obtained from the TCSPC setup, it is investigated with two test samples mentioned in chapter 3.

5.1 **Absorption and Emission Spectra of Coumarin-152**

The measured absorption (blue) and steady state emission (red) spectra of Coumarin-152 are shown in Figure 13. Coumarin-152 has a maximum absorption peak at 400nm. The excitation wavelength for the steady state and time resolved fluorescence measurements is selected to be close to this maximum absorption peak. The emission peak helps in determining the types of filters to be used in the time resolved fluorescence measurements.

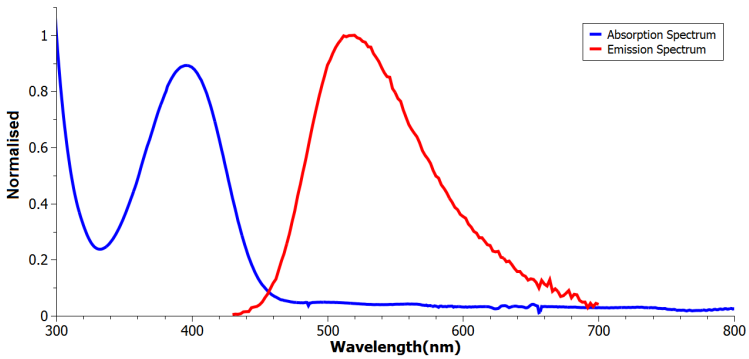

Figure 13. Absorption and Emission spectrum of the Coumarin-152 dye.

5.2 **Absorption and Emission Spectra of APFO3**

The absorption spectrum of APFO3 has peaks at 380nm and 550nm, see the blue curve in Figure 14. The steady state emission spectrum of APFO3, see the red curve in Figure 14, which has a maximum at around 700nm and indicates that it has significant emission after 700nm compared to Coumarin-152 (Figure 13). The other distinct differences of the samples are the single absorption peak of Coumarin-152 and double absorption peaks of APFO3. The polymer is not excited at 550nm as we did not have a laser of this wavelength. Excitation wavelength used for the TCSPC measurement is 405nm as both Coumarin-152 and APFO3 have absorption peaks in this wavelength region.

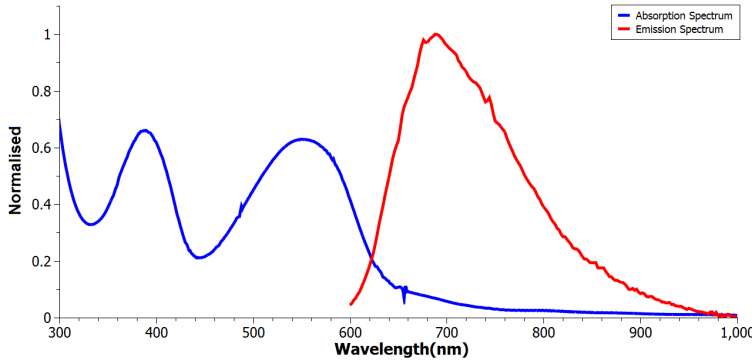

Figure 14. Absorption and Steady State Emission spectrum of APFO3 polymer

5.3 Interference Filters Absorption and Emission Spectra

In Figure 15, the absorption spectrum indicates that the first cut off filter allows all the light with wavelength longer than 572nm. The 572nm filter is suitable for blocking the excitation wavelength of 405nm, but the filter itself emits, see Figure 16. At 405nm the optical density of the filter is not very high, which allows part of the excitation light to pass through the filter. To completely block the excitation light and emission from the first filter, another cut off filter have to be used which blocked all light below 795nm.

