A Visible Light Biological Imaging System for Small Laboratories

A thesis submitted in partial fulfillment of the requirement for the degree of **Bachelor of Science** in **Physics** from the College of William and Mary in Virginia

by

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Abstract:

The field of visible light imaging has recently emerged as a new method for conducting biological research. The technique utilizes cameras, usually cooled charge-coupled device cameras, to observe both bioluminescence and fluorescence. Currently, at the College of William and Mary, a charge-coupled device camera originally designed for astronomical observations has the potential for use in such research, as its visual sensitivity makes it applicable for observing low-level fluorescence and bioluminescence. To improve image quality through the minimization of thermal noise, a cooling system was constructed and its effectiveness analyzed. In addition, tests to determine the camera's applicability as a research tool are described and the results discussed, specifically the most recent, which utilized Quantum Dots, nanocrystals surrounded by an inorganic shell that emit light at specific wavelengths.

Acknowledgements:

I would like to thank my research advisor, Professor Robert Welsh, for his enthusiasm and support for this project. As an undergraduate learning my way around the laboratory, his patience and advice were absolutely invaluable to the completion of this project. Professor Anne Reilly, my academic advisor, has also been a great help and inspiration to me academically.

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1 Introduction

The field of visible light imaging has become a valuable tool for conducting biological research. Through the use of fluorescent or bioluminescent tags, the path of various substances, including infections, can be traced through the body of an animal [3]. A major advantage to this type of research is that it can be done in vivo, without the sacrifice of an animal and with the added benefit of being able to watch biological processes as they occur. However, a factor that currently limits widespread use of this technology is the expense associated with it; current systems, such as those offered by Xenogen, Inc. and Kodak have costs ranging from $20,000 to $100,000.

Currently, a charge-coupled device (CCD) camera is being adapted for the purpose of biological imaging. The goal of this work is the development of an economical research tool that can be utilized by smaller laboratories with lower budgets. The quality of this system and its applicability in the field of visible light imaging will be reported.

2 Background

2.1 The Electromagnetic Spectrum, Visible Light, and Basic Photophysics

Light has a dual nature. It can be viewed as both a wave and a particle. One can speak of light in terms of the electromagnetic spectrum, in which light has a specific wavelength.

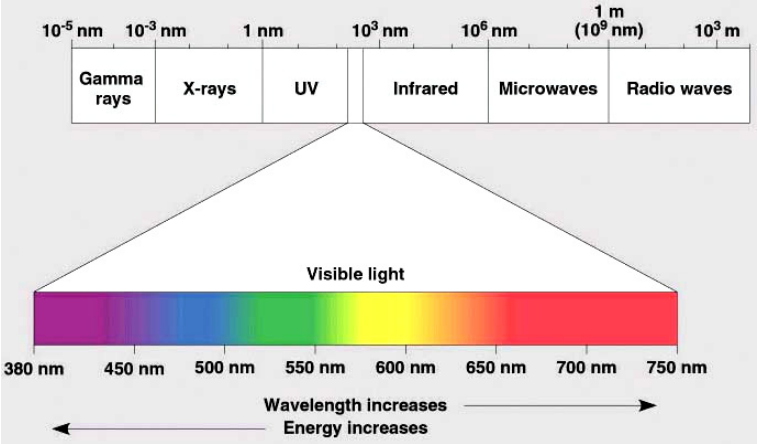

Figure 1: The Electromagnetic Spectrum.
http://cont1.edunet4u.net/ cobac2/scientist/Maxwell.html

The energy of a photon of light at a specific wavelength λ is given by

$$E = \frac{hc}{\lambda}, \tag{1}$$

where h is Planck's constant and c is the speed of light. Visible light consists of those regions on the electromagnetic spectrum which stimulate the human retina; this region falls from approximately between 400 nm and 700 nm.

When an atom absorbs a photon, the energy from the photon is transferred to the atom. One of the atom's electrons may then have the energy to make a transition to a higher energy level, called an excited state. When this electron returns to a lower energy level, it may emit energy in the form of a photon. [1].

2.2 The Photoelectric Effect and Charge-coupled Device Cameras

The photoelectric effect is the ejection of electrons from the surfaces of metals as a result of incident electromagnetic radiation, including visible light, ultraviolet light, infrared light, x-rays, etc. In 1905, Albert Einstein explained the photoelectric effect

implying the particle-like nature of light, which led to the quantum model of physics. The ejection of electrons from a metal can be described by

$$E_e = \frac{hc}{\lambda} - W_m \ , \tag{2}$$

where E_e is the energy of the electrons ejected from the material, λ is the wavelength of the incident light, and W_m is the work function of the metal. The work function is defined as the minimum energy required for electrons to be ejected from the material [1].

