Quantum Dots Imaging

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Abstract

Quantum dots are nanometer-sized semiconductor particles which are fluorescent and have proven useful in small animal medical imaging. The focus of the work has been on studying the fluorescence of quantum dots as a function of age and also to find ideal exposure time for the camera with a wide open aperture. Data and images obtained from a mouse injected with quantum dots are shown and discussed. The images include a nude albino mouse and an MMTV mouse. Cyclic RGD peptide was also injected into an MMTV mouse and images were taken in-vivo. Also some organs were extracted and images were taken ex-vivo.

Keywords: Quantum Dots, RGD peptide, conjugate

Acknowledgements

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Chapter 1

Introduction

1.1 Background on Quantum Dots

The strict definition of a quantum dot is a particle of matter so small that the addition or removal of electrons changes the properties in some useful way. By this definition, atoms are considered quantum dots, but more interesting is when multi-molecular combinations exhibit this characteristic [7]. This study will be focused on the applications of quantum dots in biomedical imaging. The quantum dots used in medical imaging are made of semi-conductor material such that when excited, they emit photons.

The semi-conducting quantum dots are nano-spheres called fluorophores, which means they are particles that absorb an unspecific spectrum of light and re-emit a specific wavelength of light [10]. The re-emitted light wavelength directly correlates to the diameter of the quantum dot; the dot's diameter is on the order of nanometers. The larger the dot, the more "red" (lower energy) the re-emitted wavelength. The dots are comprised of three parts: the core, the shell and the coating. The core contains a few thousand semi-conductor type atoms. The shell is usually zinc-sulfide which stabilizes the core [10]. The coating is used to make the dot water soluble and allows conjugation of ligands to specialize the dots.

Ligands are molecules that functionalize the dots by covalently bonding to a central metal. Usually ligands make use of electrostatic binding between a positively charged metal, the coating of the dots, and a negatively charged thiol (a sulfur and hydrogen compound).

When used with quantum dots, ligands are peptides or polypetptides which make the dots site specific. A functionalized dot can accumulate in a specific organ, gland, or cell type of choice. An example of such a ligand would be arginine-glycine-aspartic acid (RGD peptide), which can be used to bind to integrin $\alpha_v\beta_3$. An overexposure of integrin can be associated with tumor growth, specifically mammary tumors, so conjugated dots could be used to image mammary tumors in mice.

A quantum dot's florescence is due to Coulomb-correlated electron-hole pairs which get excited upon absorption of a photon light, in contrast to organic fluorophores [10]. The fluorescence of the dots allows for a non-invasive, in-vivo imaging system used in imaging organs, blood cells, vasculature, and glands of animals. Most of the research is currently done with mice, specifically nude albino mice, however imaging with non-nude mice will be described and discussed.

Since the size of the quantum dots correlates to the emitted wavelength, varying the diameter of the dots yields different emitted colors. Also, quantum dots can be excited by an unspecific wavelength of light. So different wavelength-emitting quantum dots can be excited by the same light source, enabling real-time multi-color imaging. For example, an animal could be injected with 705nm and 800nm quantum dots and excited by a single 405nm light source, and thus could provide two simultaneous color emissions. This has been discussed by Kobayashi et. al where five different colors were imaged simultaneously [6]. Multi-color imaging can be used to observe different biological functions at the same time; for example one could observe kidney and liver functions at once.

This study initially used 800nm *Qtracker* dots, manufactured by Invitrogen, which were injected into various mice to observe the effects. Qtracker dots are non-targeted quantum dots that have a polyethylene glycol (PEG) coating that minimizes nonspecific interactions. The dots exhibit good redshift and extreme fluorescence. Because the dots have the PEG coating, they are retained by the mouse and circulate for up to three months[10]. This can provide long term, real-time analysis of cellular functions. Since non-targeted Qtracker dots