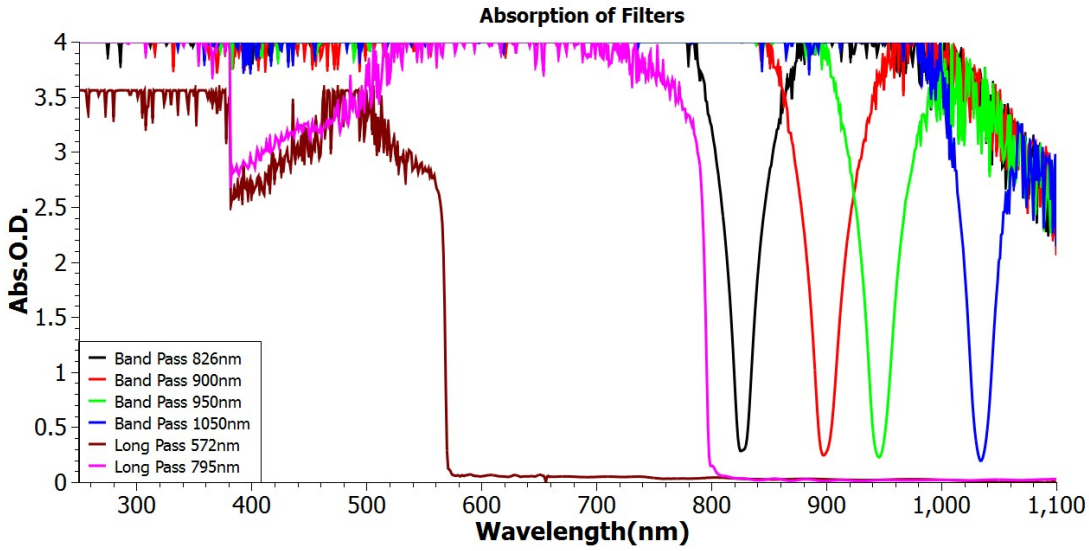

Figure 15. Absorption Spectra of cut off and band pass filters.

In Figure 15, the absorption spectrum of the 795nm cut off filter is shown. It blocks the major part of the emission from the preceding 572nm cut off filter. The 795nm cut off filter is expected to have emission as well. A further check is performed by measuring the steady state emission spectrum of this filter. Figure 16 shows that the emission peak of the 795nm cut off filter is at 750nm. The emission from the 795nm filter is very weak as the amount of excitation light, which can excite the 795nm filter, is reduced significantly by the first 572nm filter. Emission from the first cut off filter has negligible effect in exciting the 795nm filter.

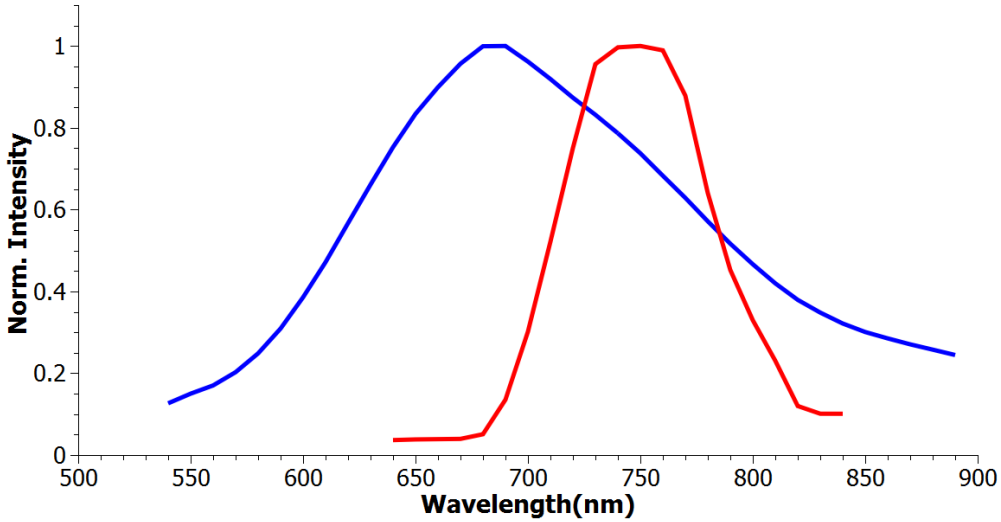

Figure 16. Steady state emission of 572nm (blue) and 795nm (red) cut off filters.

Any emission of the 795nm cut off filter is to a large extent blocked by the band pass filters that are put in front of the APD. The band-pass filters, see Figure 15, are used to spectrally resolve the emission from a sample. The band pass filters also serve as a tertiary barrier for blocking out any residual excitation light and the emission from the other long pass filter. The detected signal after the band-pass filter is mainly the sample's emission.