Charge-coupled device (CCD) cameras utilize the interactions of photons and atomic electrons to detect photons and display images in response to that detection. Most can pick up low-level light in the range of near ultraviolet light at approximately 300 nm to infrared light at approximately 1000 nm. Silicon is a semiconductor, which makes it ideal for applications of this type. Silicon has four valence electrons, and nearly all of them fall in a low energy state called the valence band. In order to excite an electron from the valence band to the conduction band, energy is required. This energy, the difference between the valence band and the conduction band, is referred to as the band gap [11]. In the case of the CCD camera, the energy is provided by incoming light from the subject of the image. After a silicon electron is excited to the conduction band, it can create an electrical current. An excited electron in pure silicon leaves behind a "hole," which is a missing covalent bond in the silicon lattice. This hole acts as a site of positive charge, and when an electric field is applied, an electron from a neighboring atom moves to fill the hole, thus leaving another hole in its place. This process continues and results in the "movement" of a hole, or a charge packet, throughout the silicon lattice, [13]. In the CCD camera, these charge packets detected on the silicon chip are converted to

numerical values, which are responsible for the relative amount of light in each pixel of the image [2].

The camera utilized in this project was produced by Santa Barbara Instrument Group, which designs CCD cameras for astronomical observation. The visual sensitivity of this model makes it applicable for observing low-level fluorescence and bioluminescence. From previous experimentation [4], it was known that this camera is able to pick up the low-level bioluminescence and the expression of fluorescence through thin layers of animal tissue.

The CCD camera used in this work is a model ST-6, and it has a Texas Instruments TC-241 CCD chip with a pixel size of 27 x 11.5 μm, an effective pixel area of 27x23 μm, and 91,000 pixels. Thus, the total photosensitive area is 55.9 mm^2 [4]. According to Santa Barbara Instrument Group, the model is able to detect light at wavelengths ranging from 400 nm to 1000 nm. The cost of the entire system is currently under $2,000.

2.3 Image Quality

One of the main obstacles that limits the camera in comparison with more expensive systems is its current inability to consistently maintain a cooled temperature at -25 °C and below. Higher temperatures generally mean higher thermal noise counts. Noise, sometimes referred to as "image mottle," gives the image a grainy appearance and reduces visibility in the image [5]. Therefore a low noise count is an important part of producing a high-quality image. Thermal noise specifically is the result of pixels in the silicon CCD chip collecting a charge regardless of whether or not they have been hit by

incoming photons. When the temperature of the CCD chip is higher, there is a greater chance that thermal events that can generate noise will occur. Thus, cooling the camera can significantly decrease the amount of thermal noise in a signal [6]. Previous experimentation shows that there is a direct relationship between the temperature of the apparatus and the amount of thermal noise, and that it can be modeled by an equation of the form counts = $\alpha\tau^{\beta}$, where α and β are parameters that depend on exposure time and source intensity [4].

Most current commercial visible light imaging systems image at consistently lower temperatures to maintain a higher image quality. The Kodak Image Station 2000MM Multimodal Imaging System images at a temperature of -29.00 °C. The Xenogen IVIS Imaging System 100 Series cools the CCD to -105.0 °C by utilizing cryogenic cooling. While this can impractical for small laboratories, it emphasizes the importance of a low signal-to-noise ratio in image quality. A goal of this project is the development of an efficient means of keeping the temperature of the CCD chip down.

The ST-6 does have its own temperature regulation system, which makes use of Peltier-effect coolers inside the camera. The CCDOPS software associated with the camera allows the user to regulate the temperature. Peltier coolers make use of the electron energy differences in different metals. If two metals of differing energies are placed in contact, the electrons in the metal with the higher average energy will move to the metals with the lower energy electrons. This system would tend toward equilibrium unless an external voltage is applied. When the applied voltage is in the direction of electrons moving from the high energy metal to the low energy metal, cooling occurs on the high energy metal side of the junction and heat is isolated on the low energy side [7].

Options for better cooling the CCD camera used here include additional Peltier coolers, liquid nitrogen, which boils at -195.65 °C, and refrigeration. Refrigeration is a possibility; however, there is the significant concern of resulting condensation on the camera and possible damage to the CCD chip and the electronics of the camera. Thus, avenues involving additional Peltier coolers and liquid nitrogen vapor were pursued in this project.

2.4 Quantum Dots

Quantum dots are particles that fluoresce with narrow emission spectra and utilize the properties of absorption and emission of light in order to provide a medium for imaging [11]. Qdot Nanocrystals, developed and manufactured by Quantum Dot Corporation, can be selected to fluoresce at specific wavelengths.

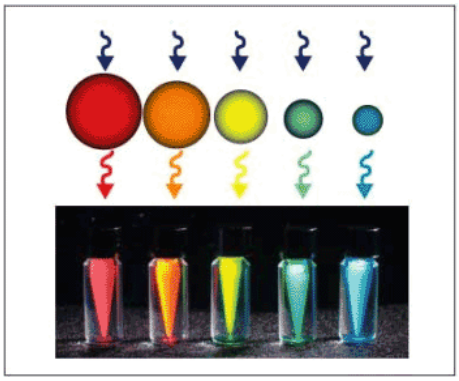

Figure 2: Vials of Quantum Dots. Each of these vials contain quantum dots excited after being place in front of an ultraviolet lamp. Each "dot" has been engineered to emit light of a specific wavelength. http://www.qdots.com

Quantum dots consist of a core composed of either cadmium sulfide (CdS), cadmium selenide (CdSe), or cadmium telluride (CdTe). Which one is utilized depends on the desired wavelength of emission: CdS is used for the wavelength range from UV to

blue, CdSe for most of the visible spectrum, CdTe for the far red and near-infrared. These cores have diameters around 10-20 nm [11]. All of these compounds are semiconductors, analogous to the silicon chip which is used in the CCD camera. When the quantum dot is stimulated by a photon, an electron is transferred to the cadmium atom from the other atom (sulphur, selenium, or tellurium), leaving a "hole" and creating a negative charge by the cadmium atom.

