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Development of an Optical System for Measuring Fluorescence Lifetimes

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ABSTRACT

This project presents the design of a cost-effective, portable, and simplified fluorescence detection system for measuring fluorescence lifetime decay as an alternative to the available methods currently in use. There are multiple systems available to detect fluorescence of a sample, but they contain multiple parts and require expensive equipment in order to function. Due to the number of parts needed, the cost of implementing those fluorescence lifetime decay measuring systems are high. The fluorescence measuring system will be simplified into four main components that are interchangeable based on the application needs.

In this project, a simple fluorescence measuring system will be used to detect a sample of quantum dots and a sample of an organic based dye and examine the results for system feasibility. Future works may include testing of various time decaying quantum dots, and testing of various other light detection devices, such as other avalanche photodiodes (APD) or photomultiplier tubes (PMTs).

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CHAPTER 1
INTRODUCTION

Quantum dots (QD) are artificial semiconductor nanocrystals made from material such as silicon and cadmium selenide. They are anywhere from 2 - 10 nm in size, and are typically a 10 - 50 atoms wide [1]. QDs have electricity carrying particles that are trapped within them. These particles exhibit interesting electronic properties due to the size of their energy band gaps [2].

The materials used and the size of the QDs usually predict their behavior. Different materials have an effect on the energy band gap, and the size of the QDs determines the fluorescent light emitted or absorbed by the crystal. The QDs absorption depends on the energy provided by the photons that come to contact with it. The energy provided needs to be high enough to move it from its ground state, which is where the QD is before it absorbs any light, to an excited state, where it has absorbed the photon's energy and is now in a state of higher energy [3]. The energy provided by the photon will enable the QD to absorb light at the frequency of its band gap, enabling the QD to reach an excited state. Likewise, the QD can emit the same frequency of light in order for it to return to its resting state. Smaller QDs emit a higher energy of light from the fluorescent spectrum, usually bluer in color, where larger QDs emit lower energy of light that are redder [2].

Quantum dots are used for various applications. They can also be used as a display technology. There are currently television with QLED display (Quantum Dot based LCD TV) which allows 30 - 40% luminance efficacy over organic light emitting diodes (OLED), lower power consumption, low-cost manufacturing, and ultra thin form factors [2][4].

They can also be used as solar cells, which replace the silicon and copper indium gallium selenide (CIGS) or CdTe. Normal solar cells can only light during the day, while QD solar cells can absorb light 24/7 as it can absorb light from ultraviolet to visible light to infrared [2].

Another application which inspired this thesis is biological imaging. The QDs can be used to track cells or to obtain an image of the cell or organ by capturing the fluorescence. Colloidal QDs are fluorescent nanoparticles that have a unique property called luminescence blinking. A single QD will undergo an instantaneous reversible transition from the ”excited” state where QD is illuminating, back to the ”ground” state where it is not radiating [5]. The lifetime decay of the QD determines how long it takes for it to transition between those two states. After exciting the QD, they will emit a fluorescent light which can be captured using various devices such as a charge-coupled camera (CCD).

This thesis is structured as follows: Chapter 2 discusses the literature review, in which the background is provided about the various other fluorescence detection systems. Chapter 3 presents the design of the circuitry used and information of the other parts in the system. Chapter 4 discusses the methodology, which discusses the sample preparation and the calibration of the system in order to obtain the fluorescence of the samples, and how the lifetime is measured. Chapter 5 shows the results of the data acquisition and analysis, which will be discussed in detail. Chapter 6 goes over the conclusion and possible future works for the simplified modular optical system.

CHAPTER 2

LITERATURE REVIEW

This chapter discusses the main ideas and concepts necessary for implementing this project, as well as exploring similar systems that measure fluorescence lifetimes. The main focus will be looking into how these systems are put together, and how they can be used in order to obtain the fluorescence lifetime decay.

2.1 Lifetime Detection

Colloidal quantum dots were proposed as an alternative to organic based dye for fluorophores, which are fluorescent chemical compounds that can re-emit light when it comes in contact with an excitation laser [6]. Multiple QDs can be excited by a single source, such as an excitation laser. QDs are also more stable when repeatedly excited, unlike organic based dyes, which will become ”bleached” after repeated excitation, and thus not produce a strong fluorescent light to be detected. It is also important to note that organic dyes and QDs have different lifetime decays [6].

Organic based dyes emit their fluorescent light as soon as it gets excited, however, as soon as the excitation light disappears, it takes a few nanoseconds for the fluorescent light to also go back to its non-excited state. Another problem that occurs when collecting measurements of the fluorescence is the existence of short-lived autofluorescence background that is found in many naturally occurring species in a specimen [6]. This, in turn, makes capturing the fluorescent light more difficult. In order to improve the applications of cellular imaging and cell

tracking, the use of QDs will greatly help with the data acquisition due to the longer decay times. This is primarily due to the autofluorescent background returning to their ”ground” state, while the QDs are still emitting their fluorescent light as it is still transitioning back to its ”ground” state.

2.2 APD Based System

A stage-scanning time-correlated single-photon counting confocal microscope was constructed at the Lawrence Berkeley National Laboratory, in Berkeley, California. Figure 2.1 shows the system's experimental setup. The setup includes homemade stage-scanning confocal microscope with a nanometer-resolution closed-loop piezo-stage scanner. The excitation laser is brought in via a fiber and a beam expander to the dichroic mirror (DC). The DC then reflects the laser light into the back focal plane of the objective lens (Ob). The fluorescence from the sample is then reflected back into the objective and passes through the dichroic mirror. The fluorescent light passes through the tube lens (TL), which sends the light into the pinhole (PH), which is used to image the fluorescent light onto the avalanche photodiode (APD) with the aid of an imaging lens (IL). The light is then passed through a bandpass filter (BP), in order to catch any of the excitation or stray light that may go through with the fluorescent light. From there, the APD is connected to the computer for data acquisition. The light pulses are controlled via the APD and the laser electronics [6].

This setup was able to almost completely eliminate the background autofluorescence, while also capturing the QDs lifetime decay.

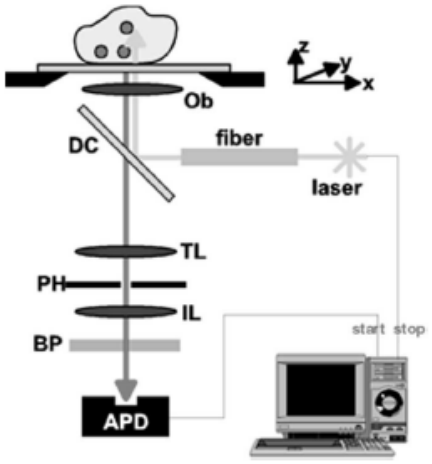


Figure 2.1: Stage-Scanning Time-Correlated Single-Photon Counting Confocal Microscope, adopted from [6].

2.3 CCD Camera Based System

A near-infrared (NIR) gated detection system was built at the GE Global Research, Niskayuna, New York, in order to evaluate gated imaging approaches for high-speed imaging of longer fluorescence decay time for molecular imaging agents in vivo [7]. Figure 2.2 shows the schematic diagram of the wide-field time-gated imaging system. The phase-locked function generators are amplified to provide the drive voltage for the light source, this being an LED array, and the image intensifier tube (IIT) photocathode modulation. The IIT phosphor is imaged by a CCD camera.

The idea of the system is to have the CCD camera take a picture after the excitation light comes to contact with the specimen, this cased being mice. The brighter the color of the image on the body of the mice, the higher the concentration of QDs is present, as shown in Figure 2.3. By adding a time-gated element to the system, the background autofluorescence will not be picked up, while the camera will take

a picture during the decay time of the QD.

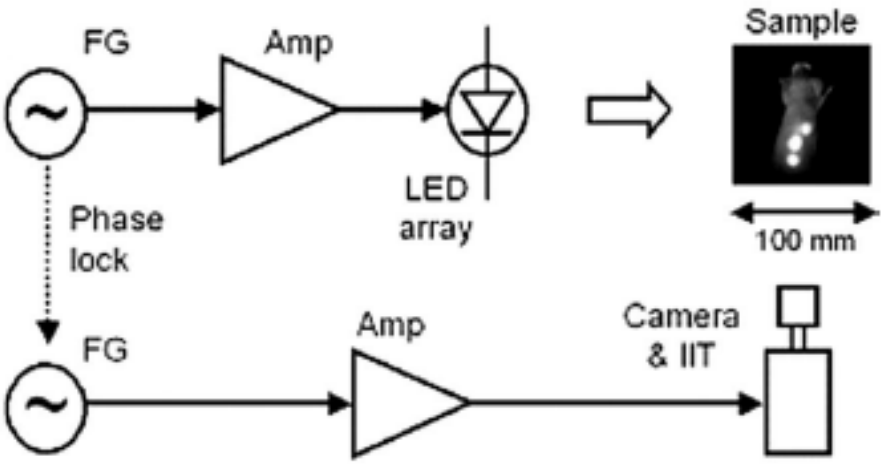


Figure 2.2: Schematic Diagram of Wide-field Time-gated Imaging System, adopted from [7].

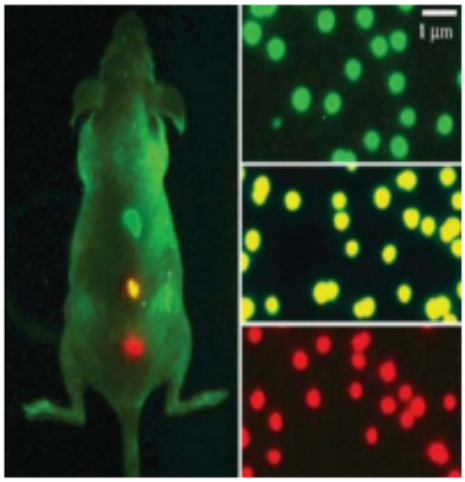


Figure 2.3: CCD Image of Quantum Dot Fluorescence From Mice, adopted from [7].

In order to collect the fluorescence lifetime decay curve for a system like this, it is necessary to capture numerous images while stepping the gate delay in order to analyze the fluorescence over time[7] [8]. However, this method is time-consuming. The article also mentions that due to the use of additional electronic

components such as a gated intensifier, RF power amplifiers, and a phase-locked function generator, it increased the relative cost and complexity compared to conventional fluorescence imaging [7].

It is important to note that although these systems were not primarily made in order to only capture fluorescent lifetime decay, these related works were used in order to understand the different methods and electronics used to generate the lifetime decay data, and how it can be optimized to produce a cost-effective system that fulfills that purpose. To that end, the optical measuring system designed will use significantly less equipment than what is shown in the other two systems in order to measure the lifetime decays of fluorescent light.

CHAPTER 3
SYSTEM DESIGN

3.1 Overview

The complete system consists of four main components. A light detecting device, such as a PMT or an APD. A laser, to excite the sample in order to measure the fluorescence. A sample holder that will allow the sample to be held in place, get excited via the laser, and allow the fluorescent light to be directed into the light detecting device. Finally, a filter that will allow the fluorescent light to pass through while blocking the excitation light.

With this system, it will be possible to measure the fluorescence lifetime decay. A filtered signal will ensure that the excitation light is not being picked up by the light detector, skewing the data.

3.2 Avalanche Photo Diode (APD)

The light detection device selected is C5658-5358 from Hamamatsu Photonics K.K, as shown in Figure 3.1. The APD was selected due to its high-speed response and high sensitivity [9]. The block diagram is shown in Figure 3.2 [10].

Table 3.1 shows the technical specifications of the C5658 APD. A wide range for the spectral response, a cutoff frequency of 1 GHz, and a gain of 100 were major factors in the selection of the APD device. The small form factor will help achieve the portability of the system.

Figure 3.1: Hamamatsu C5658 APD Module [9].

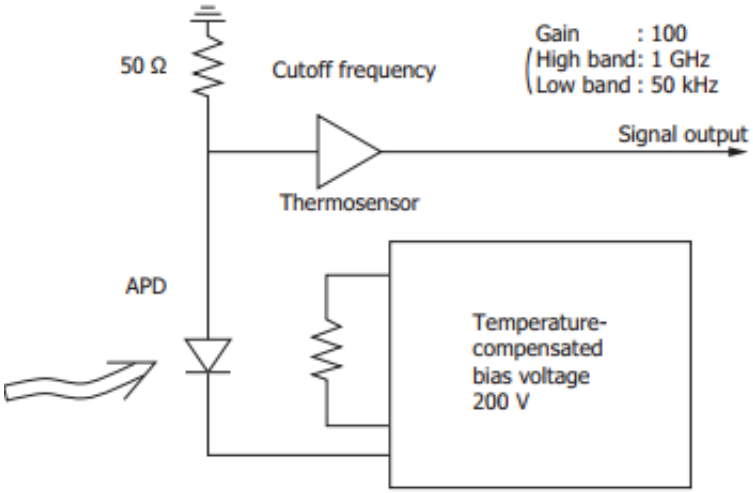


Figure 3.2: Hamamatsu C5658 APD Module Block Diagram [10].

Table 3.1: C5658 APD Specifications (Adopted from [11]).