The drawbacks of our novel geometry are firstly that band-pass filter has to be used for spectrally resolving the emission from the sample. Secondly, to remove the excitation light and emission from the cut-off filters is a challenge.

5.4 Measurement Output Modes

As mentioned earlier, the APD has two output modes: NIM OUT and TTL OUT. The output pulse from the APD is used to stop the PicoHarp timing sequence. The major gain between these two outputs is the timing resolution of the stop pulse. Difference between the two output modes is shown in Figure 17. NIM OUT gives a shorter FWHM than the TTL OUT. The IRF with the NIM OUT has a FWHM of 250ps and the IRF with the TTL OUT is 350ps. According to the specifications NIM OUT has a FWHM of 50ps and TTL OUT has a FWHM of 350ps. This indicates that we can improve our TCSPC setup timing accuracy further.

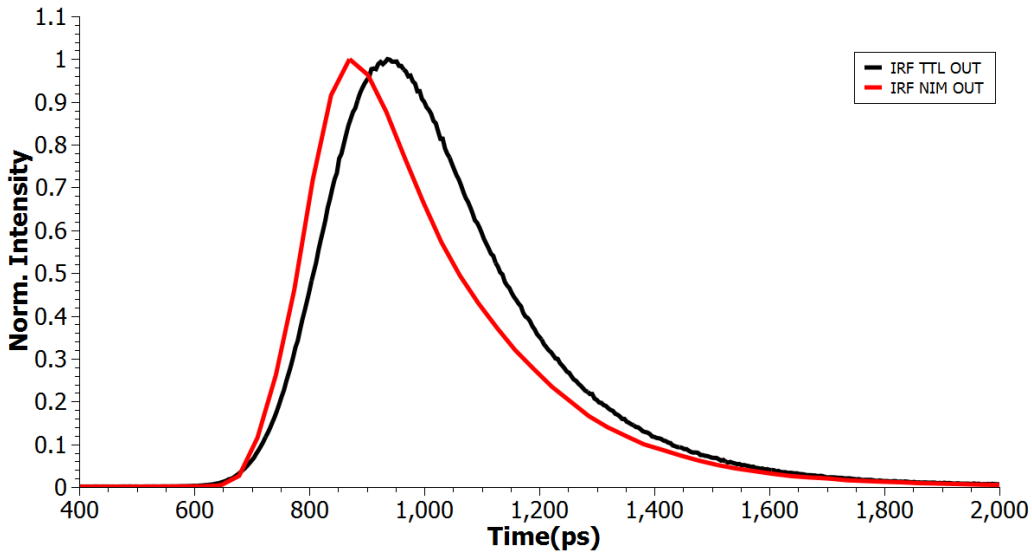

Figure 17. IRF of the system measured by scattering of the 405nm laser. TTL OUT(black) vs. NIM OUT(red).

5.5 Avalanche Photodiode Sensitivity Calibration

The APD sensitivity, in the wavelength region of our interest, is checked to characterise the overall sensitivity of the TCSPC setup. This is done by illuminating the APD and all the filters in place with a lamp. The band pass filters are changed and the counts per second are measured for each band pass filter (826nm, 900nm, 950nm and 1050nm).