flow throughout the blood stream and eventually accumulate in various organs, one can use them to study *vasculature* and overall anatomy of mice. Vasculature is the arrangement of blood vessels in the body or an organ, and how the arrangement correlates to the circulatory system. An application of quantum dots and an area of interest of this study is tumor progression and detection. Angiogenesis is the formation of new vasculature from preexisting blood vessels. Angiogenesis is a normal occurance but also is a fundamental component of tumor progression [1]. This study will try to adapt the existing equipment to detect tumor angiogenesis. This could provide a important tool for early cancer detection. An overexposure of integrin $\alpha_v\beta_3$ and Vascular Endothelial Growth Factor (VEGF) are two molecular indicators of abnormal angiogenesis [1]. Using a ligand that binds to integrin, one could potentially be able to image the abnormal vasculature.

1.1.1 The Physics Behind Quantum Dots

A photon of wavelength λ has the energy $E = \frac{hc}{\lambda}$. This energy can be transfered to an electron, to promote it to a higher energy band. When a photon enters a semiconductor atom, it can excite an electron from the valence band into the conduction band. The electron experiences a coulomb force from the negatively charged hole that it leaves behind. The electron will eventually return to the lower energy state and emit the energy in the form of another photon. A cooled CCD camera can be used to detect the photon emitted from the atom.

The *Bohr Radius of Exciton*, also known as the wavelength of exciton, in a semi-conductor is the distance between the electron-hole pair. Quantum dots are assembled in a crystal lattice; the lattice constant, denoted a_L, is the distance between unit cells in a lattice. In semi-conductors the Bohr radius of exciton can be much larger than a_L. When the dimensions of the quantum dots becomes smaller, it is possible to make the dimensions on the same order of magnitude, or even smaller, of the Bohr radius of exciton, but still larger than a_L [9]. Excitations in this structure are quantum confined, and when they are

confined in all three directions, it results in a zero-dimensional structure, or quantum Dot; the quantum confinement acts as a three dimensional potential well[9]. This stipulates that the bulk semi-conductor, i.e. the quantum dot, is forced to exhibit quantum phenomena. Thus the optical properties of quantum dots are governed by quantum confinement.

Because quantum dots are governed by quantum confinement, wavefunctions also extend throughout the semiconductor crystal lattice [8]. Since all the excitations and interactions occur within the dots, quantum dots share the same properties of bulk semiconductors and discrete molecules, i.e., discrete energy levels. Also there is large blueshift of the band gap and δ-function-like density of states [8].

1.1.2 Quantum Dots in Medical Imaging

The core of the quantum dot used for this work is Cadmium Telluride (CdTe) which emits far red to near-infrared. Red and near infrared wavelengths transmit through the skin best; when imaging tissues millimeters deep, it is desirable to avoid the major absorption peaks of blood and water [3], which are in the ultraviolet range. When the quantum dot absorbs a photon, the cadmium atom receives an electron from tellurium, thus creating a negative charge in the cadmium [4]. A high energy photon can cause the negative electron to transition to higher energy level and then to emit a specific wavelength.

Since quantum dots accept an unspecific wavelength of light and re emit a specific wavelength of light there is potential for a large Stokes shift, which characterizes the emission spectrum by a narrow emission bandwidth. This gives the potential for having multi-colored images excited by one single light source. Since quantum dots stay in circulation for an extended period of time (at least 7 weeks), it is possible to do real time monitoring of cellular processes.

1.2 Camera Used in This Study

The camera used is an SBIG-ST6, which is a cooled CCD camera. Since the camera in this work is based on a Silicon semi-conductor chip, it is important to keep the camera cool to reduce thermal noise and thus the dark count. A dark count occurs when a pixel gives a signal, not prompted by a photon, due to thermal noise. Because the images are taken in low light, the dark count will occur as pixel noise (pixel noise looks like speckles). The camera uses Peltier coolers to help regulate the temperature. A Peltier cooler makes a heat difference from electric voltage, modeled by $\dot{Q} = \Pi_{AB} I = (\pi_B - \pi_A) I$, where π is the Peltier Coeffiecent and I is the current. When a voltage is applied, heat is transfered against the temperature gradient. This will pull heat off the chip and transfer the heat to the exterior of the camera where the heat exchangers are located.