While the material composing the core roughly determines the range in which the Quantum Dots will emit light, it is the size of the dot that provides such a fine-tuned emission wavelength: the larger the core, the higher the specific wavelength of light the dot will emit. The band gap of a certain semiconductor specifies a certain wavelength of light. In order for this semiconductor to absorb light when it is reduced to the size of a quantum dot, the light it absorbs must be of a smaller wavelength and thus a higher energy. Because the incoming light has a higher energy, the electron transitions to a higher energy level, and thus a higher energy / shorter wavelength photon will be emitted. Quantum dots are engineered to be certain sizes so that they will emit at specific wavelengths [11]. In order for them to be excited, the light stimulating them must provide energy greater than the band gap.

In order to protect surface regularity and to prevent the core from becoming polluted or contaminated by chemical reactions with the external environment, a shell is put around the dot. In the dots manufactured by Quantum Dot Corporation, the shell is made of ZnS, which is unreactive and improves the quantum yield at the surface of the dot [11]. Quantum yield is defined as the percentage of incoming photons resulting in the

creation of hole-electron pairs in the semiconductor. A 100% quantum yield would mean that every incoming photon resulted in an emitted photon.

The zinc sulfide shell of the Dot is covered with a coating. The inner coating consists of organic ligands covalently attached to the surface of the shell, which further stabilizes the shell. These organic ligands are firmly attached to the outer coating, which is hydrophobic where it interacts with the inner coating and hydrophilic on the outside, which allows for solubility of the quantum dots in buffers. It is this solubility that allows the Quantum Dots to be such valuable tools for biological research, in that they are undegraded by various environments.

The use of Quantum Dots for in vivo imaging has been demonstrated through Xenogen's IVIS Imaging System 200, which has shown the distribution of Quantum Dots through the body of a mouse after injection through the tail vein [12]. Also, a Photometrics C258 cooled CCD camera was utilized for the visualization of 755 nm quantum dots in nude mice, pictured below, 10 minutes after injection through the tail vein [8]:

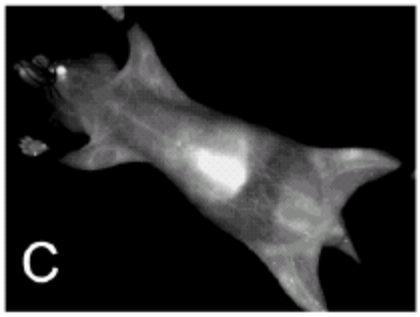

Figure 3: Quantum dots in-vivo in a nude mouse. B. Ballou, B. Lagerholm, L. Ernst, M. Bruchez, and A. Waggoner, "Noninvasive Imaging of Quantum Dots in Mice," Bioconjugate Chemistry **15**, 79-86 (2004).

2.5 Optics of Quantum Dot Imaging

In order to obtain constant excitation (and thus emission) of light from the quantum dots, they must be excited by incoming light and their radiation subsequently detected by the CCD camera. This presents a challenge in the experimental set-up of the detection system, as one wants the camera to see the light the quantum dots emit but not the exciting light stimulating the emission. The CCD camera used here is a monochrome device, as it cannot distinguish between the varying wavelengths of light that register on its chip. Therefore, one must use a device external to the camera to establish selective detection of light. The use of interference filters provides a solution to this problem [8].

Optical filters are defined by the part of the spectrum which they pass or exclude [9]. By selecting the correct interference filter, one can effectively limit the "vision" of a device such as a camera by preventing light of certain wavelengths from reaching the detector. Bandpass interference filters allow light of only specific wavelengths to pass through, and they reflect all other wavelengths of light.

An interference filter can be fabricated as a multilayer thin film device [10]. Light passes through two reflecting layers that are coated with a substrate. The distance between the two reflecting layers determines which wavelengths are allowed to pass and which are reflected through destructive interference. The gap between the two layers is called a spacer. A spacer can be a thin film of a dielectric material. The thickness of the spacer depends on the wavelength of light one wants to transmit:

$$d_{spacer} = \frac{1}{2}\lambda_{peak},$$

where λ_{peak} is the peak wavelength one wants to be transmitted through the filter.

The two layers on either side of the spacer are composed of several film layers, each of which has a thickness of ¼ the desired wavelength. The compositions of these layers alternate between materials of a high and low index of refraction. Typically zinc sulfide serves as the high index material and cryolite (Na_3AlF_6) is the low index material. This series of layers is referred to as a stack. The combination of two stacks and the spacer comprise a "one cavity" bandpass filter [10].

A one cavity bandpass filter, however, does not provide the sharpest contrast between wavelengths of light, and thus one may employ multiple cavity bandpass filters which can serve to fine-tune the filter's selectivity. Multiple cavity bandpass filters consist of several one cavity bandpass filters stacked together. Finally, to ensure only the passage of light of the desired wavelength, a blocking layer is added to the filter, which utilizes the principles of destructive interference to prevent "shoot through" of light not in the desired band. The blocking layers and passband layers are covered with a glass subtrate and sealed in a protective metal case with optical epoxy [10].