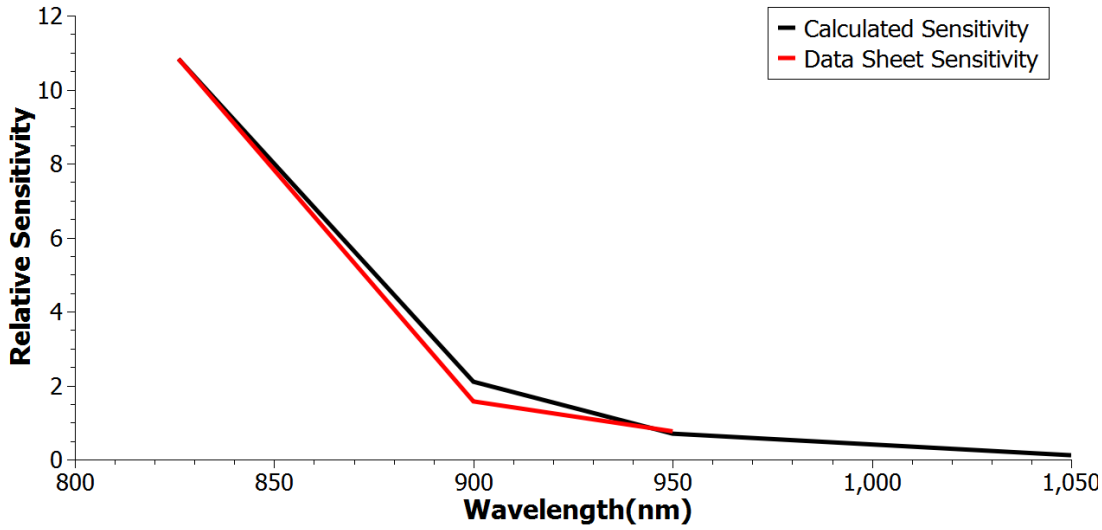

Figure 18. The calculated sensitivity of the APD compared to the data sheet normalised at 826nm.

In Figure 18 the relative sensitivity curves indicate the relation between the calculated sensitivity and the manufacturer's specifications. In Table 1, the data is given for the measurement made. The O.D. from the absorption spectrum is used to calculate the transmission of the band pass filters. This transmission, the measured counts per second, and the lamps counts per second for the specific wavelength (calibration data of lamp) are used to calculate sensitivity of the APD. The model expression used to calculate this sensitivity is given in Eq. 8.

$$S = \frac{T \cdot M_{CPS}}{L_{CPS}} \tag{8}$$

S is the calculated sensitivity, T is the transmission, M_{CPS} is the measured counts and L_{CPS} is the counts from the calibration data of the lamp.

Table1. Measured data to estimate the sensitivity of APD. Optical density, O.D, and transmission, T, of the filters is shown. Measured counts, M_{CPS}, and calibrated lamp counts, L_{CPS}, are given along with estimated sensitivity, S, and the manufacturer specification.

Filters	O.D.	T	M_{CPS}	L_{CPS}	S	APD Data Sheet
826nm	0.28	0.52	27000	614.70	10.83	10.83
900nm	0.24	0.57	22000	1299.96	2.10	1.57
950nm	0.25	0.55	18000	2055.04	0.69	0.76
1050nm	0.19	0.64	15000	3646.96	0.15	-NA-

5.6 **Time Resolved Fluorescence Measurements**

In this section the results obtained from the lifetime measurement performed on the test sample Coumarin-152 and APFO3 are presented. The excitation wavelength is 405nm and it is selected based on the common absorption peak of Coumarin-152 and APFO3. A band-pass filter at 826nm is used to measure the fluorescence decay curve of Coumarine-152 in Figure 19 (blue) fitted with the Instrument Response Function (IRF) (red). The fitted curve (black) is overlapping with the decay curve and after the deconvolution the lifetime of Coumarin-152 is extracted. The lifetime that is obtained from the measurement is 1.69ns and the published lifetime of Coumarin-152 is 1.63ns [9]. This result is in good agreement with the published lifetime. The fitting function used, Eq. 4, for Coumarin-152 is a single exponent. The lifetime obtained using single exponential fitting of Coumarin-152 shows that it is only a single type chromophore fluorescing.