Chapter 2

Intial Results

2.1 Experimental Setup

The experimental setup was created by Kevin Smith and Anne Guzzi, William and Mary graduates who did research in the field. The current setup is comprised of a cooled CCD camera that was designed for astronomy but adapted for medical imaging, a light-tight wood cabinet, blue emitting LED's, and a laptop computer used to capture the images (figure 2.1). The current total cost of the setup is under $2000 [4].

An obstacle in imaging with this setup is exciting the dots with a certain wavelength of light while only detecting the re-emitted light. Guzzi solved this problem by employing a 800nm band pass filter, which covers the camera lens and only allows the 800 nm light through while excluding the short wavelength excitation light. The end goal of the project is to show that this low cost setup can be applied to biological research and can yield results comparable to higher cost commercial setups such as those by Xenogen and Kodak.

Currently the camera used is made by the Santa Barbara Instrument Group(SBIG) and is the model SBIG-ST6 which is equipped to image in gray scale. In the future, acquiring a color wheel for the setup would yield color, and eventually multi-color, images. The SBIG-ST6 can detect wavelengths ranging from 400 nm to 1000 nm, which covers the full spectrum of quantum dots made by Invitrogen.

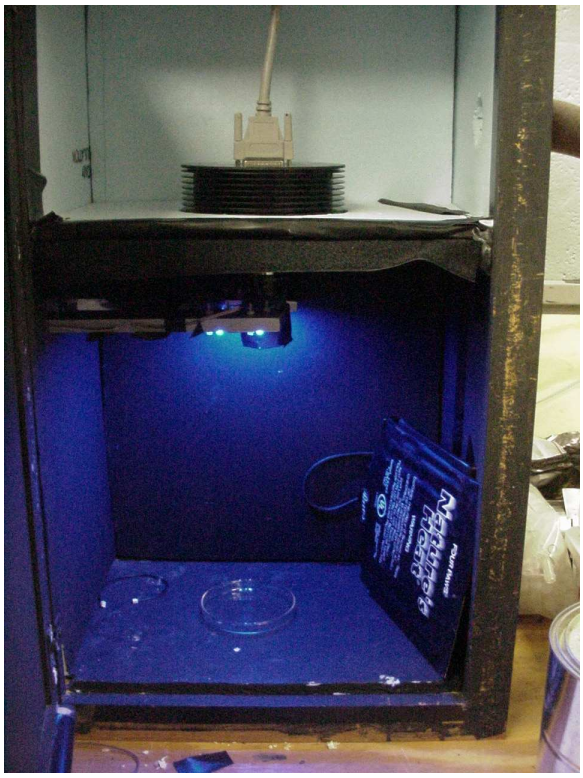

Figure 2.1: Inside of imaging cabinet, the camera is sitting in the top

2.2 Calibration of Equipment

A setup procedure involves focusing the camera on a calibration test sheet, which has different size fonts (figure 2.2). One of the difficulties is in opening the aperture of the camera as wide as possible(figure 2.1) while still being able to not only keep the camera in focus but also to regulate the exposure time so that the images are not over exposed. Adding an auxiliary lens brought the camera into closeup focus, yet still allowed for the aperture to be opened as wide as possible. Having the aperture all the way open is important because one needs to be able to detect the lowest level of quantum dots which is achieved by detecting the smallest amount of light.

For calibration trials Qtracker 800nm non-targeted quantum dots from Invitrogen were used. Also the 800nm bandpass filter was fitted to the camera. An interesting question that presented itself in this study is: how long do quantum dots hold their fluorescence if properly

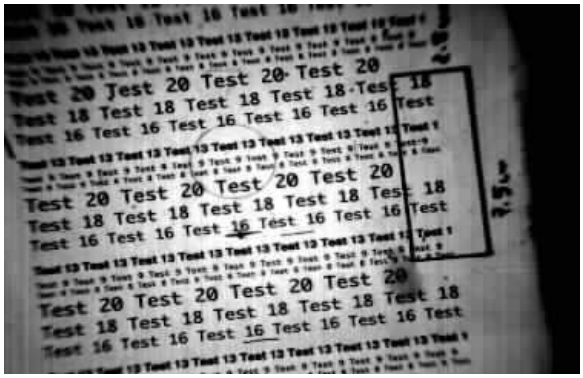

Figure 2.2: Test sheet for initial focus

maintained at $2 - 8\,\deg C$? Quantum dots from August of 2005 were used to compare their fluorescence against new quantum dots.

