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Design of Bench Top Fluorescent Microscope for Quantification of Quantum dots (QDs) Novel Fluorescent Label

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Abstract
A simple and Reliable Bench top microscope is constructed to detect the fluorescence intensity for the quantum dot. Which can act as fluorescent tag for a sensitive fluorescent studies like sandwich enzyme-linked immunosorbent assay(sELISA). The Cadmium Telluride (CdTe) capped with MPA (3-Mercaptopropionic acid) is a quantum dot with fluorescent signal output (Excitation: 490nm, Emission: 515nm) used. It is a bifunctional molecule containing both carboxylic acid and thiol group. Hence used in serval applications in medical field. Fluorescent microscope plays a major role, there is a real demand in the medical field targeting the public health centers for a Bench-top device, which finds its tremendous application in quantifying the analyte in the sELISA by measuring the fluorescence intensities.

Keywords: Fluorescent tag, sandwich enzyme-linked immunosorbent assay, Fluorescent microscope

1.Introduction

Fluorescence is used in the life sciences generally as a non-destructive way of tracking or analyzing biological molecules .Some proteins or small molecules in cells are naturally fluorescent, is called intrinsic fluorescence or auto fluorescence (chlorophyll Green fluorescent protein).Alternatively, specific or general proteins, nucleic acids, lipids or small molecules can be "labeled" with an extrinsic fluorophore, a fluorescent dye which can be a small molecule, protein or a quantum dot.

Quantum dots(QDs)represent a new type of fluorescent nanocrystal and are considered as ideal labels for ELISA and even for LCS(lateral- flow immune chromatographic strips) use due to their unique properties, such as broad adsorption, narrow and symmetric photoluminescence spectra(easily excitable), strong luminescence(highly sensitive) and robust photo stability.[1] These excellent properties render QDs as robust reporters for developing highly sensitive ELISA and LCS capable of simultaneous quantification multiple analytes .Quantum dots (QDs) represent a new type of fluorescent Label.

2. Method

Hardware Setup: Here work is carried on fabrication and as well design of bench-top fluorescence unit with Excitation: 490nm, Emission: 515nm.This custom, miniaturized fluorescence based system can be further used for in situ real time quantification of protein analyte in the sELISA with QDs as fluorescent tag [2,4].

The Proposed system employs the following Hardware:
2.1 Source:5mm UV LED (InGaN)RL5-UV0430-400 with Package of 5 mm (T-1 3/4).
5mm through hole, 400nm wavelengthth,40mW radiant power and 30 degree viewing angle.

2.2 Lens: Plano Convex lens of 9mm diameter used for converging the UV rays form LED and focussing on Short pass dichoric filter (excitation Filter).

2.3 Excitation filter (short pass dichoric filter**):**400nm Dichroic Short pass filter with 12.5mm diameter and Transmission Wavelength(nm) 325-385 from Edmund Optics Inc.

2.4 Dichroic beam splitter: Beamsplitter:505dclp 110687Chroma Technology, scan range of 380.0nm to 750.0nm is used to split input light into two separate parts based on wavelength.

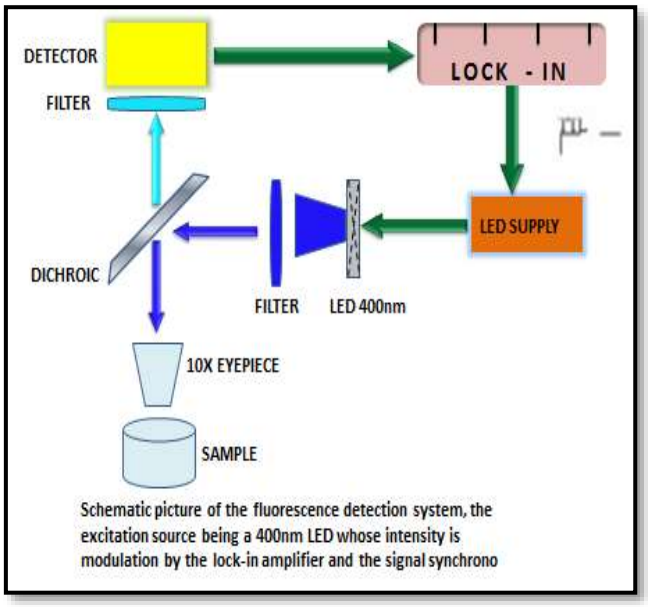
2.5 Emission Filter (Long pass Filter): high performance OD 4.0 long pass filter500 nm is used from Edmund Optics Inc.