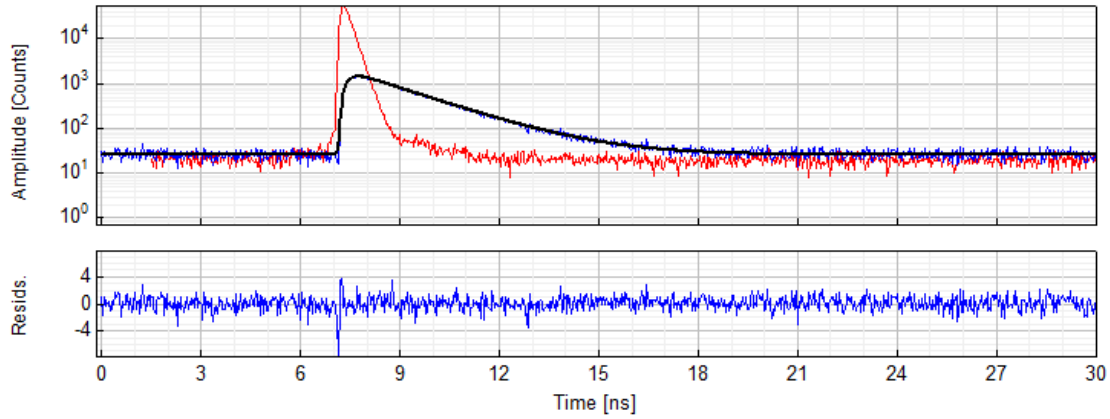

Figure 19. Fluorescence decay curve of Coumarin-152, excited at 405nm. IRF (red) and the residual of the fit (blue) are shown in the figure above.

The measured fluorescence decay curves of APF03 at wavelengths 826nm, 900nm, 950nm and 1050nm, are shown in Figures 20, 21, 22 and 23 respectively. APFO3 has a maximum emission at around 700nm, see Figure 14. The intensity of fluorescence is found decreasing as the wavelength of the band-pass filters is red shifted. One way to have a good signal to noise ratio for different band-pass filter measurement is to vary the integration time. The trade off is between low excitation intensity and integration time. If the excitation intensity is too low although sample degradation is minimised but the integration time is to increase for a good signal to noise ratio.

Besides, APFO3 is expected to have a non exponential decay due to existence of different fluorescing moieties. These moieties might have distinct lifetimes. In Figure 20, the non exponential decay of the APFO3 polymer is shown. The fitting with the IRF is done iteratively. Initially, the curve is fitted with a single exponent but the fit curve did not follow the decay curve. The decay is then fitted with two exponents, Eq. 9. The fit is improved as it followed the decay trace quite well. So, the shape and the fitting with two components suggest that using the TCSPC setup we can resolve non exponential decays.

$$A(t) = A_1 e^{-\frac{t}{\tau_1}} + A_2 e^{-\frac{t}{\tau_2}} \qquad (9)$$

The fluorescence decay curves measured at 900nm and 950 nm are shown in Figure 21 and 22, respectively. The integration time for both these measurements is same i.e. 300 seconds and a decrease in amplitude (counts) is clearly seen. For the 1050nm band-pass filter the integration time has to be increased as the APF03 has less

emission at this wavelength. The setup characterization is then interesting when there is almost no emission from APFO3 at 1050nm. It is interesting that APD still registers the counts indicating the very high sensitivity of the setup. Although, Figure 23 shows that the amplitude (counts) is below a 1000 counts which is comparable to the amplitudes of the other measurements at 826nm, 900nm and 950nm. The integration time for this measurement is 600 seconds to get signal to noise ratio equivalent to other measurements.

Also, the Coumarin-152 is measured using the 1050nm band-pass filter. And even though the dye has no significant emission there counts are still registered. This shows the very high sensitivity of the setup as well of the APD.