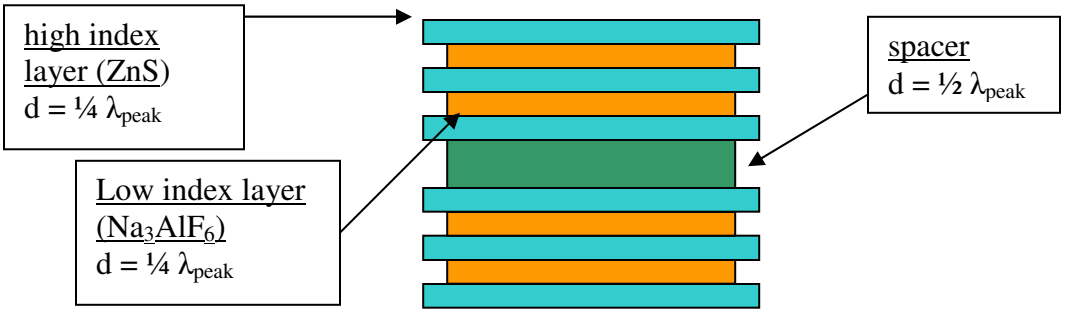

Figure 4: A single-cavity bandpass filter

In imaging quantum dots, one seeks to maximize the Stokes shift, which is defined as the difference in wavelengths between the absorption and emission spectra

[10]. With a large Stokes shift, one can be sure to clearly separate the light absorbed by the quantum dots and the light emitted by the quantum dots through the use of interference filters.

3 Experiment: Constructing a Cooling System for the Camera

3.1 Peltier Coolers: Experimental Set-up

In a test intended to attempt to draw more heat away from the camera, supplemental Peltier coolers were purchased. These Peltier coolers, like the ones inside the ST-6, make use of the electron energy differences in different metals [7].

After connecting a Peltier cooler to a power supply and supplying voltage it was noticed that the isolation of the heat did not last long, and after several minutes the entire Peltier cooler began to warm, unable to dissipate all the heat produced. In order to help conduct some of the heat away from the Peltier coolers, aluminum heat sinks were designed. The Peltier coolers, hot side connected to the aluminum sticks (Figure 3) were pressed, cold side down, to the heat radiator at the top of the CCD camera to draw heat away from the camera. A voltage difference of 3.5 V was applied to the Peltier coolers, and data were collected examining the heat gain in the electronics starting from -30 °C, the lowest temperature the camera had thus far attained.

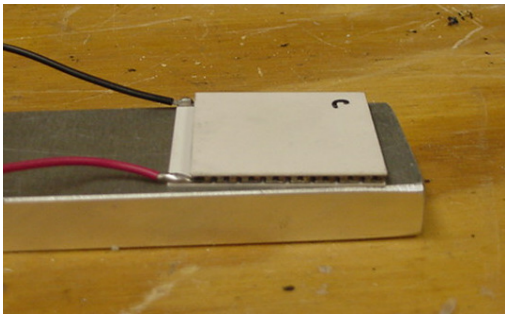

Figure 5: The Peltier cooler, connected to a voltage source, laying, hot side down, on an aluminum heat sink, in order to conduct heat away from the Peltier cooler.

3.2 Liquid Nitrogen Circulation: Experimental Set-up

Liquid nitrogen has several properties which make it a viable option for cooling the CCD. In its gas form, it is both very cold and very "dry," and thus will deposit minimal condensation on the camera if it is used in moderation. When considering how to utilize liquid nitrogen evaporate, one must take into consideration the amount of cooling the camera can tolerate without condensation, which would harm the electronics, as evidenced from a previous experiment:

A method of cooling utilizing liquid nitrogen had been tested previously. Two dewers containing liquid nitrogen were set up directly below the camera, which was supported by a ring stand and two aluminum sinks in contact with the liquid nitrogen. Without any temperature regulation by the CCD, the liquid nitrogen cooled the CCD chip to a low of -102 °C, and the temperature then stabilized at -85 °C. However, frost collected on both the camera and the aluminum sticks. This resulted in condensation on and within the camera. The camera was opened up, and allowed to dry out, and no harm appears to have resulted.

However, this raised concerns as to how to safely cool the camera without the danger of damaging it through condensation. Discussion with Mr. Alan Holmes of Santa Barbara Instrument Group made it clear that care must be taken not to *overcool* the camera in order to avoid the precipitation of excess moisture that could harm the electronics. Taking that into consideration, the following system utilizing liquid nitrogen evaporate was designed and tested:

The camera was removed from its original large plywood black box, and a new box was constructed, using a wall cabinet of approximate dimensions of 68 cm x 33.5 cm x 27 cm. The box was lined with Styrofoam, a shelf installed in the middle and the bottom half and door painted flat black to prevent reflection of light. Cracks were sealed and a frame was built around the inside of the door to prevent light from entering, thus making the box light-tight. A hole was put in the middle of the shelf so that the larger, top part of the camera could rest on the shelf and the lens could go through into the bottom half of the cabinet, where the object being imaged would be placed. The wire that connected the CCD camera with the central processing unit (CPU) was put through a hole in the top of the cabinet.