2.6 Detector: A highly sensitive phototransistor is used as detector with a lens ACL 108, and further the phototransistor was connected to Lock- in amplifier to measure output of very small currents and voltages.

2.7 Lock-in amplifier:SR830 Lock-in amplifier is used with a carrier wave of 1 KHz square wave

3. System setup: The hardware components mentioned above **2.1** to **2.7** is assembled according to the schematic diagram [3] shown in the Figure 1.

Figure 1 :The Schematic diagram of the fluorescence microscope



3.1 Design Part
The Design of the fluorescent microscope was carried with the Inventor CAD software, the preliminary designs are as shown below before and after fabrication.

Figure 2. The 3D designsof the microscope with the x- axis,y- axis and z- axis movemen

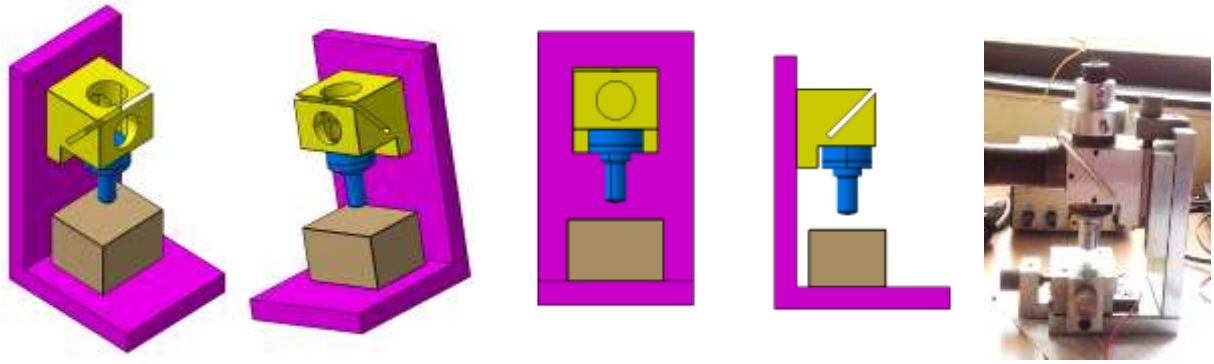
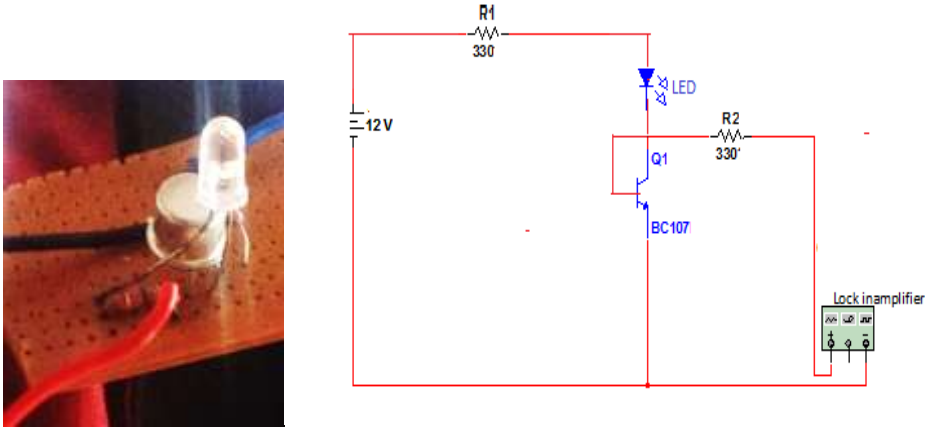
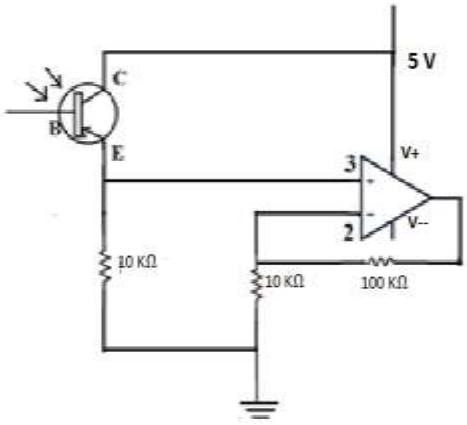


Figure 3: The circuit diagram representing UV LED source and the Lock- in amplifier.