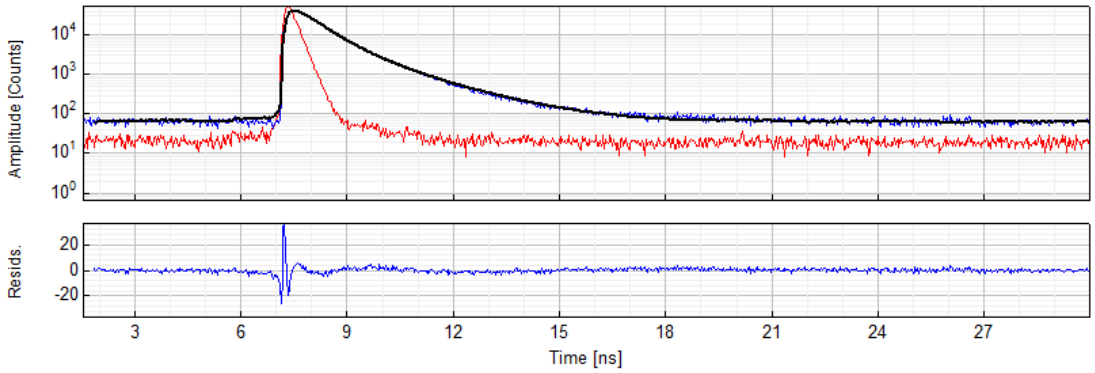

Figure 20. APFO3 fluorescence decay (non exponential) measured with 826nm band-pass filter. IRF (red), Fitted curve (black) and residual of the fit (blue).

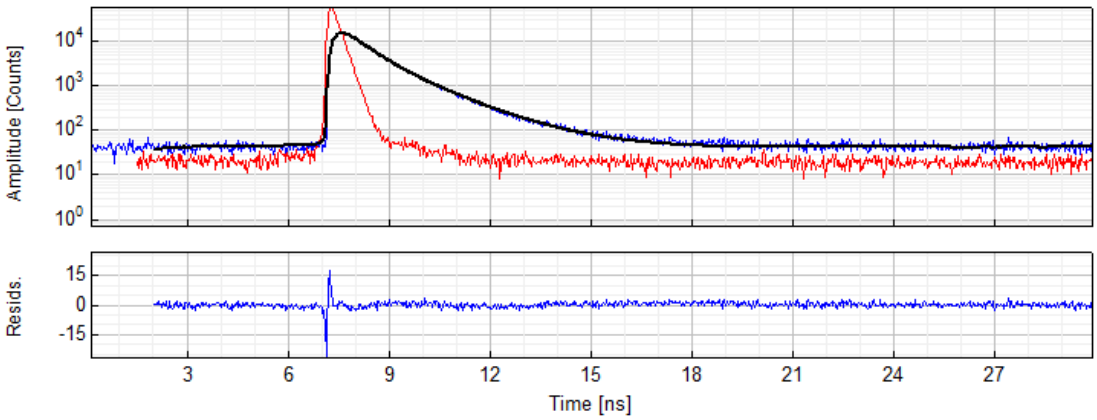

Figure 21. APFO3 fluorescence decay curve measured with 900nm band-pass filter.

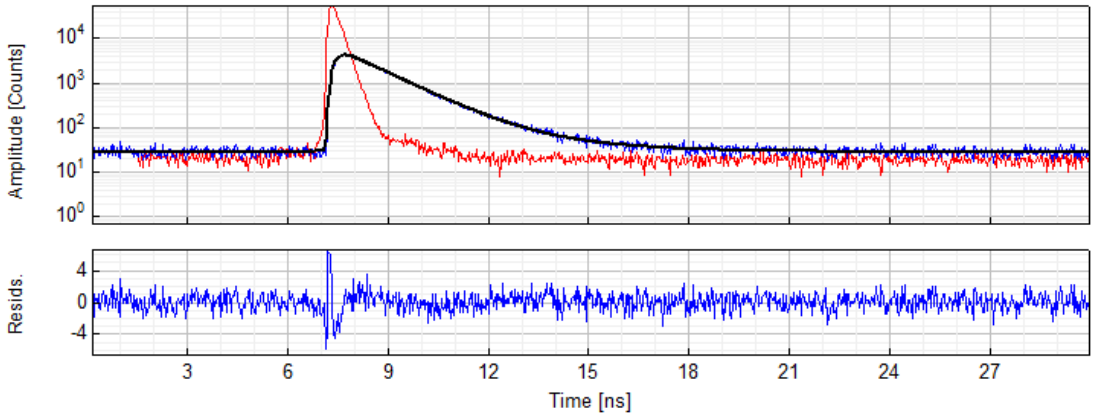

Figure 22. APFO3 fluorescence decay curve measured with 950nm band-pass filter.