An original test of a method using liquid nitrogen evaporate to cool the camera consisted of connecting a long foam tube to the top of a liquid nitrogen dewer. Inside the dewer, a 20 Ω resister was connected to a power supply and approximately 5 V was applied to the resistor. However, after a few trials and no temperature change on the inside of the box, it was determined that the liquid nitrogen was not evaporating quickly enough. The design was altered to the one pictured below:

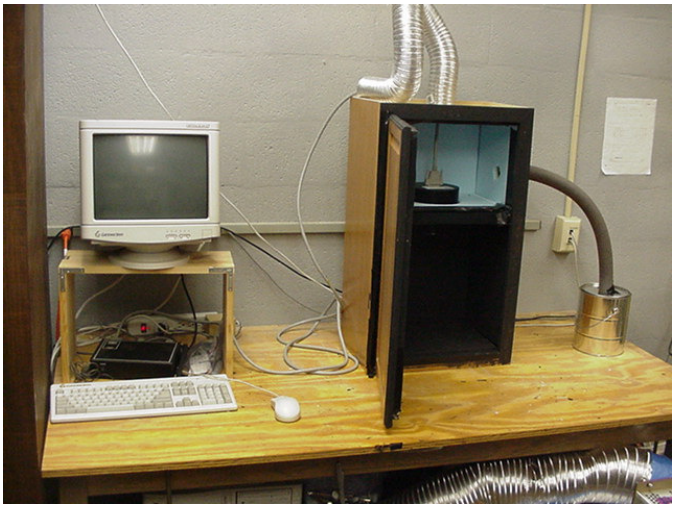

Figure 6: The final set-up the liquid nitrogen evaporate cooling system. The liquid nitrogen boils in the metal can and the cold gas passes through the gray foam tube and into the upper chamber of the cabinet, where the camera is located. The cold vapor moves over the camera, drawing heat away, and then leaves the upper chamber through the venting tube at the top.

The liquid nitrogen dewer was replaced by a clean, dry metal can of volume .0123 m^3 filled to approximately ¾ of its volume with liquid nitrogen. The liquid nitrogen boils off at room temperature and the metal can, unlike the liquid nitrogen dewer, exerts enough pressure on the evaporate so that it is forced through the foam tube.

3.3 Results and Analysis

Our results for each of the methods (no cooling, Peltier coolers, and liquid nitrogen evaporate) are presented in the graph below. The Peltier coolers provided a slight improvement, but not enough to practically improve the image quality of the camera.

The method of circulating liquid nitrogen evaporate using the pressure exerted by the sealed paint can, however, provided an ideal solution to the problem of cooling the charge-coupled device camera. The lowest temperature the camera had safely reached

was -30 °C, but the liquid nitrogen evaporate brought it down to -50 °C, with no visible condensation on the camera.

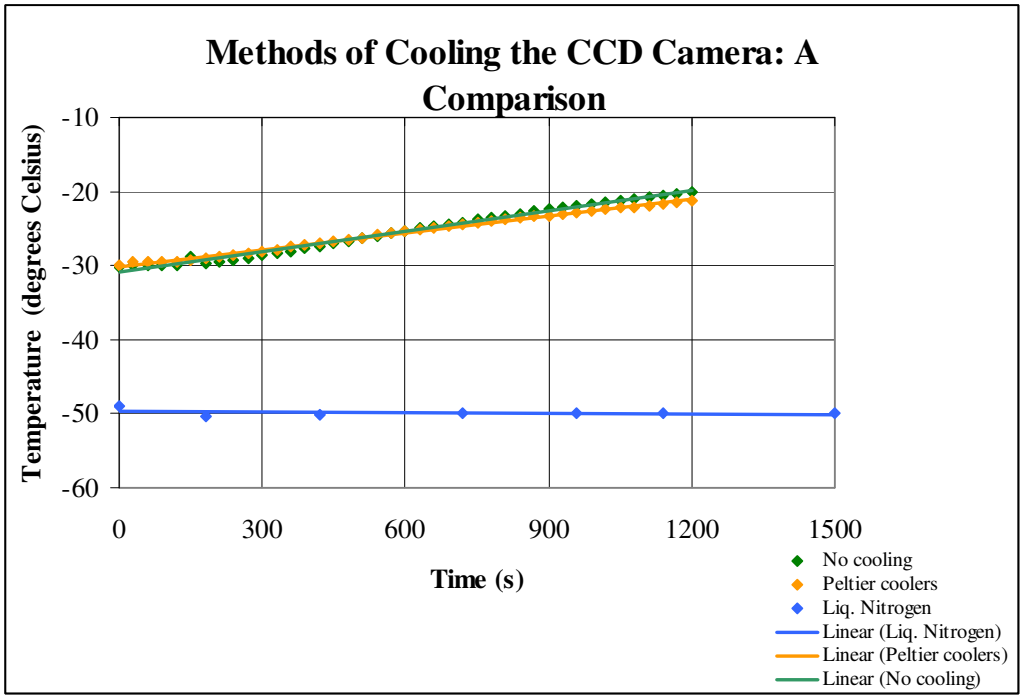

Figure 7: Results of Various Methods for Cooling the Camera

4 Experiment: The Camera as a Tool for Biological Research

4.1 Quantum Dots: Experimental Set-up

In designing an experimental set-up for the uses of quantum dots, one must take into account the optical parameters of such a system. To stimulate emission of the quantum dots, one utilizes high energy (short wavelength) light. Thus, one selects quantum dots that emit at a lower energy (longer wavelength) light than the exciting source.