First the older dots were imaged. A T was drawn on a Petri dish using a quantity of $\leq 1/10\mu$L, and imaged using five different exposure times: $0.01s$, $0.1s$, $1s$, $5s$, and $10s$ (figure 2.3). The dots could not be seen at $0.01s$ and could faintly be seen at $0.1s$ exposure times. The T was clearly seen at $1.0s$ and $10.0s$ exposure times, but the $10.0s$ exposure yielded a very bright emission of the quantum dots. However the T started to blur into a spot. The $5.0s$ exposure time generated the best light detection with good resolution quality.

Then a *period* symbol of $\leq 1/10\mu$L, of new dots, was placed next to the T to compare fluorescence (figure 2.3). Exposure times of $.1s$, $1s$, $5s$, $10s$ were used. A $10\mu L$ capillary was used to measure the quantity of quantum dots, which is of limited accuracy. The T or the *period* could have a higher volume of quantum dots than the other, and thus would be brighter. However, to the accuracy needed, the quantum dots that were in a refrigerator for over two years are not only still fluorescent, but gave an image as bright as new dots. Thus it was determined that the calibration tests could make use of the old dots.

The quantum dots were then diluted to see the effect of a 10:1 solution and a 100:1 solution (figure 2.4). Regular tap water was used for the dilution and exposure times were $1s$, $10s$, and $100s$. The 10:1 dilution could barely seen at $1s$, and it became noticeable at a $10s$ exposure time. The $100:1$ dilution was not even noticeable at $100s$. An I shape was