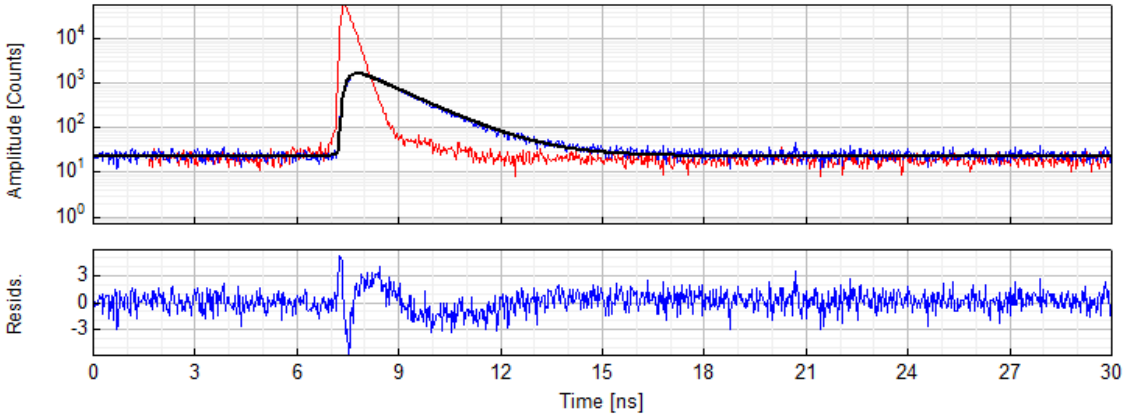

Figure 23.APFO3 fluorescence decay curve measured with 1050nm band-pass filter

These experiments have been performed twice to check the reproducibility of the measurement. In Table 2, the fitted data of these two measurements are given. The lifetimes and amplitude components are similar for repeated measurements.

Table 2. The reproducibility of measurements performed on APFO3

Filters	**826nm**		**900nm**		**950nm**		**1050nm**
Measurement	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**
Amplitude	0.73	0.70	0.68	0.58	0.88	0.57	0.48
Lifetime(τ/ns)	1.55	1.65	1.34	1.00	1.94	2.00	1.21
Amplitude	0.27	0.30	0.32	0.42	0.12	0.43	0.52
Lifetime(τ/ns)	0.69	0.72	0.50	0.30	0.90	1.30	0.80

6. Errors and Uncertainties

Error in science is not a mistake. It is the uncertainty related to any measurement. Any measurement done is not an exact one. The accuracy and precision determine a measurements credibility or certainty. There are two major categories in which the error can be classified: Systematic and Random .

Systematic error is defined as an error, which results from non calibration of instrument. On the other hand, a random error is caused by unpredictable variation in measurements. The variations in measurements due to random error are dealt in a statistical manner .

6.1 Errors and Uncertainties in TCSPC

Although the fluorescence experiments are easy to perform with this technique but we should be aware of possibility of errors and inaccuracies. Major errors in this TCSPC setup are: Pile up, background counts, synchronisation, and noise pattern. All these errors lead to an inaccurate measurement. To minimise these errors we need to define them.

6.1.1 Pile Up

The detection and consequent loss of a second photon in one signal period is called pile up. Detection or loss of a second photon usually occurs in the later part of the signal and hence the decay curve is influenced. A decay curve, which is distorted by pile up can be noticed if the counting rate is higher than repetition rate .

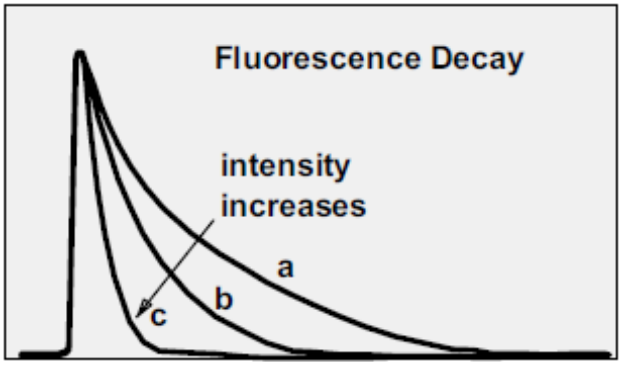

Figure 24. Effect of pile-up on recorded curves. Curve (a) is the correct one. Curves (b) and (c) are distorted by pile up .