For purposes of this work, QTracker™ 800 Non-targeted quantum dots were selected. They emit light at 800 nm, the longest wavelength emission offered, which is in the near-infrared region of the electromagnetic spectrum. This is well within the CCD camera's range of detection, which ranges from approximately 300 nm – 1000 nm. Non-targeted Quantum Dots are specifically developed to reduce interactions with other molecules.

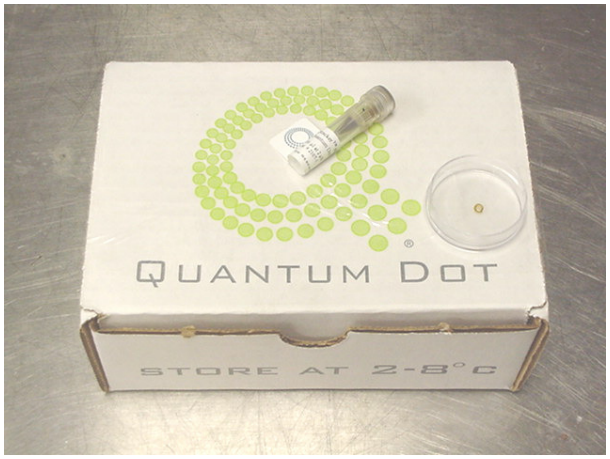

Figure 8: vial with 190 µL QTracker™ 800 Non-targeted quantum dots, and 10 µL "dot" in Petri dish (right) used for test images (200 µL total).

Filters were purchased from Edmund Industrial Optics for use in this set-up. These included an 800 nm bandpass interference filter, a red dichroic filter, a blue dichroic filter, and a cyan dichroic filter.

The basic set-up for the quantum dot imaging project is diagrammed below in Figure 7:

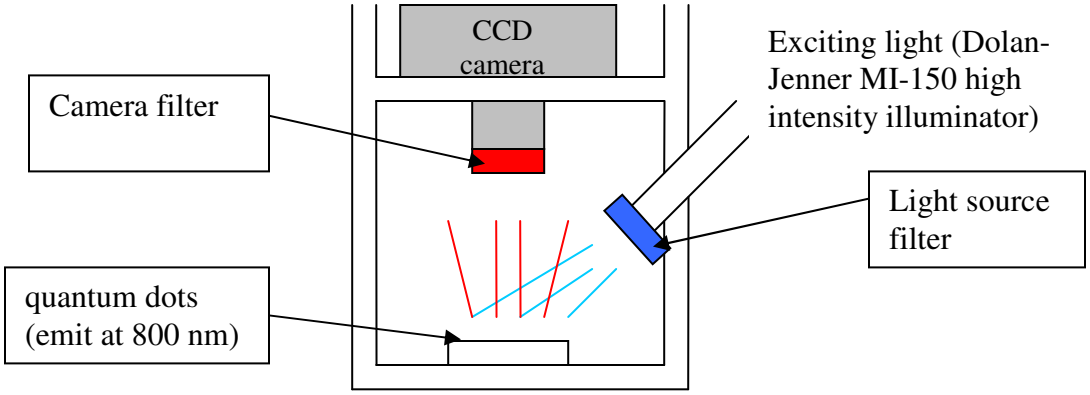

Figure 9: Optical Set-up of quantum dot imaging system

In choosing filters for the set-up, one hopes to maximize the amount of light exciting the quantum dots and the amount of light from the quantum dots being registered by the camera. In order to attempt to collect the maximum light from the quantum dots, we chose to try the red dichroic filter on the camera, which reflects light of wavelengths shorter than 575 nm, and the blue dichroic filter on the light source, which reflects light of wavelengths longer than 525 nm. The cyan filter was deemed as passing wavelengths too close to those the red dichroic filter was allowing to pass. The Stokes shift for this first set-up was: 800 nm – 525 nm = 275 nm

The images taken with this combination of filters were largely unsuccessful and it became clear that some strength in the signal would have to be sacrificed in order to get an image. Thus, the blue dichroic filter was removed from the exciting light source, and replaced by a 405 nm bandpass interference filter.

It was through this optical setup that the following image was obtained, confirming the ability of this set-up to detect quantum dots:

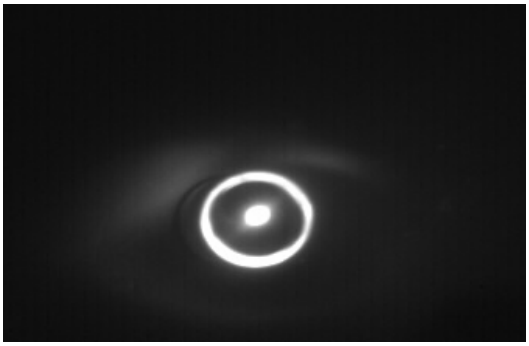

Figure 10: Image obtained of quantum dots, utilizing the red dichroic filter on the camera and a 405 nm bandpass filter on the exciting light source.