Parameters	**Ratings**	**Unit**
Maximum Input Light Intensity (DC)	+13.5	V
Spectral Response	400 to 1000	nm
Active Area	ϕ 0.5 (With SMA optical fiber connector)	mm
Cutoff Frequency (-3dB) High	1	GHz
Cutoff Frequency (-3dB) Low	50	KHz
Gain	100	-
APD Radiant Sensitivity	50	50A/W (at 800 nm M = 100)
Output Impedance	50	Ω
Supply Voltage	+12 +/-0.1 ; MAX +13.5	V
Current Consumption	100	mA
Dimensions	60x50x28	mm

3.2.1 Power Supply Circuit

To power the APD, a 12 V supply with a tolerance of +/- 0.1 V or 0.83% is required. In order to achieve this, a voltage regulator circuit will be designed. The LM7812 by Fairchild Semiconductor is selected [12]. Figure 3.3 shows the voltage regulator circuit recommended by the manufacturer.

The LM7812 is supplied with a 15 V input supply. The output voltage recorded was 11.84 V. This was unacceptable, so the circuit must be re-designed using Figure 3.4.

Equation 3.1 shows the calculation needed to get the output voltage closer to 12 V [12]. Equation 3.2 shows the expected current through R_1 [12]. I_Q is 5 mA typical, but due to the temperature of the IC during operation, it will be closer to 5.6 - 6 mA [12].

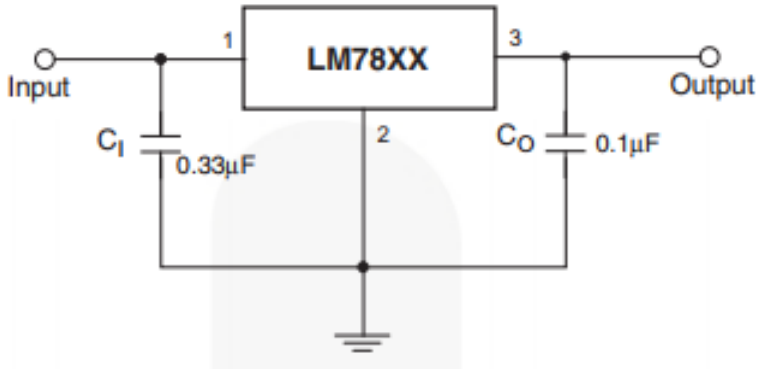


Figure 3.3: Voltage Regulator Circuit from Fairchild Semiconductor [12].

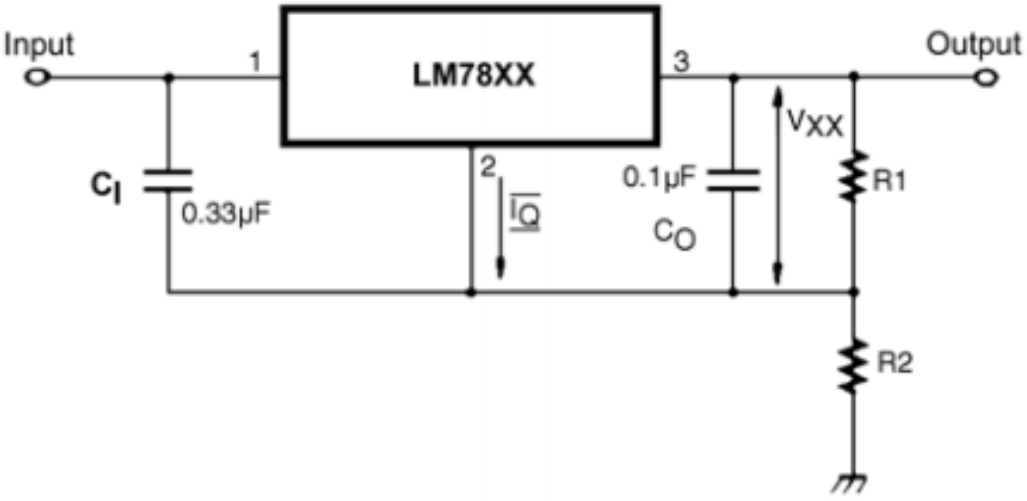


Figure 3.4: Circuit for Increasing Output Voltage [12].

$$V_o = V_{XX}(1 + R_2/R_1) + I_Q R_2 \tag{3.1}$$

$$I_{R1} \geq 5I_Q \tag{3.2}$$

V_{xx} is substituted for the output voltage of the IC used. Nominally, it V_{xx} is 12V, but 11.84 V will be used as it is the measured voltage. R_1 is selected to be 470Ω. The power dissipation from R1 is 0.336 W, as shown in Equations 3.4 and 3.5. The current is calculated to be 0.28 A, as shown in Equation 3.3.

$$I_{R1} \geq 5 * 5.6mA \geq 280\ mA \tag{3.3}$$

$$P = IV \tag{3.4}$$

$$P = 0.028\ A * 12V = 0.336\ W \tag{3.5}$$

The 470Ω resistor selected for the circuit only supports a power dissipation of 1/4W maximum. To rectify this, five 2.2kΩ is put in parallel in order to distribute the power dissipation evenly between each resistor. The equivalent resistance will equal to 440Ω. With this data, R_2 will need to be calculated using Equation 3.1. Equation 3.6 and 3.7 show the calculations for R_2.

$$12V = 11.84V * (1 + R_2/440\Omega) + 0.0056A * R_2 \tag{3.6}$$

Solving for R_2:

$$R_2 = 4.92\Omega \approx 4.7\Omega \tag{3.7}$$

The complete power circuit was simulated using LTSpice. Figure 3.5 shows the circuit design and Figure 3.6 shows the output voltage simulation.

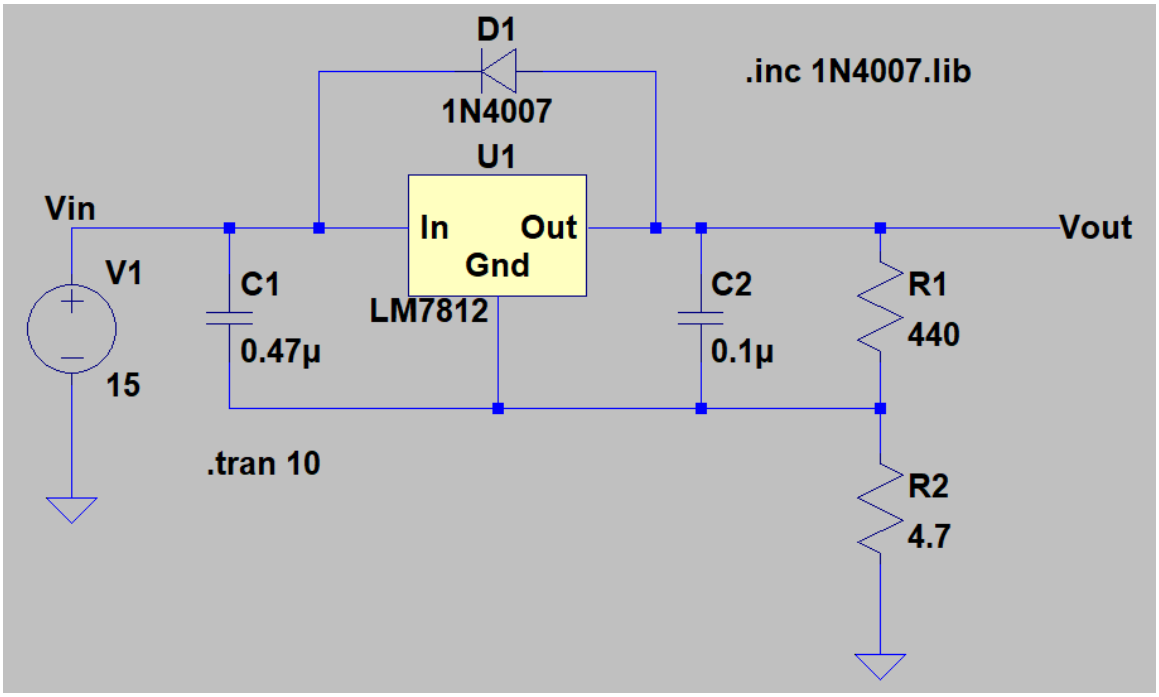


Figure 3.5: APD Power Circuit Simulated Via LTSpice XVII.

The circuit includes a diode D1, the 1N4007, and two capacitors C1 and C2. C1 is used to provide sufficient AC bypassing. C2 improves transient response. The diode provides a low impedance path to prevent the capacitor from discharging back into the output of the regulator [13]. The output voltage is a little over 12V to compensate for the power dissipated. After constructing the circuit on the prototyping PCB board purchased from Amazon [14] and testing the circuit, the output voltage with the load was measured to be 11.95 V, which is within the APD limit. The current was measured to be approximately 120 mA.

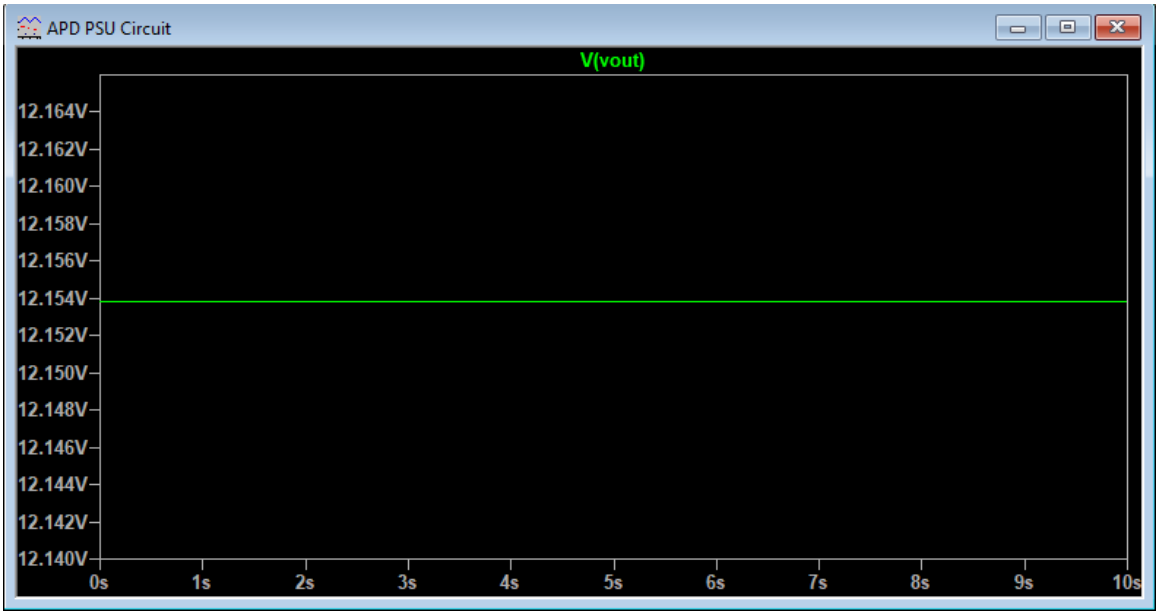


Figure 3.6: APD Power Circuit Output Voltage.

3.3 Laser

The D405-20 20 mW laser from US-Lasers Inc. was selected due to the accessibility and the output power. The C5658-5358 APD module has a maximum Input Light Intensity (DC) of 10 mW. The laser must output enough power to excite the sample to be able to produce a strong enough fluorescence in order for the APD to be able to output a signal. The 20 mW limit is also imposed in case the light from the laser is not filtered and enters directly into the APD. There are losses expected due to the mounting method and the lack of a focusing lens to the optical fibers. This will help prevent permanent damage to the APD. The laser is in a TO-18 5.6 MM package as shown in Figure 3.8 [15].

Table 3.2 shows the operating characteristics of the D405-20 laser. To achieve an optical power output of 20 mW, the laser must have a 5.0 volt drop across the diode, and a 50 mA current running through the circuit.

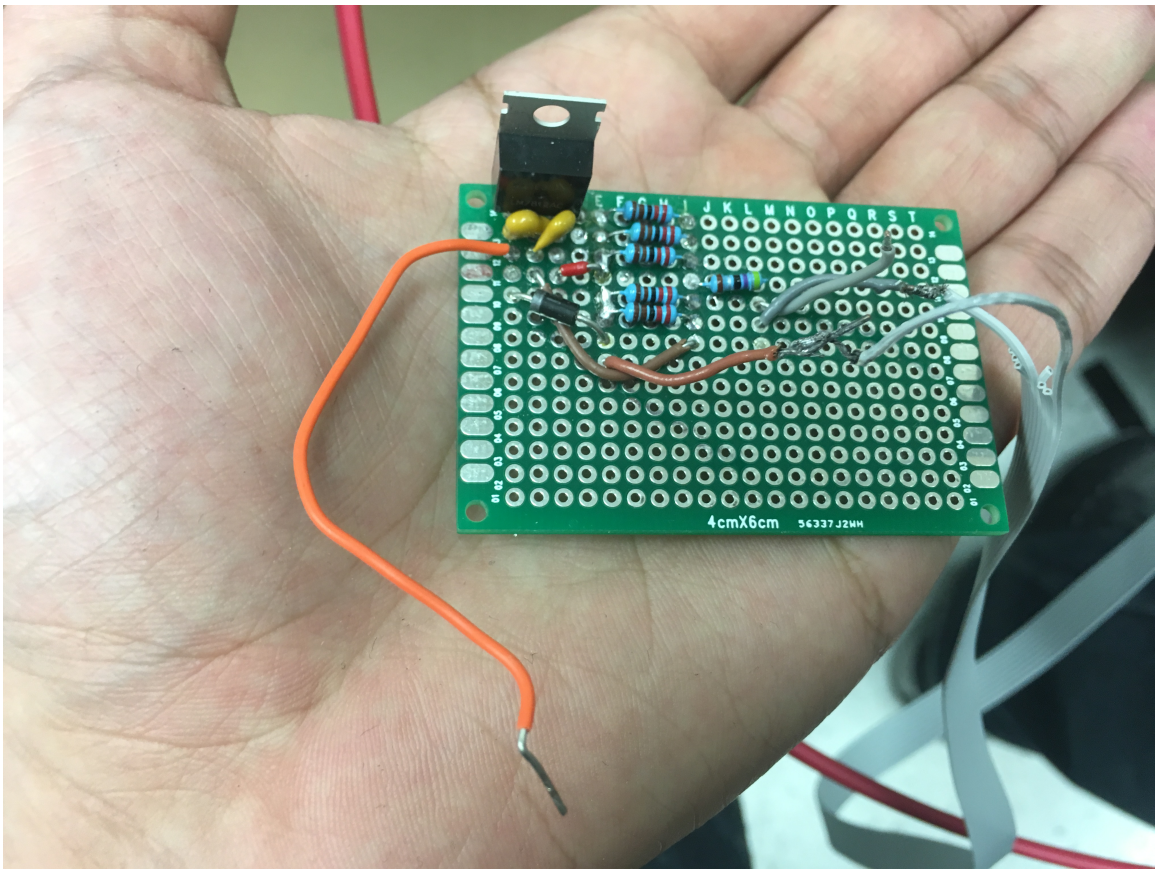


Figure 3.7: APD Power Circuit PCB.