As presented in Figure 1 the light from the UV LED with the circuit diagram shown in Figure 3is impinged upon the excitation filter. The excitation filter selects 400nm and blocks the unwanted wavelength. The selected wavelength after passing through the excitation filter, reach the dichromatic beam splitting mirror with scan range 380.0nm to 750.nm. It reflects shorter wavelength light 400nm and efficiently passes longer wavelength light greater than 500nm. The dichromatic beam splitter is tilted at a 45-degree angle with respect to the incoming excitation light and reflects this illumination at a 90-degree angle directly through the objective optical system and onto the specimen CdTe. Fluorescence emission produced by the illuminated CdTe is gathered by the objective, now serving in its usual image-forming function. As the emitted light consists of longer wavelengths than the excitation illumination, it is able to pass through the dichromatic mirror upward into the phototransistor detector through emission band pass filter [3].
At the receiver end phototransistor was used with Operational Amplifier to increase the efficiency as show below in Figure 4.

Figure 4: The Receiver Circuit consisting of Phototransistor and LM 358 IC



The receiver part was done more simple using photo transistor as shown in Figure 4. The signal strength was further increased by 100 using LM385 IC. Thus the weak signal from the photo transistor is amplified (100 times) and then fed to lock-in amplifier to obtain the outputs as shown in the Table1.

4. EXPERIMENTAL SETUP

CdTe capped with MPA (3-Mercaptopropionic acid) was procured from Physics dept. IISc Bangalore [5]. The fluorescence intensity of several samples of QDs in different concentration was tested. For the study, different dilution were prepared with concentration of 10µ L,20 µ L from the stock solution.The amplified signal was fed to the Lock-in amplifier and the following set of reading for the QDs were taken.

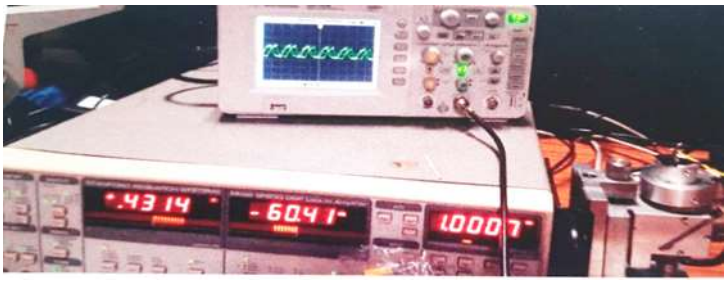


Table 1: Fluorescence intensity for various concentrations of CdTe quantum dot.

Material	Volume	Voltage (volts)
U.V LED Source (off)	10 µL	0.0026
Dark background	10 µL	0.3450
Quantam dot CdTe	10 µL	0.3671
Quantam dot CdTe (dry)	20µ L	0.4274
Quantam dot CdTe	20µ L	0.4592