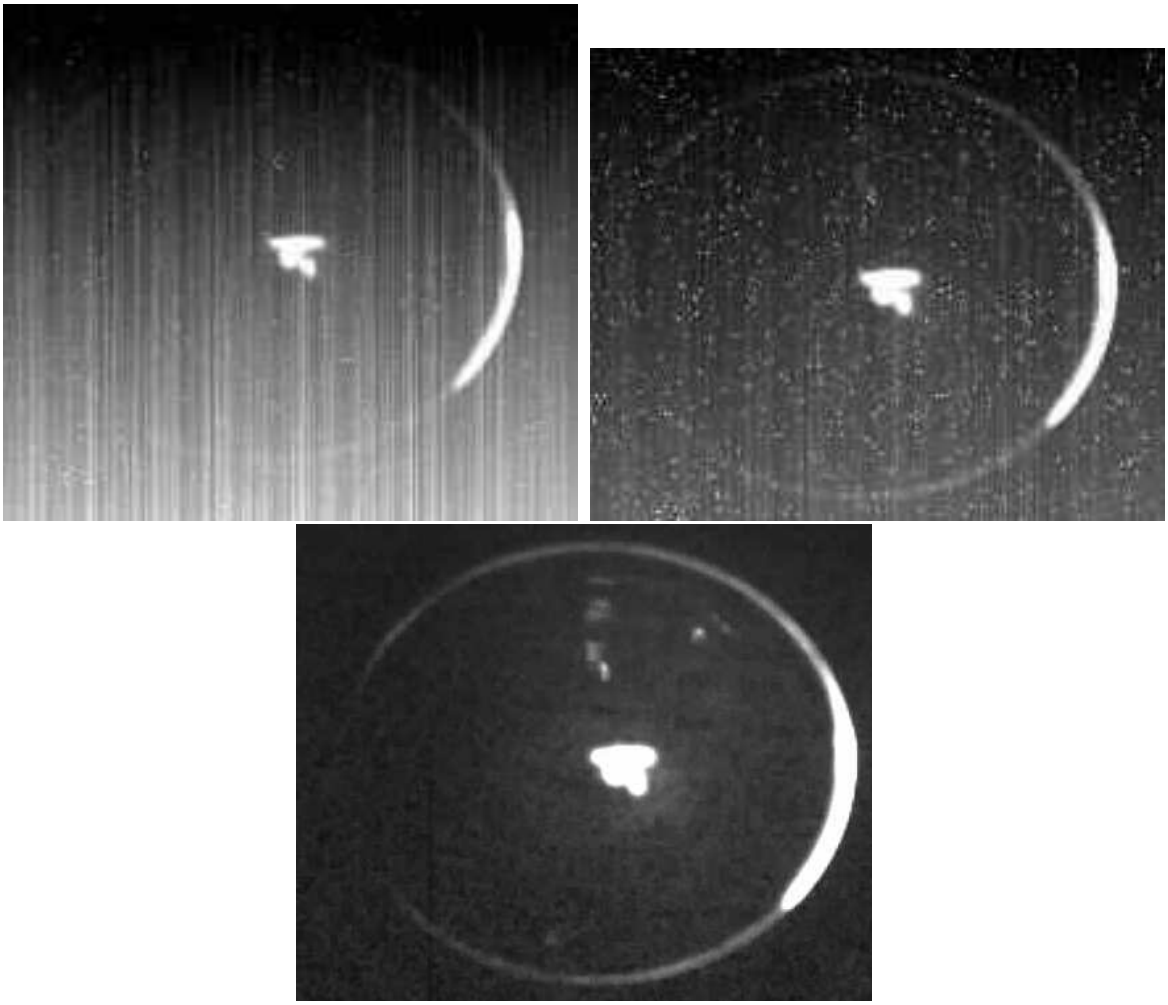

Figure 2.3: 'T'next to period at 1s (top left), 5s (top right), dark exposure at 1s (bottom middle)

used for the $10:1$ solution and an A shape for the $100:1$ solution. After a short time, the water used to dilute the quantum dots evaporated. Thus after a few moments of imaging the patterns, were equivalent to imaging a very small quantity of dots.

Remark: After imaging mice injected with quantum dots it was noticed that very bright regions of quantum dots masked less bright regions. Thus since the higher quantity of quantum dots in the $1:1$ and the $10:1$ marks were a lot brighter than the 100:1 mark, the brighter regions could have masked the emission by the 100:1 solution. To get a truly accurate representation of the fluorescence of the 100:1 solution, it would be preferable to image each different solution in a separate petri dish and then compare the luminescence.