Table 3.2: Laser Specifications (Adopted from [16]).

Characteristics	Symbols	Min	Typ	Max.	Unit
Optical Power	Po	-	20	-	mW
Threshold Current	Ith	-	25	50	mA
Operating Current	Iop	-	50	75	mA
Operating Voltage	Vop	-	5.0	6.5	V

3.3.1 Laser Mount

To mount the laser, the CP02 threaded cage plate from Thorlabs was selected as shown in Figure 3.9(a) [17]. The laser diode will be held in an SM1 series 5.6mm

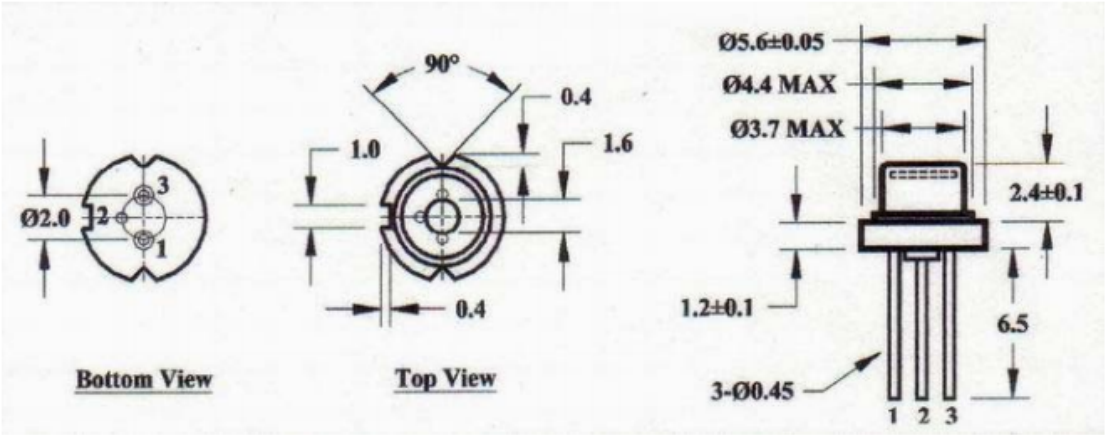


Figure 3.8: TO-18 5.6MM package [15].

cage plate, as shown in Figure 3.9(b), and will be placed as close as possible to the SM1SMA fiber adapter plate with an external SM1 connector which holds optical fiber, as shown in Figure 3.10. There are losses expected with this mounting method, but the design will be tested to see if this method will suffice.

3.3.2 Laser Driver Circuit

To drive the laser at the required output power, a laser driver circuit must be designed to meet the typical requirements as listed in Table 3.2.

There are two approaches to this design. A constant voltage source circuit, where the circuit is designed to drop 5 volts across the laser diode. Another method is a constant current source (CCS) circuit where the current flowing through the diode is the same no matter what the load is.

Between the two approaches, option 2 is the safest. A circuit that is designed to control the voltage will not limit the amount of current that will flow through

(a) CP02 Threaded Cage Plate.

(b) SM1 Series 5.6mm Laser Diode Mount.

Figure 3.9: Cage Mounts [17].

Figure 3.10: SM1SMA Fiber Adapter Plate.

the circuit. Due to the possible variations in the load, the current can fluctuate and give either an insufficient amount of current or overload the laser to the point of permanent damage.

A CCS circuit will provide the same amount of current flowing through the laser diode regardless of the load. This will also ensure the appropriate voltage drop across the diode. It is the safest between the two options as the circuit will be designed to never go over a specific current, thus preventing fluctuations of the current and permanent damage that may result due to overloading the diode.

An LM317T by STMicroelectronics is selected for the CCS circuit. Figure 3.11 shows the current regulator circuit recommended by the manufacturer.

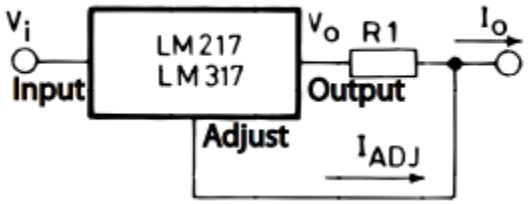


Figure 3.11: Current Regulator Circuit from STMircroelectronic's Datasheet [13].

Equation 3.8 determines the output current Io.

$$I_o = (V_{REF}/R_1) + I_{ADJ} = 1.25V/R_1 \tag{3.8}$$

For the CCS design, 50 mA is required to get an output power of 20 mW. The resistors used in this circuit have a tolerance of +/-1%. The laser diode shows a typical current consumption of 50 mA. It is good practice to design for a 5% - 10% of the typical current in order to compensate for variances listed in the data sheet. The values shown in the data sheet are usually the 3 sigma value. Using Equation 3.8, Equation 3.9 shows the calculation to get a current of roughly 55mA. With 56.8 mA, it is guaranteed that the circuit will drive the laser with an output power to 20 mW.

$$I_o = (V_{REF}/R_1) + I_{ADJ} = 1.25V/22\Omega = 56.8mA \tag{3.9}$$

The circuit for the laser driver has been designed and simulated using LTSpice XVII [18], as shown in Figure 3.12 and Figure 3.13. C1 is necessary as an input bypass capacitor, which will help deliver a clean voltage supply to the input of the LT317T IC [13]. A 12 V supply is used for the input. A dropout voltage of 1.5 - 2.5 is required in order for the circuit to operate properly. With 12 V supply, 5 V will be dropped across the diode, 1.23 V will be dropped across R1, and 5.77 V will be dropped across U1, which will dissipate 0.323 Watts. This is well within the operating limit of the IC [13].

Figure 3.14 is the assembled circuit on a Prototype PCB board purchased from Amazon.com [14]. The wires connecting to the diode are twisted in order to cancel electromagnetic interference (EMI) from external sources. The current through the

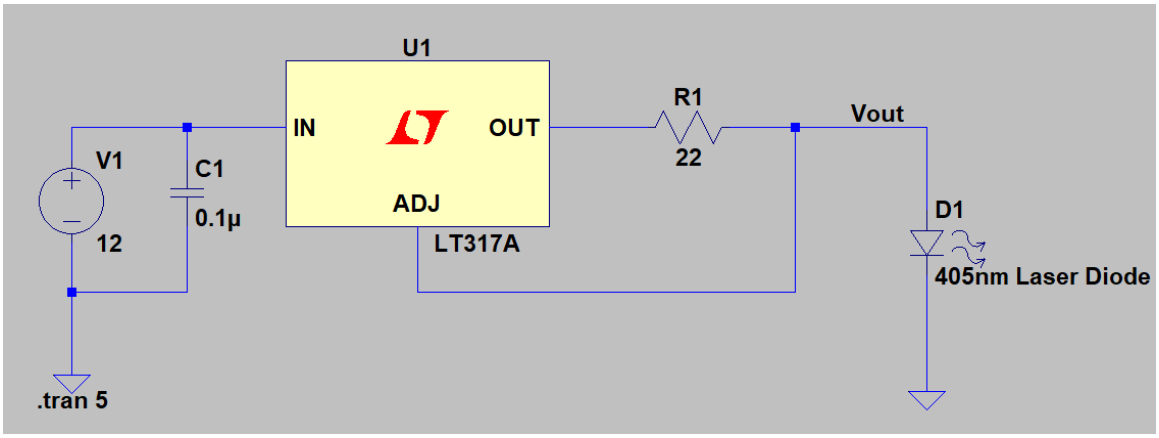


Figure 3.12: Laser Driver Circuit Designed in LTSpice.

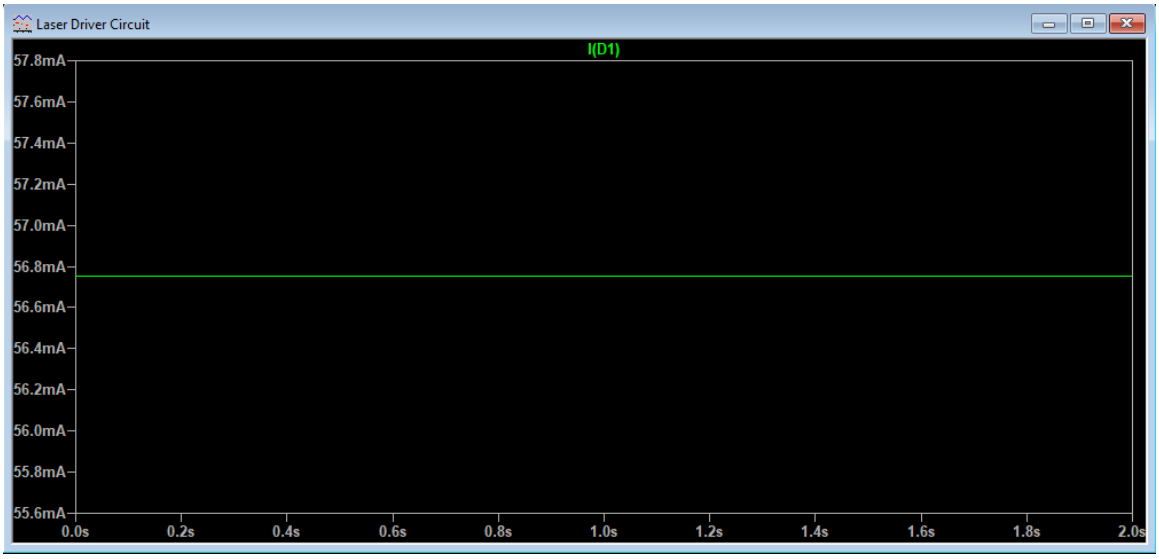


Figure 3.13: Current Simulation of Laser Driver Circuit.

laser diode has been measured to be approximately 53 mA. The voltage drop across the laser diode was measured to be approximately 5.07 V.

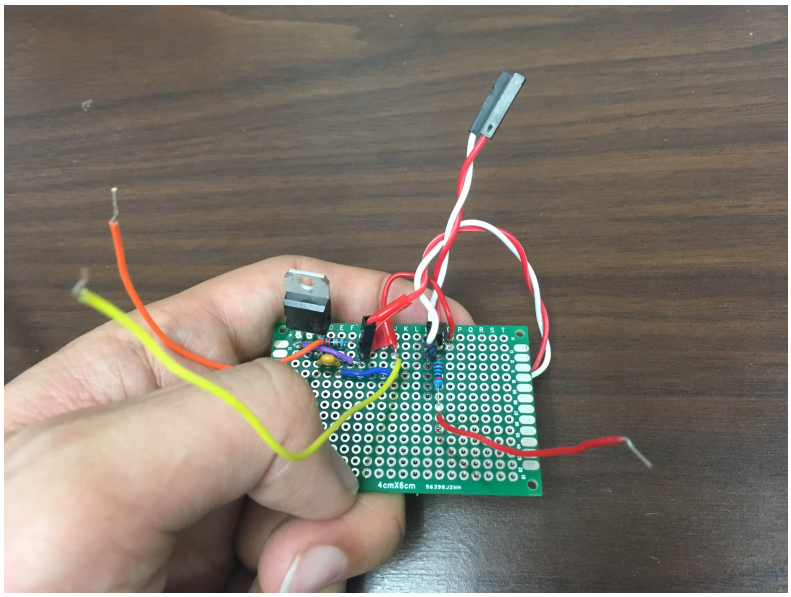


Figure 3.14: Laser Circuit Driver PCB.

3.3.3 Controlling the Laser

To obtain a proper fluorescent measurement, the excitation laser must pulse at a certain frequency in order to examine the decay. To pulse the laser, an N-channel bipolar junction transistor (BJT) is used as a switch in order to turn on and off the laser. Figure 3.15 shows the laser driver circuit with the BJT added in a switch configuration. This configuration alternates between the saturation and cut-off region [19].