If the detector sees thousands of photons per laser pulse, due to pile up the decay curve obtained could give lifetimes shorter than the detector transit time spread. To mention that multiphoton detection and pile up are two different effects. Multiphoton detection can occur if the CFD threshold amplitude is above a single-photon pulse amplitude .

6.1.2 Background Counts

Dark counts influence the measurement of TCSPC because it is the first photon that is detected and stored. So, if a thermal photon is detected before the fluorescence photon then the detector does not see the fluorescence photon. This distorts the fluorescence decay curve. To reduce the dark count rate the APD could be cooled .

6.1.3 Synchronisation

Accurate timing between the start and stop pulse is important and hence a measurement mode called Time-Tagged Time-Resolved data acquisition (TTTR). TTTR tags the timing of excitation pulse to the detection of the photon by the APD. Using this mode we still lose photons due to the dead time of the APD. The dead-time means the APD is unable to detect another photon or next photon for a certain time. Each recorded photon causes a dead-time and another photon can not be detected during this time so it is lost .

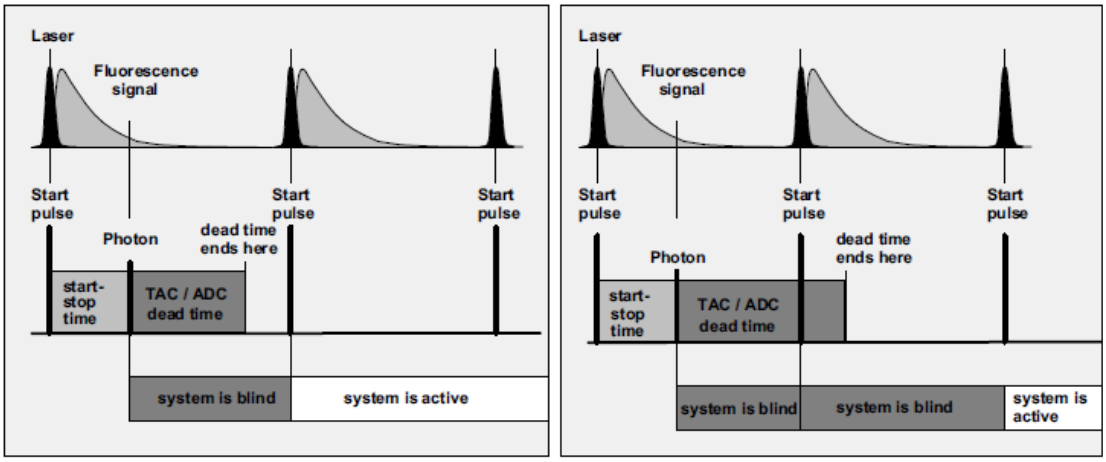

Figure 25. Repetition rate and dead-time of APD. Left the APD is active for longer due to shorter processing time. Right the APD is inactive for longer due to increased processing time, so it misses a cycle completely .

The dead-time of APD is also related to the repetition rate. Figure 25, shows how the dead-time and repetition rate are interlinked. If the dead-time of APD is short higher repetition rate can be employed but if its longer then lower repetition rate has to be used.

6.1.4 Noise Pattern

TCSPC works by counting photons, so in principle it is a digital technique. Another noise source, apart from the dark counts, is the counting noise. The counting noise is governed by the Poisson noise distribution. This counting noise is defined by the standard deviation of a data point which is the square-root of the data point. The noise is of Poisson type has an influence on the dynamic range. The SymPhoTime Software used for analysis uses the square root noise model to de-convolute the decay curve with IRF to extract the fluorescence lifetime.

6 Conclusion

We presented a home built TCSPC setup that allows fluorescence measurements with high time resolution, high life time accuracy with single and multi exponential decay functions and high sensitivity. The optimized instrumental response function of our system is 250ps. The sensitivity of integrated setup is found decreasing at red emission relative to 826nm. The setup is novel in the sense that the geometry to collect fluorescence is simple and also the use of very low density of excitation photons. The latter is of advantage as it slows down sample photo-degradation. We have also demonstrated the setup verification and have been applied to study the solar cell materials. The measured life times are also in good agreement with published data.

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