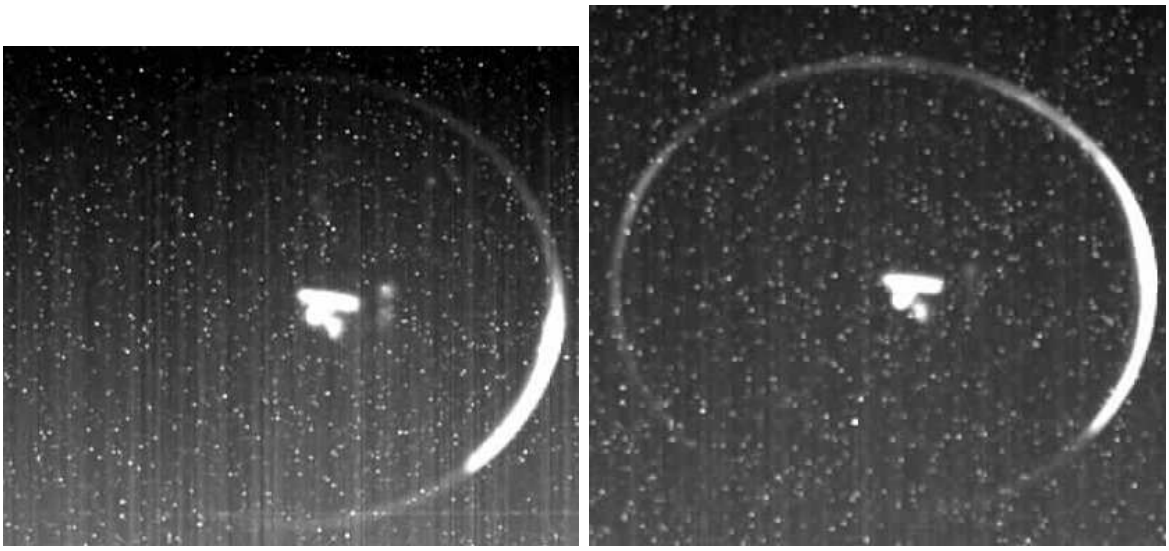

Figure 2.4: 10-1 solution at 5s exposure (left), 100-1 at 10-1 at 10s exposure (right)

Chapter 3

Tracking Quantum Dots on a Period of Seven Weeks

The goal of this series of images has been to track the circulation of $800nm$ Qtracker dots in an albino nude mouse for an extended period of time. This experiment has not been previously done in this lab. However, tracking accumulation of the Qtracker dots could be useful, to provide a control for extended imaging.

When analyzing site-specific imaging, a natural accumulation of dots in a certain organ or gland, could provide a false reading for the targeted imaging. For example, a goal for this study is to image cancer, specifically mammary tumors. In this case, the quantum dots would be conjugated with thiolated RGD peptide, which would specifically target an over expression of integrin $\alpha_v\beta_3$. Naturally the quantum dots accumulate in the upper lymphatic system which (Figure 3.5), if not taken into account, could be confused with a false positive for breast cancer.

Tracking Qtracker dots for an extended period of time is also useful as a purely academic pursuit. It is valuable to learn all of the properties of the dots, including how the dots act on a long term basis.

3.1 Initial injection: Quantum Dot Imaging of a Nude Albino Mouse

Initially a nude albino mouse of 17g was imaged. The mouse was anesthetized with $0.15mL$ of Sodium Pentobarbital and and after 45 minutes another $0.05mL$ of anesthesia was administered. After the mouse was anesthetized, it was injected, intravenously through the tail, with 40μL of quantum dots in solution with $60\mu L$ of $1.4M$ saline solution. However, it is estimated that only 50μL of total solution was injected because there was an embolism and the injection was stopped.

Seven pictures were taken at $5s$ exposure times after injection, which were separated by about ten minutes each. Immediately after injection, a picture was taken (Figure 4.6). After the fourth exposure the dark frame feature was added because it made the resolution noticeably better. A dark frame is a noise-supression picture taken with the shutter closed; that image is then subtracted from the exposure with the shutter open. This corrects for charge leakage on long exposure times. After about 20 minutes, spread of the quantum dots was noticed, with concentration in the ear lobe and the gut(Figure 3.2). However, still, a majority of quantum dots were in the tail. Since the concentration of quantum dots was so high in the tail, the brightness of the tail masked the quantum dots in the rest of the body. At minute 50, a black piece of felt was used to cover the tail and shield the photons emitted from that region (Figure 3.3); (the tail is of little biological interest in this research). This reduced the contrast problems caused by the very bright concentration of the quantum dots in the tail.

For the first 20 minutes, most of the quantum dots did not appear to flow freely through the mouse. One would expect immediately after the dots were injected that they would flow freely in the blood stream. However the dots stayed in the hind region(Figure 3.2). It is believed that the dots were absorbed in the musculature before circulating and that the embolism mentioned earlier inhibited flow, causing a blockage and slow dispersion. Also, given the size of the dots, it is possible that the dots were dispersed through the lymphatic

Figure 3.1: 17g mouse with with about 20 μL of quantum dots after the initial injection

system. Then the dots would be carried to the thoracic artery where they would be taken to the heart and distributed to the blood stream.