A 2N3904 is used as it has a rise and fall time of 35 ns. This is sufficient for lifetime decays that are longer than that time. For measuring faster-decaying particles, a 2N5179 is used as it has a switching frequency of up to 2 GHz. This will be used in order to measure the organic dye.

R2 is used to limit the current going through the base. 5 V is used to guarantee the saturation of the device. When the base voltage goes over 0.65 V, the transistor

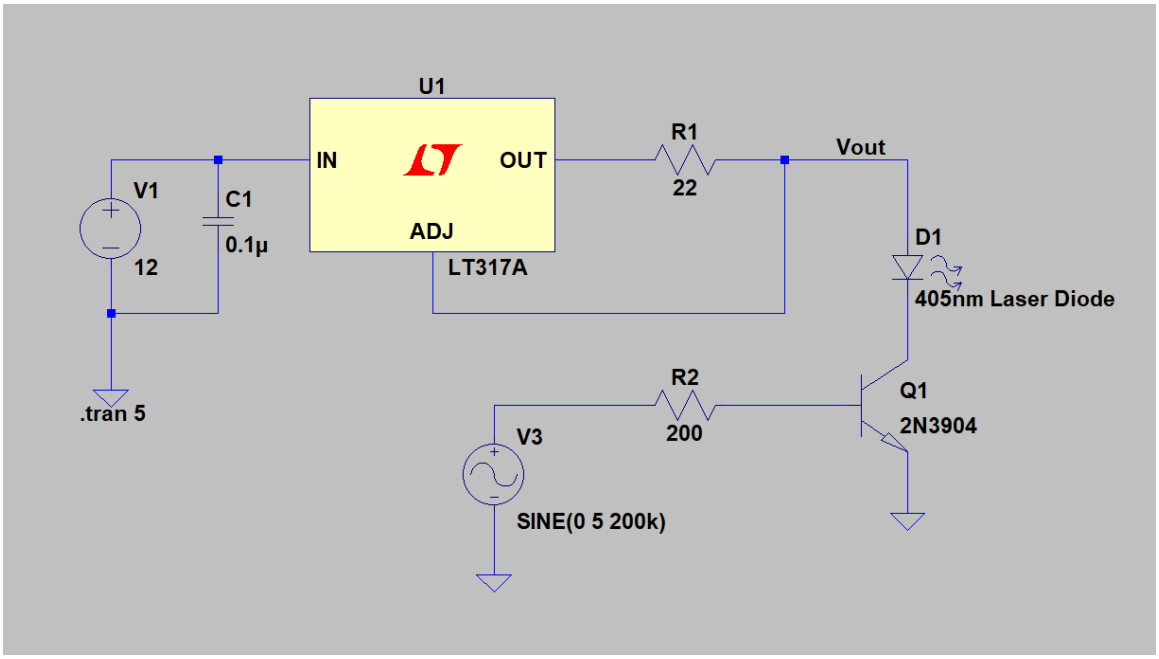


Figure 3.15: Laser Driver with BJT as a switch.

will complete the circuit and turn off the laser. When the base voltage goes below 0.65 V, the circuit will be ”open” and leave the laser on.

3.4 Sample Holder

A sample holder is required to hold the micro-cuvette in place. The FOFMS In-Line Fiber Optic Fiber mount was selected as the sample holder, as shown in Figure 3.16. Figure 3.17 shows the beam path through the In-Line Fiber filter holder.

The FOFMS features two removable filter holders. For this design, one of the filter holders will be removed and replaced with the sample, which is in a micro cuvette, as shown in Figure 3.16. The excitation light will enter one side of the FOFMS mount, pass through the sample and the filter to the other end of the

Figure 3.16: FOFMS System from Thorlabs [20].

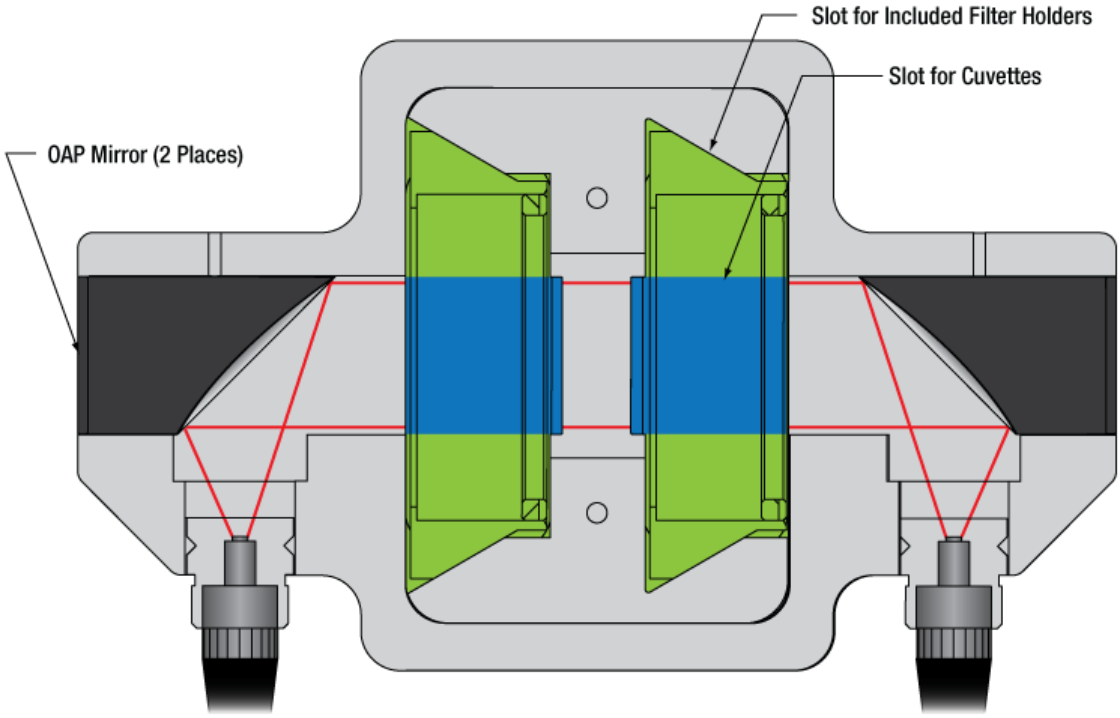


Figure 3.17: Beam Path of the FOFMS System [20].

mount, and into the APD to read the fluorescent light.

The FOFMS features SMA connectors on both ends in order to hold the optical fibers. The system uses two off-axis parabolic (OAP) mirrors with silver coatings designed for wavelengths from 450 nm to 20μm for protection. It is expected to

have an insertion loss of ≤ 2dB for a 200μm-1000μm core, ≤ 0.39 NA fiber within the range of the specified wavelength [20].

The system is mounted using a bottom-located 1/4-20 (M6) tap which mounts onto a ϕ1/2" post or post holder base, which is shown in Figure 3.18.

Figure 3.18: FOFMS Mounted to an Optical Table [20].

3.5 Filter

The excitation laser is using 405 nm wavelength, which is a blue color. The expected fluorescence from the quantum dots is yellow to orange. That is a wavelength of over 500 nm. In order to separate the excitation laser from the fluorescence, a 500 nm long pass filter will be used between the fiber going to the APD and the sample. This will ensure that the blue excitation light will be filtered out and only light from a wavelength of 500 nm and above will pass through. The 0500 FEL filter from Thorlabs will be used, as shown in Figure 3.19.

Figure 3.19: 1ϕ Long Pass Filter, Cut-on Wavelength of 500nm [21].

This filter features a durable dielectric coating that withstands normal cleaning and handling. It is recommended by Thorlabs to use for emission filters in fluorescence applications [21]. As these filters are not threaded, they require retaining rings in order to fit into the sample holder, which is also sold by Thorlabs.

3.6 Complete System

The complete system was built using the design outlined in Chapter 3, as shown in Figure 3.20.

The end of the APD will be connected to an oscilloscope as the data acquisition device. The InfiniiVision MSO-X 3104T mixed signal oscilloscope from Keysight Technologies was used as it has a bandwidth of 1 GHz, which is the same as the APD limit [22].

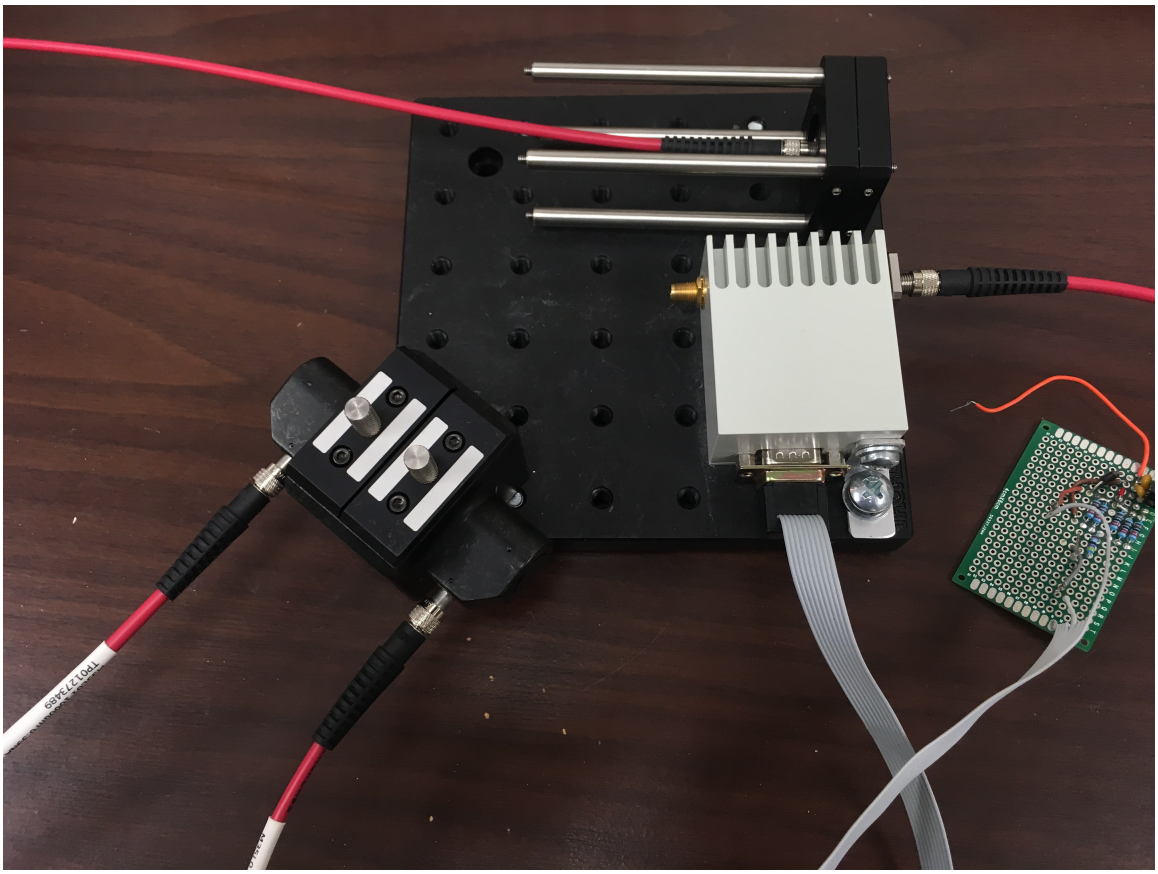


Figure 3.20: Complete Optical Fluorescence Lifetime Measuring Device.

CHAPTER 4
METHODOLOGY

4.1 Sample Selection

The cadmium-free silver-indium-sulfide (Ag-In-S or AIS) chalcopyrite QDs was chosen due to the bright fluorescence and low toxicity [23]. This QD is used to be internalized into U-87 brain tumor cells. This QD emits a yellow light and has an expected lifetime decay of approximately 350 - 450 nm depending on doping [24]. The system will measure the lifetime decay of this sample and compare it the expected decay.

The Coumarin 153 organic based dye will be used to test the detection of a higher speed signal, and a different wavelength. It has a fall time of approximately 4.3 +/- 0.2 nm [25]. The system will capture the high-speed waveform and the data will be analyzed to determine if the system provides acceptable data. The expected emission when diluted in ethanol is 532 nm, which is a green light.

4.2 Detection Calibration

Calibration is required in order to accurately capture the lifetime of the colloidal quantum dot. The limits of the APD was tested by inputting an excitation laser at a frequency of 50 KHz and 1 MHz, as shown in Figure 4.1 and Figure 4.2 respectively. The 50 KHz signal is extremely noisy, to measuring on the low end of the device limit is not recommended. The 1 MHz signal was significantly more stable. The system will turn on with the sample and the excitation laser will clearly be seen

going through the sample. Figure 4.3 shows the system with Coumarin 153 being excited.