The dot in the middle of the picture in Figure 8 is the 10 μL in the middle of the Petri dish in Figure 6. The reflection of the Petri dish is the figure encircling the quantum dot. One can also see a light ring of light around the reflection of the dish, which is consistent with the edge of the light used to excite the quantum dots. Thus the camera was detecting some of the exciting light, to a small degree.

In order to improve the image, the red dichroic filter on the camera was replaced with the 800 nm bandpass filter, and the following image was obtained:

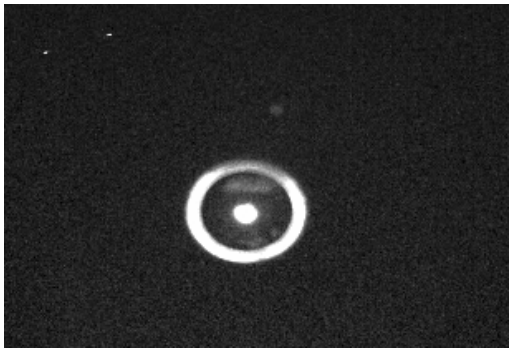

Figure 11: Image obtained of quantum dots, utilizing the 800 nm bandpass filter on the camera and a 405 nm bandpass filter on the exciting light source.

The light from the exciting source has been substantially reduced in this image, as the Stokes shift is increased to: 800 nm – 405 nm = 395 nm.

In order to remove the "halo" seen in the image and prevent reflection that could distort the image or inaccurately show the response of the quantum dots, the rim of the Petri dish was removed and covered with black tape. The following image was then obtained:

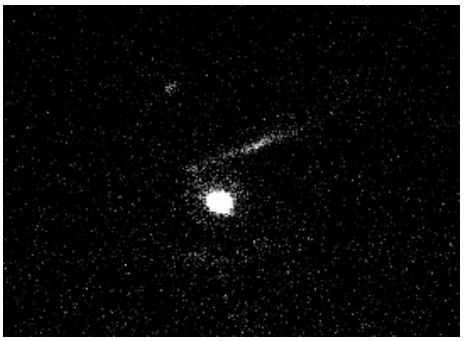

Figure 12: Image obtained of quantum dots, utilizing the 800 nm bandpass filter on the camera and a 405 nm bandpass filter on the exciting light source. The rim of the Petri dish is removed and has been covered with black tape to prevent reflection. The small line of the light above the dot in the image, however, is likely a reflection of the edge of the tape covering the Petri dish.

4.2 Quantum Dots: Results and Analysis

The table below compares the results from the images taken under different conditions. The numbers on the left refer to the Figure # of the respective images:

	CCD filter	**MI-150 filter**	**Stokes shift (nm)**	**Highest value (# photon "hits"), Dots**	**Average value, Petri dish reflection**	**Average value, background**
10	Red dichroic	450 nm bandpass	275	3944.52	1683.72	655.93
22	800 nm bandpass	450 nm bandpass	395	710.86	220.80	107.82
12	800 nm bandpass	450 nm bandpass	395	514.16	-	102.79

The average number of photon hits from a certain part of the image was determined using CCDOps, the image analysis program that accompanies the ST-6. In performing the analysis, the highest number from each of the regions was utilized.

One can see that in Image 1, a great deal of the light that appeared to be coming from the quantum dot was actually due to reflection from the Petri dish and light from the source that was reaching the camera. Subsequent adjustments to the filters and reduction of the reflection-causing Petri dish allowed one to detect only the actual light being emitted from the quantum dot.

An important part of in vivo biological imaging is dealing with the optical properties of animal tissue. In order to get a general idea of how well this system images quantum dots through tissue, clear microscope slides had been dipped in paraffin and allowed to dry. Paraffin has optical properties similar to that of animal tissue of a similar thickness. The slides were dipped in hot paraffin various times, so that each had a different thickness. Images were taken using the following set-up:

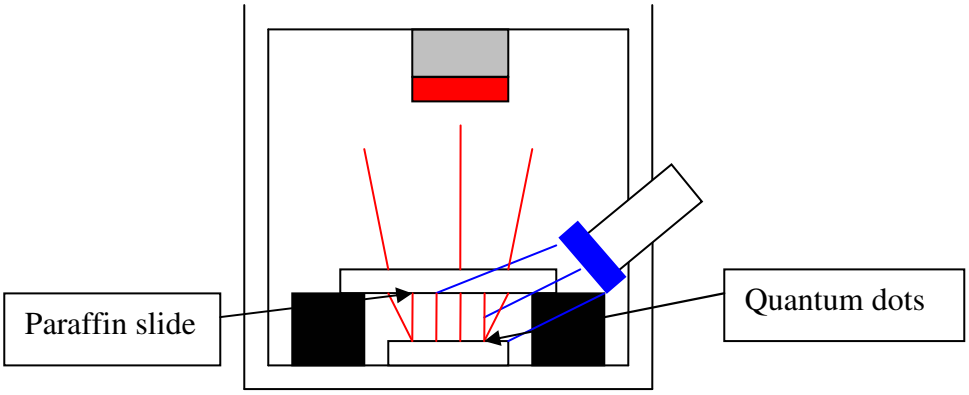

Figure 13: A diagram of the set-up used to determine the amount of light from the dots that would pass through various thicknesses of paraffin on a microscope slide. The distance from the dots to the paraffin slide is 6.5 cm. Of note: The exciting light (blue) does not pass through the paraffin slide. Only the emitted (red) light passes through the slide.