This experiment gives insight into the dispersion of quantum dots in small animal imaging after tail vein injection. It also shows the lymphatic concentrations and circulations. The mouse will be imaged again after 13 days and then again after 7 weeks. It is projected by the manufacturer that the quantum dots stay in circulation for three months. It is intended to exploit the accumulation of quantum dots on a scale of weeks and months, and observe the changes in accumulation and circulation.

3.2 Images Taken Thirteen Days After Injection

In this portion of the experiment, images were taken thirteen days after the initial injection. The exposure times were varied, because the emission intensity had decreased since the initial injection. Initially the pictures, were fuzzy and unclear so the contrast was varied to determine where the dots were accumulating. At first all of the exposure times were at $5s$,

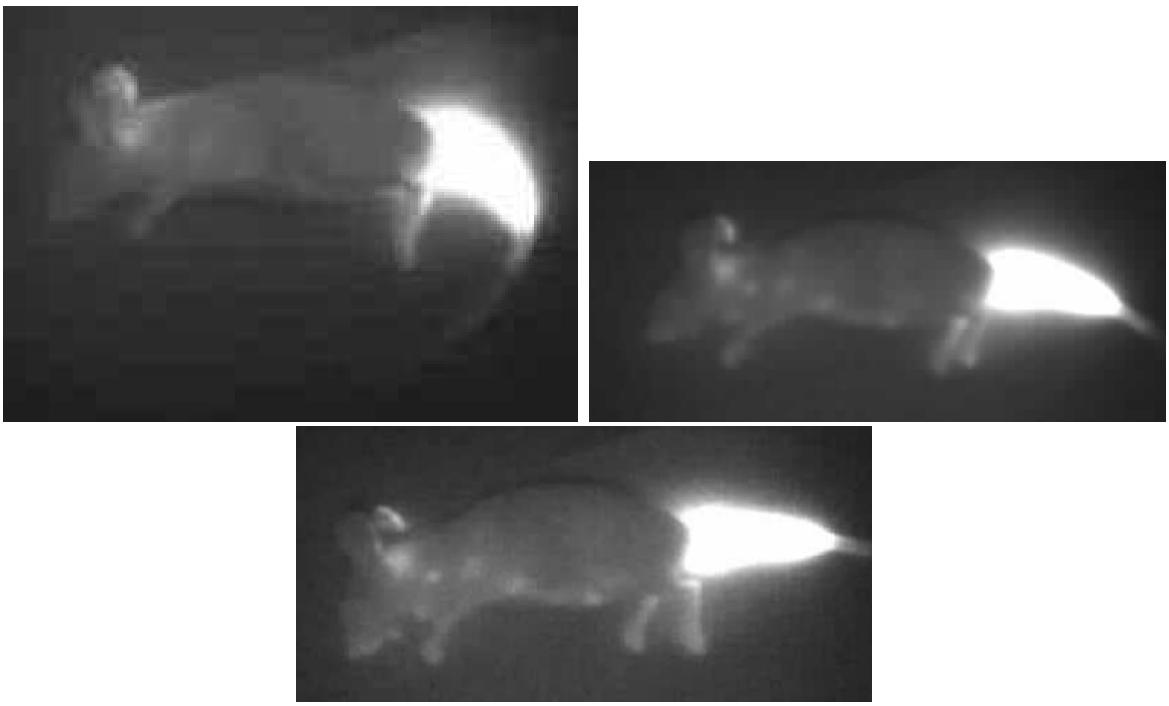

Figure 3.2: 20 minutes elapsed(Top Left), 30 minutes elapsed(Top Right), 60 minutes elapsed(Lower Center)

however, it was determined that longer exposure times improved the images. The camera appears to have an automatic gain adjustment feature that adjusted how much light the picture exposed. It was determined that the best exposure time was 150 seconds.

It should be noted that the mouse was anesthetized with $.15mL$ of Sodium Pentobarbital but a fair amount failed to enter the injection site so another $.05mL$ was administered. This was sufficient to keep the mouse sedated for about 45 minutes.