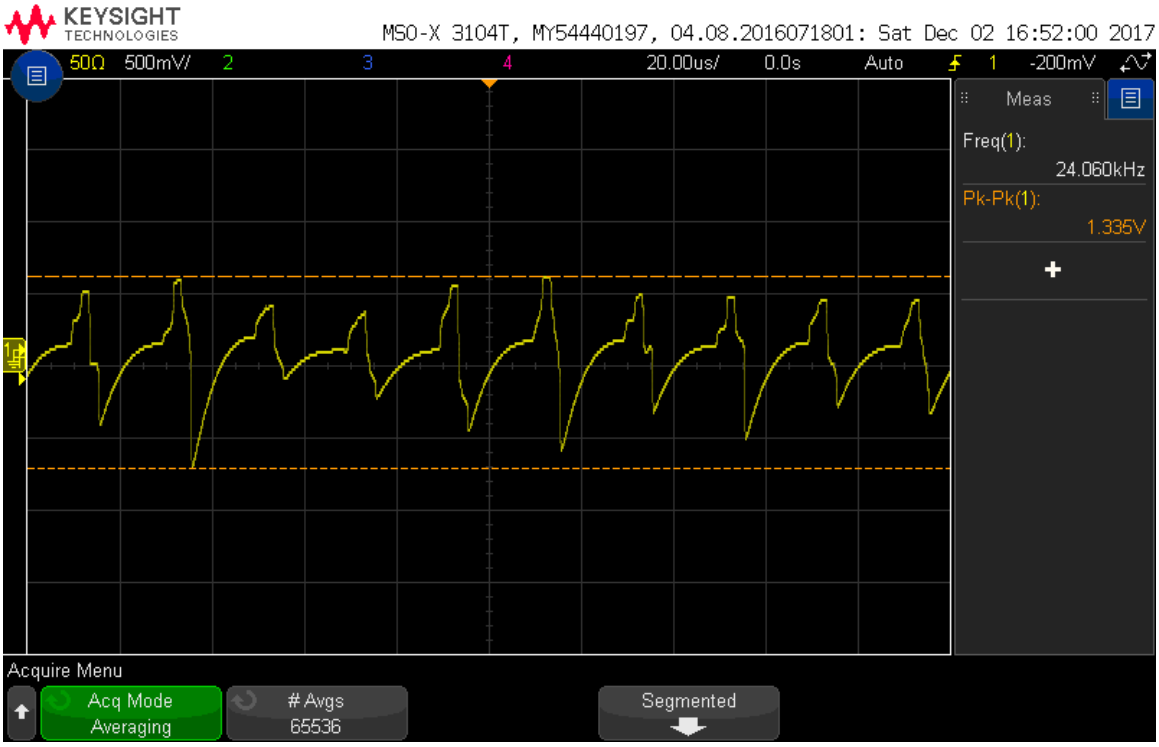


Figure 4.1: 50 KHz Excitation Frequency.

The oscilloscope is then set to a 50Ω impedance on the probe, as per the data sheet of the APD shown in Table 3.1. In order to get an accurate signal reading of the APD, the acquire mode of the oscilloscope is set to averaging mode and is set to the maximum number of points, in this case, 65536 points. This filters out the noise and allows the oscilloscope to capture the data properly. The trigger has to be sweep manually across the signal in order to get a flat peak after the rise and a flat signal after the decay. This allows reliable data to be collected as is it will show how the signal behaves during excitation and after the decay. This calibration has to be done constantly after changing the settings from the function generator.

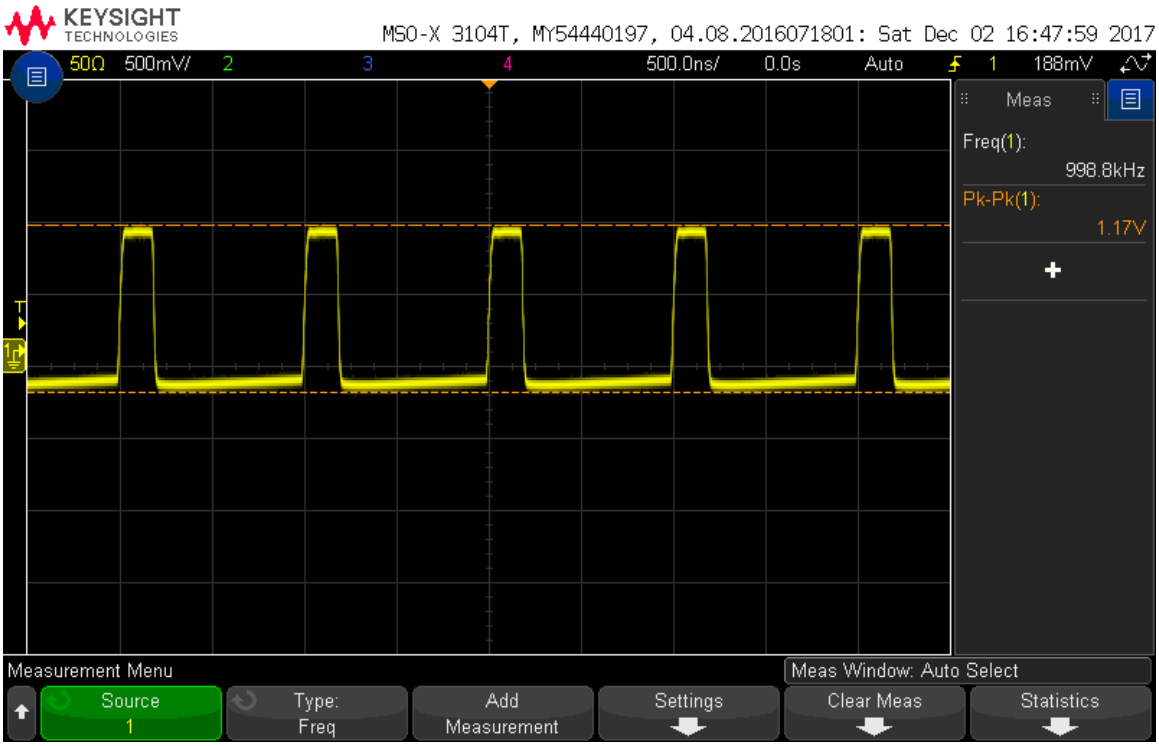


Figure 4.2: 1 MHz Excitation Frequency.

4.3 Function Generator

The function generator is used to the drive the laser circuit designed in Chapter 3. The frequency, duty cycle, amplitude, offset, and signal waveform needs to be adjusted in order to obtain reliable data.

For the QD tested in this paper, it was found that a square wave at a frequency of 350 KHz pulsed at 20% duty cycle produces the lifetime decay waveform that will be captured. A voltage amplitude of 5 V was used in order to allow enough current through the base resistor in order to fully saturate the transistor. At that amplitude, the square wave by default goes from +/- 2.5 volts, which is 5 volts peak to peak. This is a problem due to the transistor needing a positive voltage drop of 0.65 V seen across the base and emitter in order to bias the transistor. To

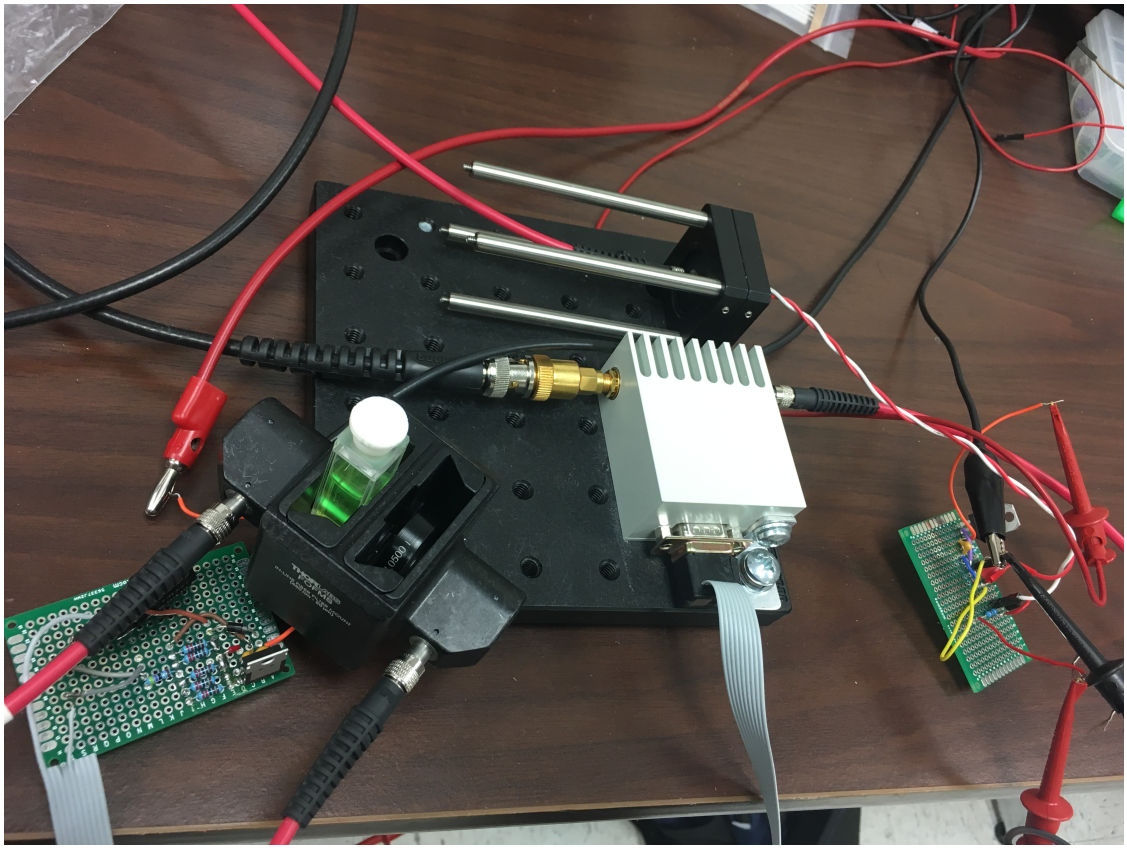


Figure 4.3: Optical Fluorescence System Exciting a Sample of Coumarin 153.

rectify this, a DC offset voltage of 2.5 volts is added to the waveform in order to bring the square wave range from 0 to +5 V. This will prevent damage to the transistor as a large enough negative voltage applied to the base will damage it. It is important to note that it would be beneficial to have the offset as close to the 0.65 V forward voltage required to turn on the transistor without biasing it. This will help keep the laser on and off for a longer duration of the square wave, which will compensate for the slower rise and fall times of the transistor.

For the Coumarin 153 sample, the frequency of the square wave is 650 KHz, at an amplitude of 5 V, and a 0 volt offset.

4.3.1 Excitation Laser

The excitation laser waveform is determined by the function generator. Using the oscilloscope, both the output of the function generator and the output of the APD can be captured at the same time, in order to see how the changes of the settings can affect the APD reading. The most noticeable differences will be encountered when changing the frequency, amplitude, and duty cycle of the waveform. Figure 4.4 shows the input signal of the laser vs. the output of the APD.

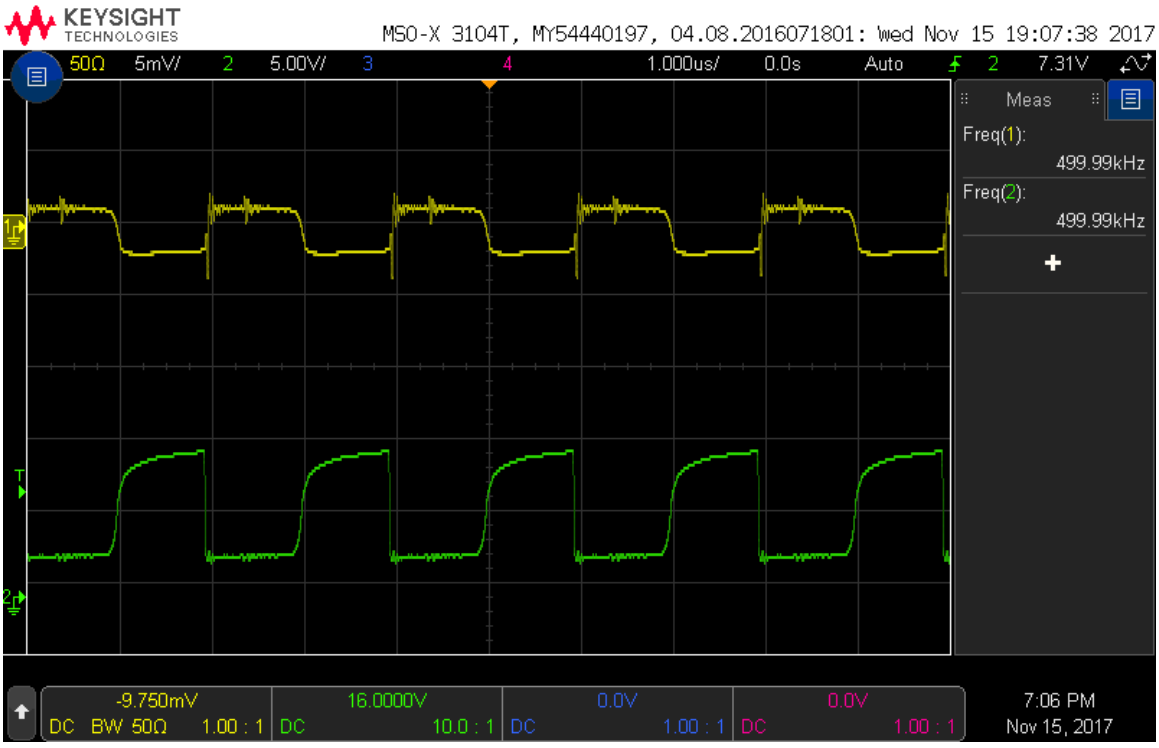


Figure 4.4: Excitation Laser Waveform (Green) vs. APD Output (Yellow).