2.00 second exposures were taken at 0 °C. The images were analyzed using CCDOps, and average photon counts from the area of the slide were taken. Below is a sample image:

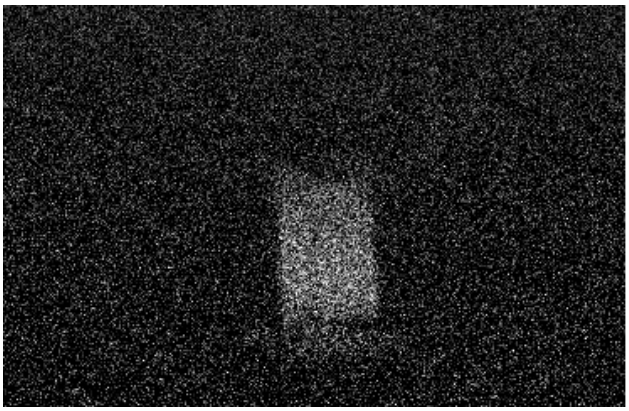

Figure 14: A sample image from the trial with one full slide of paraffin. Though there is considerable diffusion from behind the paraffin, one can clearly distinguish the light coming from the dot.

The results were as follows:

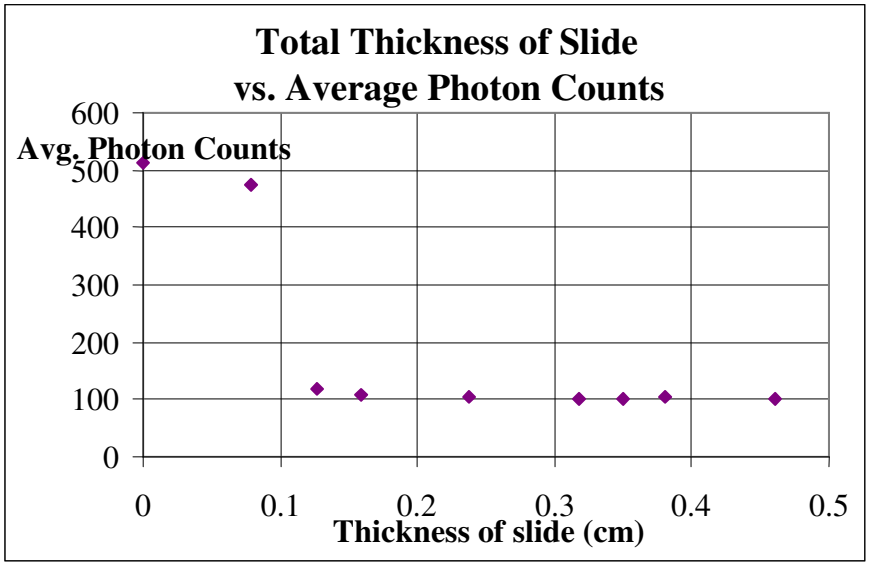

Figure 15: Thickness of Slide vs. Average Photon Counts. The first point, at a slide thickness of 0, was taken with no slide between the dot and the camera. The second point, at a slide thickness of 0.08 cm, represents the thickness of only the glass slide, with no paraffin on it. The third data point represents the first slide actually dipped in paraffin.

Thus, "Total Thickness of Slide" refers to the total amount of material between the dot and the camera, 0.08 cm of that being the blank slide and the rest comprised of paraffin.

One can see that there is a minor drop in visibility when a glass slide is placed between the dot and the camera. There is a significant drop when the first paraffin slide is used, and as shown in Figure 12, there is considerable diffusion through the material. Subsequent images show that the thickness of the paraffin, up to nearly 0.5 cm, does not make a major difference in visibility. Background was generally at around 80 photon counts. One can expect even more contrast with background when the CCD camera is cooled using the liquid nitrogen system described earlier.

Conclusion

The goal of this project was twofold: first, to find an economical but successful method of cooling the camera; and second, to show that this camera can be used to conduct biological research.

The first goal was met: liquid nitrogen is an inexpensive commodity, and the camera attained and held a temperature of -50 °C. Since no condensation was apparent on the camera after 25 minutes, at which point the metal can of liquid nitrogen ran out, one could presumably refill the can and continue to cool the camera for longer using the apparatus.

The camera's ability to see quantum dots both in the open and behind a barrier simulating animal tissue shows the instrument's promise for the future. Quantum dots can be purchased as a part of a conjugation kit, in which they bind to specific substances in an animal's body. This can provide valuable information contributing to the study of

tumors and the spread of infections in animals. The ultimate realization of this second goal will be to eventually see the quantum dots *in vivo* in an animal.

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