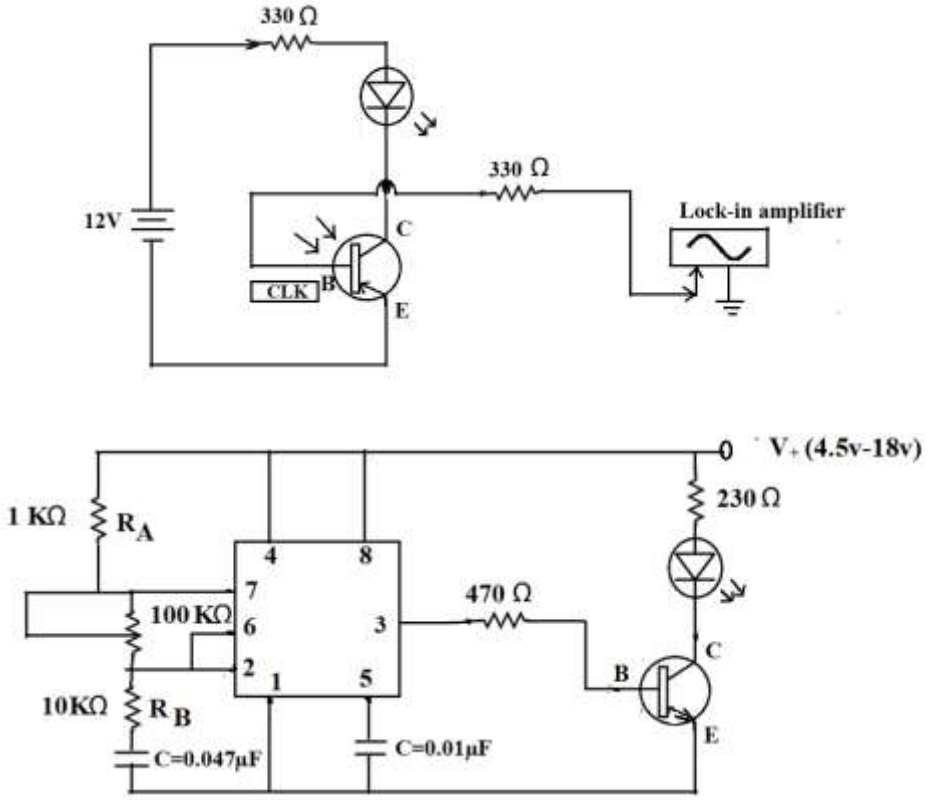
5. Replacement circuits

Replacement circuits for the input and output measured with lock-in amplifier.

Using lock-in amplifier in the Bench-top microscope is more expensive and voluminous. Lock –in Amplifier can be replaced with simple circuits making it more user friendly and more economical and Portable.

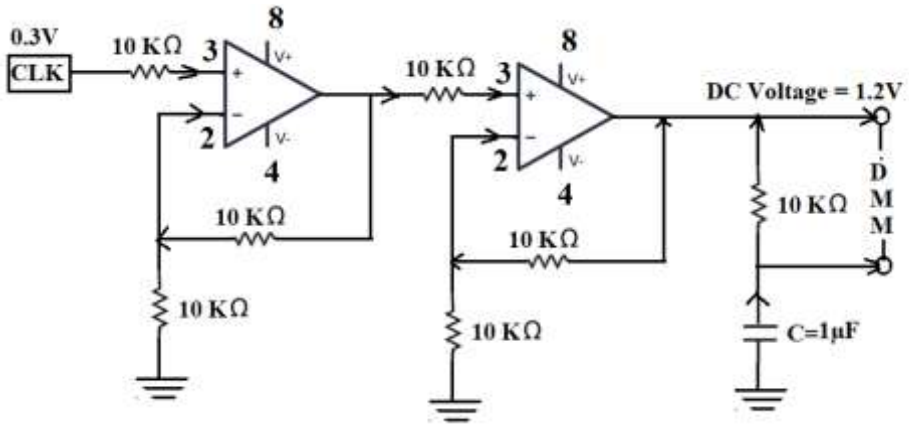
At the Input:

Figure 5: UV LED source and function obtained from lock-in amplifier ***Verses*** replacement circuit for generating the square wave frequency 1KHz. Using 555 Timer IC. [Frequency= $1.44/(R_A+2R_B)$ C]



At the output: The amplification of the output signal obtained from the detector, phototransistor as shown in Figure 4 can be further amplified with the below circuit and the amplified voltages can be measured using Multimeter. **C=0.047μF.**

Figure 6: The circuit diagram showing the amplified output can be measured using Digital Multimeter (DMM)



The first stage amplification with the LM 358 is with the gain 2 for the second stage gain 2 with total gain of 4. The input signal for the photo transistor observed is 0.3 Volts. The total D.C output voltage measureable at DMM is 4x0.3 Volts=1.2 Volts. The output capacitor of 1μF and resistance of 10KΩ was used to obtain D.C voltages as shown in the Figure 6.

6. CONCLUSION

Bench-top fluorescent microscope is built with simple circuits and is highly economical. Further the designed and fabricated fluorescent microscope can be used for the quantification of the analyate for sELISA where the detection of antibody labeled with QD will be allowed to bind selectively to the captured antigen. Fluorescent CdTe quantum dots act as a novel fluorescent tag.

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