Slight ringing was present in the output waveform of the function generator, however, some of the ringing did not affect the APD as the transistor was completely biased and fluctuations due to the ringing did not register to the APD. The APD is affected by the laser driver circuit, which is causing reflection due to a

possible impedance mismatch. Adding a capacitor to the laser driver circuit at the adjust pin to ground will filter out some of the noise. This was done for the Coumarin 153 sample.

4.4 Data Acquisition

The system will attempt to detect the lifetime decay of the QD used with different concentrations of the sample. 5.5 mg of the QD is used and is diluted with 6 ml of Tetrahydrofuran (THF), 99%, stab, with 250-350 ppm butylated hydroxytoluene (BHT). The waveform is then captured by saving both the image of the oscilloscope and the .csv data. The .csv data is used as a method to filter out the noisy signal by removing oddities found in the signal that might be due to the circuit or possible stray light that may have entered with the sample.

Once the waveform and the .csv data is saved, 3 ml of that sample will be taken and be further diluted by another 3 ml of THF. This process will repeat until the waveform is barely noticeable. This test will show if the system is feasible, and the sensitivity of the light detection device, the APD.

The same test will be done with the green organic based dye. The 2 mg of Coumarin 153 will be diluted with 6 ml of ethanol. The image and .csv data will be captured, 3 ml of the sample will then be diluted by 3 ml of ethanol.

The microcuvette is rinsed with acetone and dried completely each time the sample is removed and awaiting the diluted sample. This will ensure that there is no residue left by the more concentrated sample that can affect the fluorescent light emitted from the diluted sample.

In order to measure the lifetime, Equation 4.1 is used.

$$V(t) = A_0 * e^{t/\tau} \tag{4.1}$$

V(t) is the amplitude of the voltage measured by the oscilloscope at time t. A_0 is the maximum amplitude of the signal. τ is the lifetime that will be calculated, which will be obtained by setting the time t equal to τ. This will give us a voltage on the oscilloscope equal to 36.79% of the max amplitude A_0.

CHAPTER 5

RESULTS AND DATA ANALYSIS

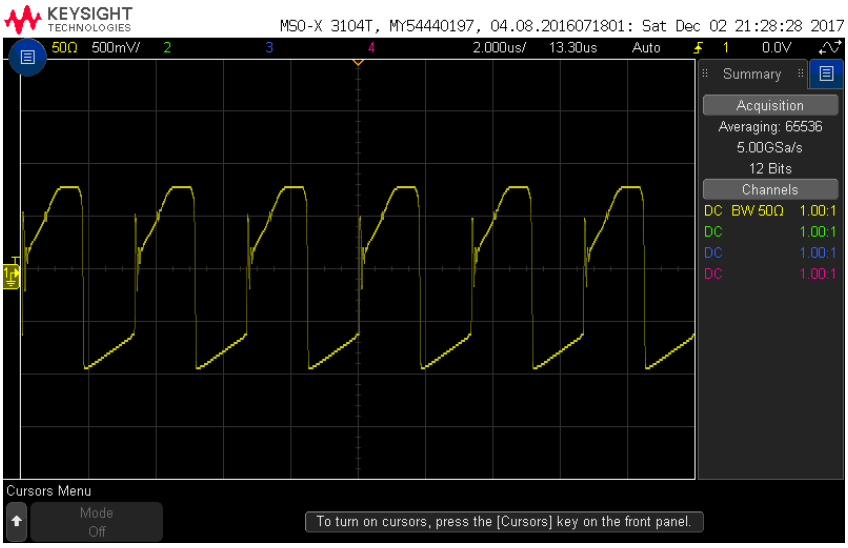
5.1 Overview

After the data collection presented in the methodology section is completed, the data will be analyzed by comparing the lifetime decay of the fluorescent light to the expected known decay value. The fluorescent light acquired will also be compared to the excitation light signal, this will ensure that the fluorescent light obtained is due to the laser and is not a result of stray light or other sources. Both samples will be diluted multiple times to test the overall sensitivity of the system, and to examine if the lifetime decay of the colloidal QD sample is different based on the concentration.

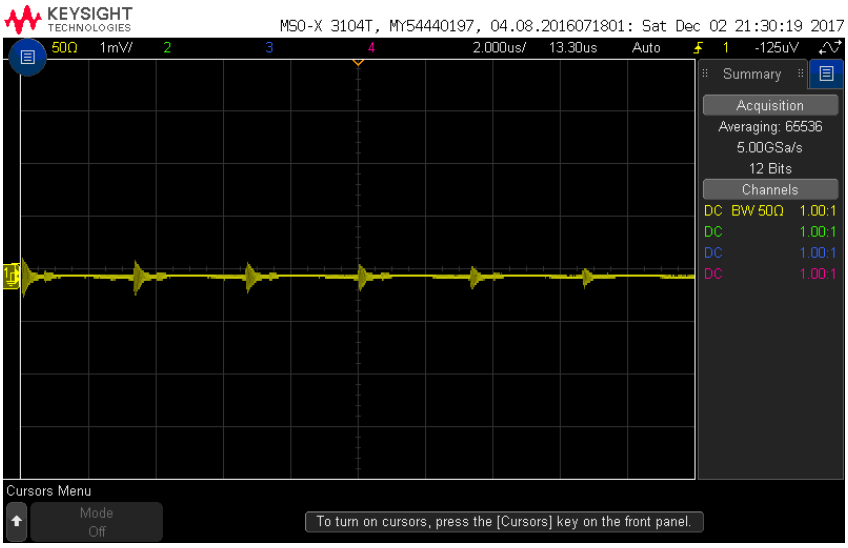
5.2 Quantum Dots

5.2.1 Laser

Figure 5.1(a) shows the input excitation laser that was calibrated to give the best waveform for to capture the QD lifetime decay. The rise time is affected by the circuit designed that turns on and off the transistor. A capacitor could be added to the adjust pin of the current regulator in order to smooth out the pulse. The rise time of the excitation laser will affect the rise time of the fluorescent light. However, as the purpose of this system is to capture the QD lifetime decay, the delay in the rise time is acceptable.



(a) APD Output of Excitation Laser.



(b) APD Output of Excitation Laser Going Through the Filter.

Figure 5.1: APD Output of Input Laser for the Quantum Dots.

5.2.2 Laser and filter

The input excitation laser is then fed straight into the longpass filter. It is expected that the APD will give no waveform due to the laser being approximately 100 nm below the cut-on frequency of the filter. Figure 5.1(b) shows the output of the APD.

5.2.3 Laser and THF

Figure 5.2 shows the APD output with a sample of only THF. This is to show that the THF that is diluting the QDs is not affected by the excitation laser. The rise time is further affected and the amplitude is reduced due to the refraction of light, but the signal is mostly unchanged.

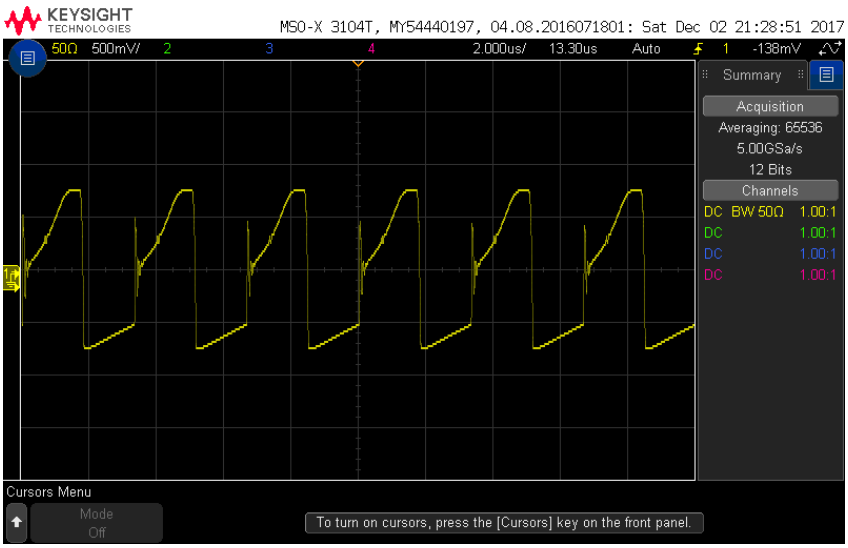


Figure 5.2: APD Output of Excitation Laser Going Through the THF Sample.

5.2.4 Laser, THF, and Filter

Figure 5.3 shows the APD output of the excitation laser going into the THF sample and the filter. The ringing present is most likely due to the circuit not properly having the impedance matched. It is also likely that it is influenced by the slow rise time present in Figure 5.1 (a) and Figure 5.2

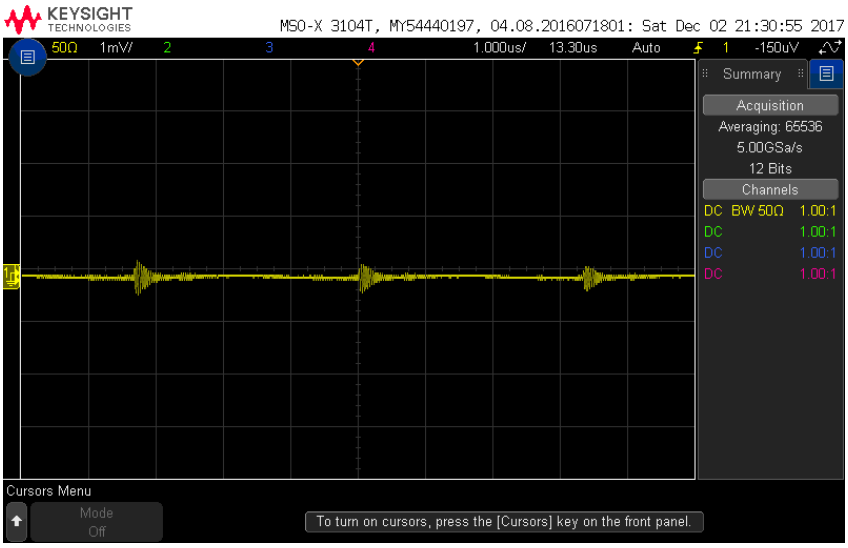
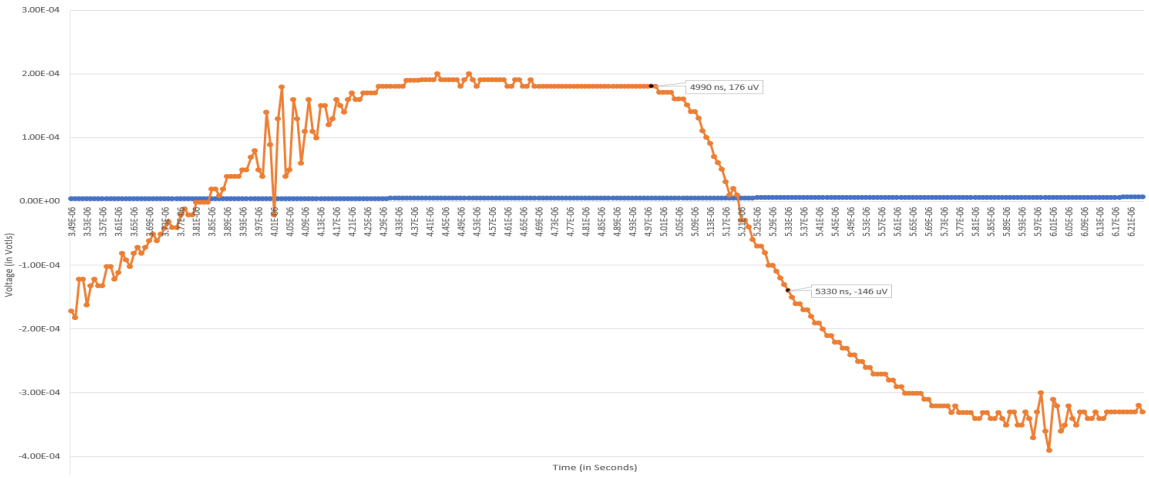


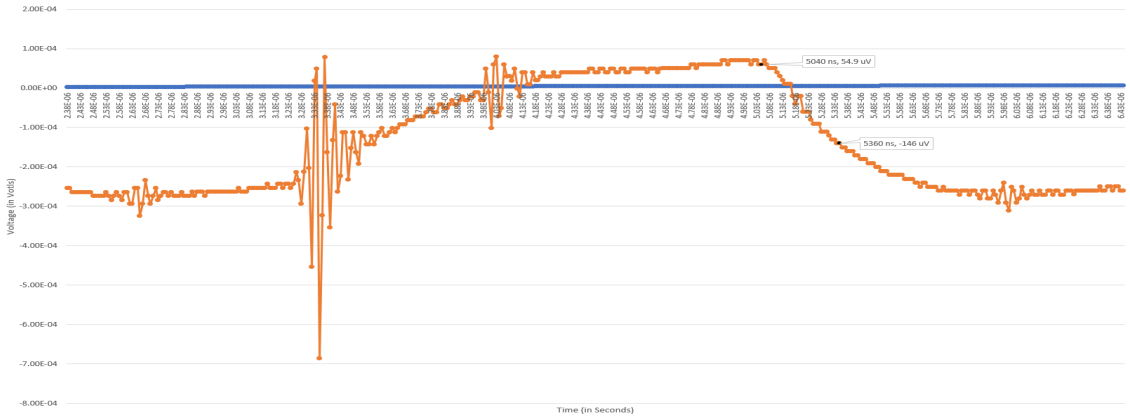
Figure 5.3: APD Output of Excitation Laser Through the THF Sample and Filter.

5.2.5 Undiluted Sample

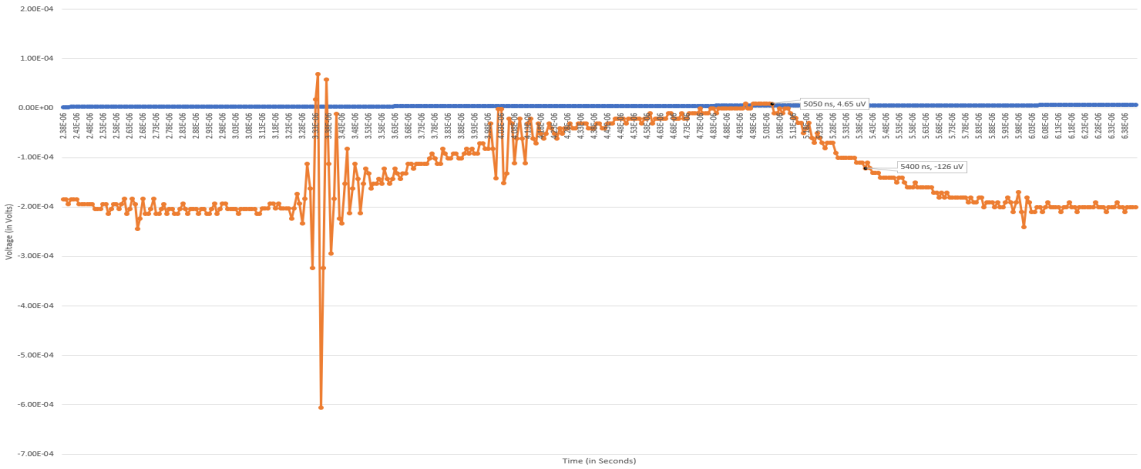
The undiluted QD sample is expected to yield the best waveform due to the high concentration of the QDs present. Figure 5.4(a) shows the APD output of the undiluted QD sample. The slow rise time is due to the input of the excitation laser. The flat top of the signal shows the QD has been fully excited and does not continue to rise anymore. The flat bottom shows that there has been ample time for the fluorescence to completely decay. The calibration has been done correctly and the lifetime decay can be properly acquired. The lifetime decay was measured and calculated to be approximately 340 nm, which is within the range of the lifetime decay expected by the QD sample.



(a) APD Output of Undiluted Quantum Dot Sample.



(b) APD Output of Quantum Dot Sample Diluted One Time.



(c) APD Output of Quantum Dot Sample Diluted Two Times.

Figure 5.4: APD Output of Quantum Dots with Various Concentrations.

5.2.6 First Dilution

As mentioned in the methodology section, 3 ml of the undiluted QD sample is diluted with 3 ml of THF. Figure 5.4(b) shows the APD output of the QD sample diluted once. The amplitude is reduced however the lifetime decay can still be captured. The lifetime decay of the sample was measured and calculated to be 320 nm, which is a 30 nm difference or a less than 10% from the first measurement. This shows the even though the QDs present in the sample is halved, the lifetime decay is not affected.

5.2.7 Second Dilution

Figure 5.4(c) shows the APD output of the QD sample diluted a second time. The amplitude is greatly reduced, but the lifetime decay can still be acquired. The lifetime decay was measured and calculated to be approximately 350 ns. The difference is negligible and proves that the decay is not affected by the concentration.

Table 5.1 shows the tabulated data of the QD fluorescence that was measured. The amplitude is quite low, which is due to the sensitivity of the APD. The lifetime however, appears to be very close to what is expected according to the literature. This QD sample is expected to have a lifetime decay of 350-450 nm. The measurements obtained shows a lifetime decay of 320 - 350 nm. The system has successfully measured the fluorescent lifetime decay of this QD sample.

Table 5.1: Quantum Dot Data Comparison

Sample #	Concentration	Lifetime (ns)	$\Delta\ \tau$ from expected	Pk - Pk (mV)
Undiluted	1	340	2.857%	0.513
1st Dilution	1/2	320	8.571%	0.332
2nd Dilution	1/4	350	0%	0.211

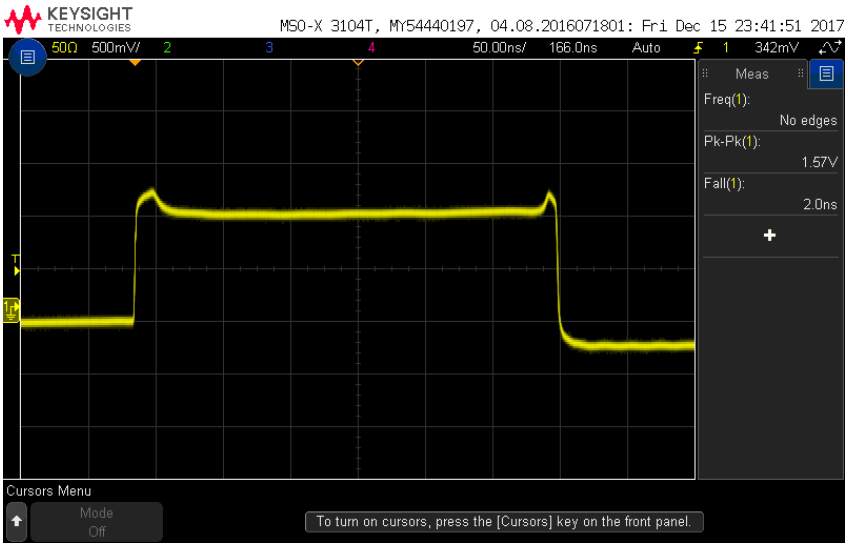
5.3 Organic Dye

5.3.1 Laser

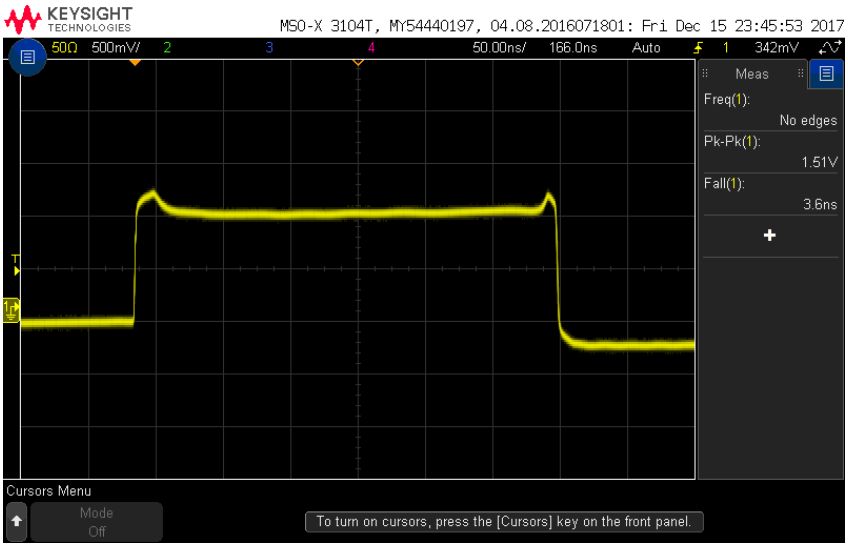
The data collected for the Coumarin 153 organic dye will be analyzed at a higher frequency in order to further test the high-speed detection of the APD. The undiluted sample produces the strongest fluorescent light. Figure 5.5(a) shows the calibrated excitation laser. The waveform exhibits a sharp rise and fall times, which is ideal. The amplitude is measured to be 1.57 V.

5.3.2 Laser and Ethanol

Figure 5.5(b) shows the excitation laser going through a sample of ethanol. The amplitude has been reduced by approximately 60 mV and the rise time has increased by approximately 1 - 2 ns. A capacitor has been added to the laser driver circuit at the adj pin to ground. Although it cleans the signal, there is an overshoot on both ends of the circuit, and this is most likely due to an impedance mismatch.



(a) APD Output of 650 KHz Excitation Laser.



(b) APD Output of Excitation Laser Going Through the Sample of Ethanol.

Figure 5.5: APD Output of Input Laser for the Organic Dye.

5.3.3 Laser, Ethanol, and Filter

Figure 5.6 shows the excitation laser going through the sample of ethanol and through the filter. It can clearly be seen that the ethanol does not produce a distinct signal through the filter. This noise can be due to the circuit.

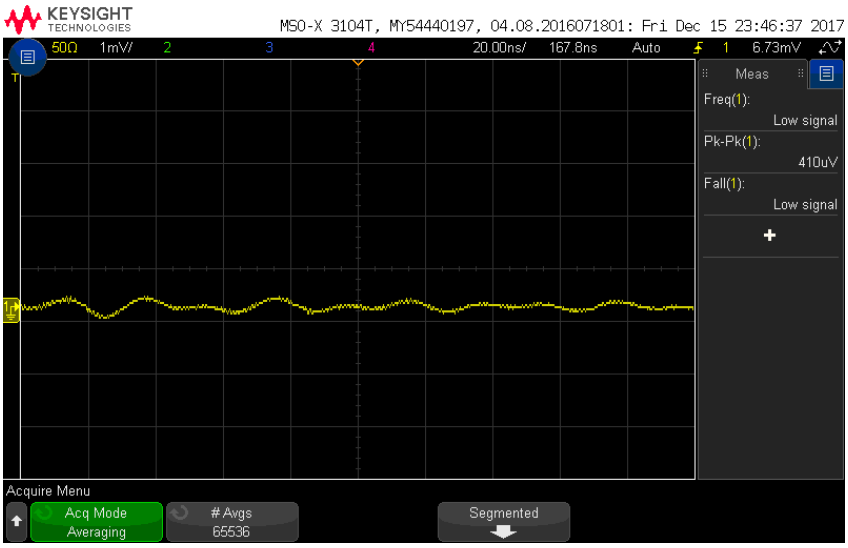


Figure 5.6: APD Output of Excitation Laser Going Through the Sample of Ethanol and Through the Filter.

5.3.4 Laser and Dye

Figure 5.7 shows the APD output of the excitation laser going through the undiluted sample of the organic dye without the filter. The excitation laser is mostly absorbed by the sample, and approximately 35 mV peak to peak voltage was able to be detected. The amplitude has dropped by approximately 97.68%.

5.3.5 Undiluted Dye

After completing calibration and confirming that the excitation laser is providing a sufficient waveform for the APD to operate, the excitation laser passed through undiluted organic dye sample and through the filter, as shown in Figure 5.8(a). Figure 5.8(b) shows the .csv data plotted of the falling edge. The lifetime is measured and calculated to be approximately 9 ns, which is higher than the 4.5 ns lifetime

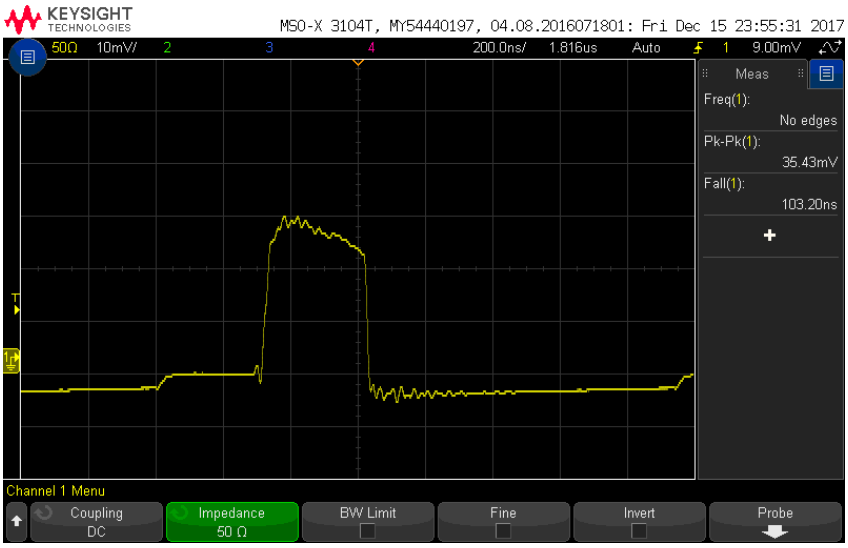


Figure 5.7: APD Output of Excitation Laser Going Through the Undiluted Dye.

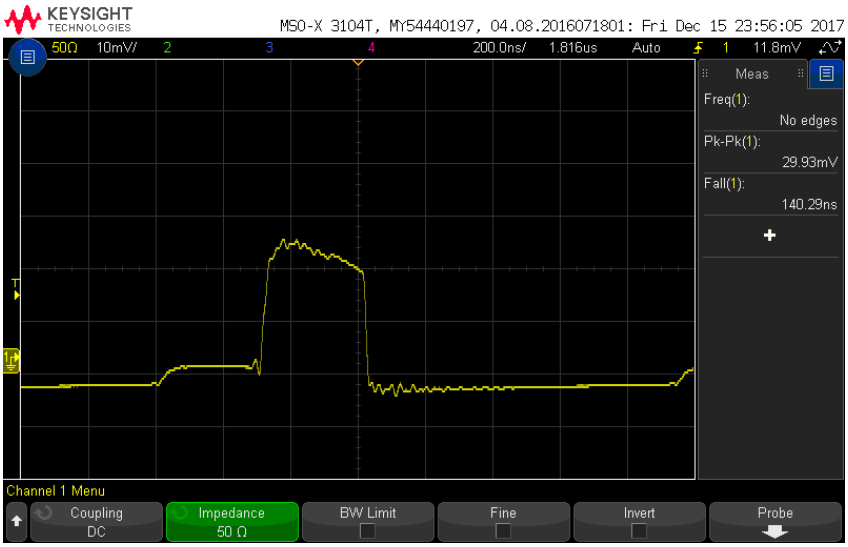
expected.

5.3.6 Second Dilution

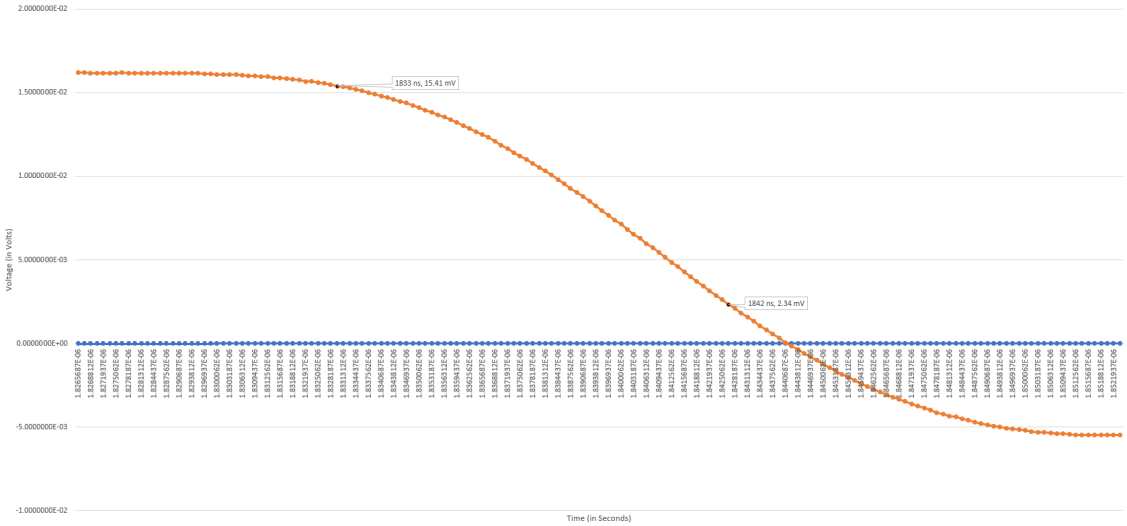
The sample is then diluted by taking 3 ml of the concentrated sample and adding 3ml of ethanol. This is done twice due since it will take multiple dilutions for the organic dye to weaken its fluorescence. After a second dilution, lifetime is measured and calculated to be approximately 9 ns. The waveform is shown in Figure 5.9(a).

5.3.7 Fourth Dilution

After a fourth dilution, the amplitude has negligibly changed, within 0.02 of a mV. The lifetime was measured and calculated to be approximately 8 ns, and the

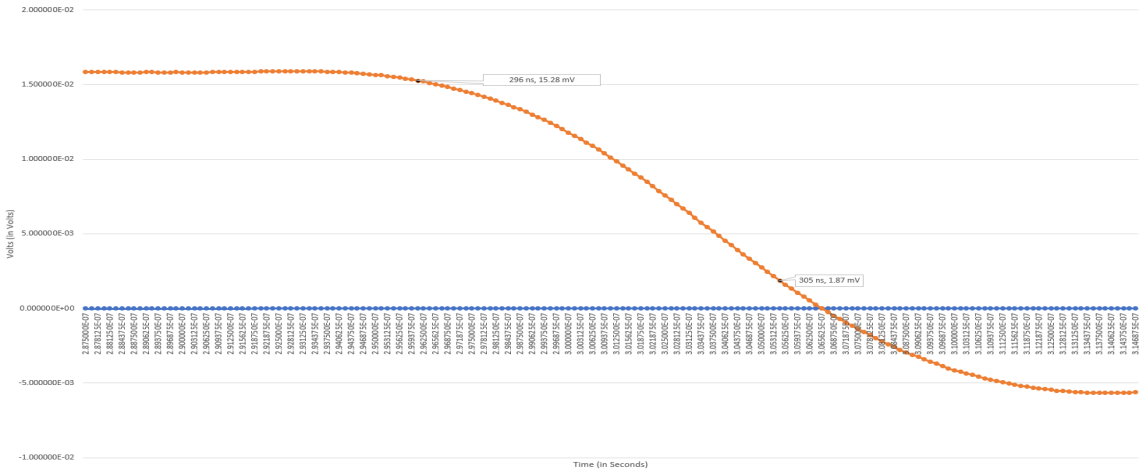


(a) APD Output of Excitation Laser Going Through the Undiluted Organic Dye and Filter.

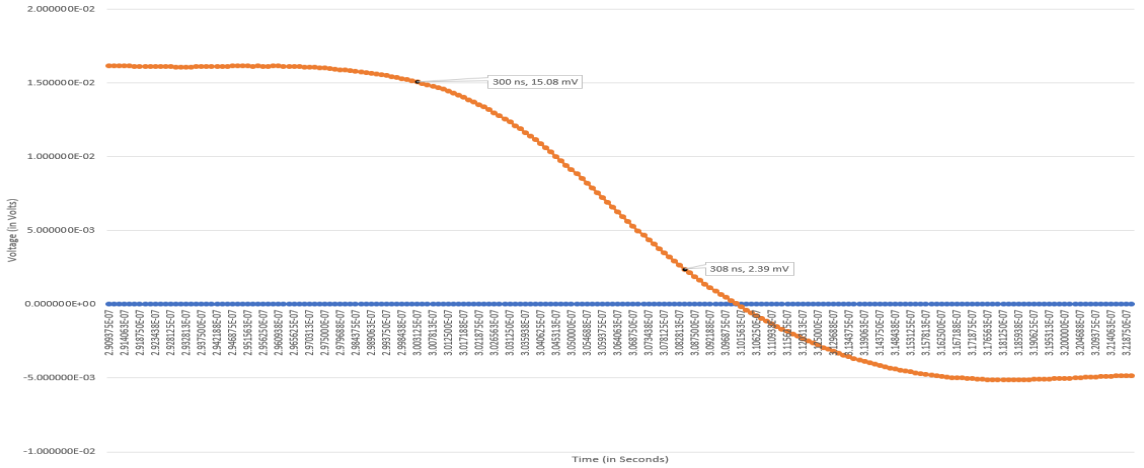


(b) APD Output of Undiluted Organic Dye.

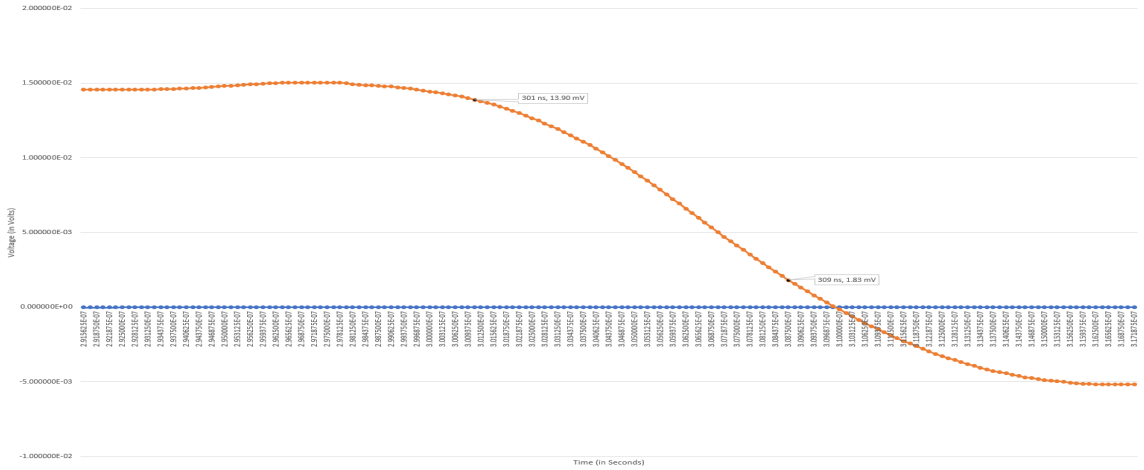
Figure 5.8: APD Output of Laser for Organic Dye.



(a) APD Output of Organic Dye After Two Dilutions.



(b) APD Output of Organic Dye After Four Dilutions.



(c) APD Output of Organic Dye After Five Dilutions.

Figure 5.9: APD Outputs of Organic Dye with Various Dye Concentrations.

waveform is shown in Figure 5.9(b). The lifetime has negligibly changed by 1 ns, but the trend is showing that the lifetime is unaffected by the concentration of the dye.

5.3.8 Fifth Dilution

After a fifth dilution, the amplitude dropped by another 0.02 mv. The lifetime has been measured to be 8 ns, and the waveform is shown in Figure 5.9(c). The fifth dilution means the sample is at 1/32 the concentration of the original sample.

The results of the data acquired from all of the dye samples are tabulated in Table 5.2.

Table 5.2: Organic Dye Data Comparison

Sample #	**Concentration**	**Fall Time (ns)**	**Pk - Pk (mV)**
Undiluted	1	9	21.46
2nd Dilution	1/4	9	21.45
4th Dilution	1/16	8	21.22
5th Dilution	1/32	8	20.16

Based on the data collected, it shows the lifetime is not affected by the concentration of the sample. The amplitude is marginally affected by the concentration, with a difference of 1.51 mV from the undiluted sample and the 1/32 concentration sample. The waveform has followed the excitation signal properly, however, there is noise present in the signal. The lifetime is double the expected 4 - 4.5 nm. This is most likely due to the laser switching on and off at the same time the decay is happening, and it is causing inaccuracies in the reading. It might also be caused by the noise present in the circuit.

CHAPTER 6

CONCLUSION AND FUTURE WORKS

This thesis presented a cost-effective, simple system for measuring fluorescence lifetime decay by examining similar works that captured fluorescence imaging. The system was built and optimized in order to exclusively measure the lifetime decay of either a quantum dot sample or an organic based dye. This system is drastically simpler than the other methods mentioned in Chapter 2, due to the lack of the use of a microscope and other imaging tools such as a tube lens or a dichromatic mirror. The system managed to measure the fluorescent lifetime decay while separating the excitation laser from the APD. This system will prove to be beneficial for measuring the lifetime QDs and other fluorescent lifetimes as long as they are decaying longer than the laser. The system's modular design will allow flexibility when measuring, as one can change the filter used, the excitation input, and the method of how the data is acquired, whether it is an oscilloscope or a dedicated data acquisition (DAQ) system. The system has accurately measured the quantum dot's lifetime decay, and although there were some small variances, it fell within the expected decay. When measuring the organic based dye, however, the system was not switching the laser fast enough to get an accurate measurement of the lifetime decay. There is also a possibility that the filter used was being excited as well and emitted it's own fluorescence, hence the increase decay time of the dye. Upon contacting Thorlabs Inc. about the excitation of the filter, a premium hard-coated long pass filter was recommended to use in order to prevent the excitation of the filter [26].

The cost of the overall system was approximately $3,500, this is without including the data acquisition device. A cheaper oscilloscope can be used, as data

collection was also tested by limiting the bandwidth to 20 MHz resulting in acceptable results. Other systems and equipment cost significantly more. For example, the Optika B-383FL Trinocular Fluorescence Microscope from New York Microscope Company costs approximately $6,513, and that is just for the microscope only [27]). It is important to note, however, that this system is clearly limited. The PicoQuant PDM series single-photon avalanche diode have significantly faster response times. Its counting output is as low as 250 ps, and its timing output can be as low as 50 ps. Clearly, a large advantage compared to the current system limited to above 10-20 ns lifetime decay requirement [28]. The PicoHarp 300 from PicoQuant is also another good choice for very fast fluorescent lifetime measurements, with a variety of additional features and software [29]. The device costs upwards of $17,000. If the application requires detecting quantum dots with long lifetimes such as the one presented in this paper, then the system proposed would be a cheaper alternative.

The APD used was not very sensitive, requiring the oscilloscope used to zoom in to the 1 mV per division range. However, this did help filter out stray light as it did not seem to affect the APD output whether the testing environment was in an unlit room or not. The circuit did include ringing which needed to be filtered out in order to measure the lifetime decay without with a higher signal to noise ratio. However, the system was able to capture the decay without the ringing being an issue for the QD.

Since the system is fully modular, future work includes testing with different APDs available in the market. There are various APDs with different frequency responses and fluorescence detection. The APD can be replaced with a PMT and test to see if it yields a signal with higher amplitude, compared to the 1 mV range

that was seen when using the APD. The data acquisition can be changed by using a DAQ or similar devices. The filters are easily interchangeable when using the Thorlabs FOFMS sample holder. Using a bandpass filter to target the specific fluorescence emission would most likely yield a clean, sharp signal with no noise, as it will filter out any potential stray light that would enter the light measuring device. A filter that will not be excited from either the excitation or fluorescent light is highly recommended.

The system can be improved by modifying the circuit in order to clean the signal used for driving the laser. The excitation input signal had a misshapen waveform, where a sharp square wave is ideal. Testing using a MOSFET or a Schottky BJT would most likely yield a sharper rise and fall times, within 1 - 2 ns, due to their faster switching times. Replacing the function generator with another frequency generator can be explored to examine the changes on the waveform.

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