In Figure 3.4, the mouse was on its side and it was noticed that there was luminescence that occurred in the heart, lungs and bladder. Also there was a line running diagonally across its body. It appeared that the line could either be the liver or the spleen. Also, shown in Figure 3.4, the mouse was placed on it's other side. There was no luminescence where the spleen would be, so it was concluded that there was an accumulation in the liver. It was also noticed that the tail was still very bright with quantum dots.

In Figure 3.5, the ventral area has been imaged. In the upper left picture, the exposure time was $50s$ and the tail was covered with black felt. There is an excess of light that distorts

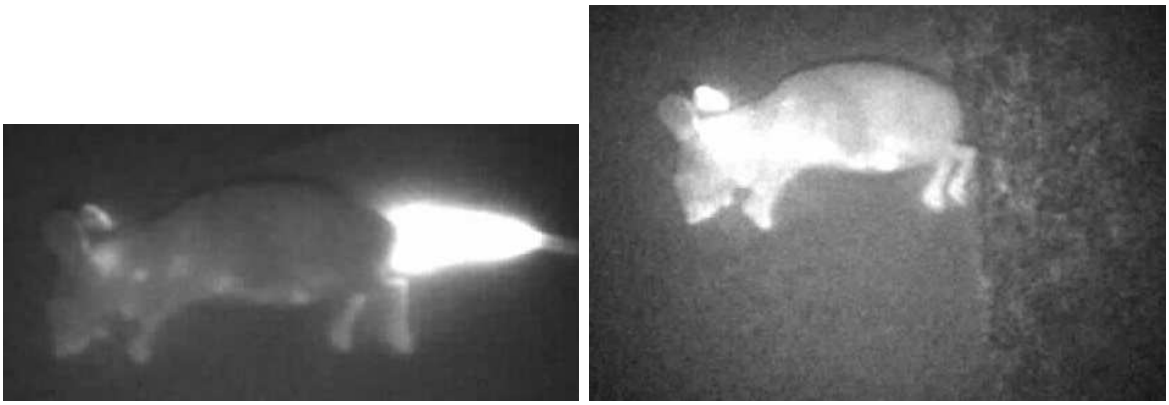

Figure 3.3: An hour after initial injection, the tail uncovered(left), tail covered(right)

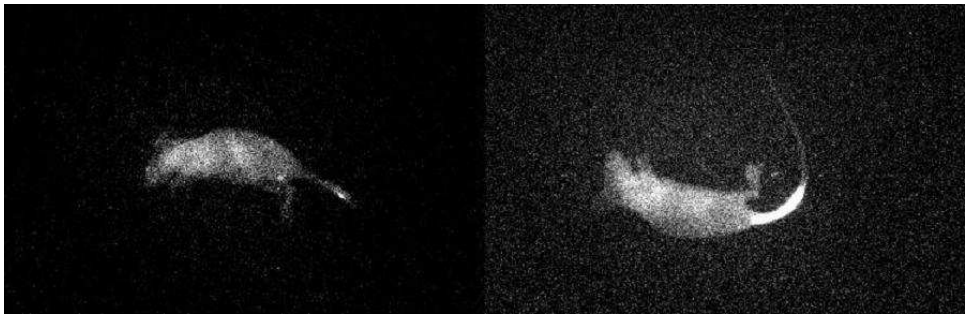

Figure 3.4: Left:On Right Side 5s exposure. Right:On Left Side 5s exposure.

the resolution and it is difficult to determine where the light is emitted from. In the upper right picture the contrast has been digitally reduced, which isolated the emission source of light. It is clear from this figure that there is major accumulation in the liver. There is also accumulation in the upper lymphatic system and under the arms. Also there is accumulation in the bladder.

The lower middle picture, of Figure 3.5, is imaged at a $150s$ exposure time. This has a far better resolution than the shorter exposure time. No digital modifications were done with the picture (post exposure). This exhibits the best detail and gives the contrast of the pictures previously taken. Also, the resolution is much better, and since the contrast is not reduced it results in a better outline of the mouse to give better points of reference. This enhances the detail for better anatomical analysis, which is the main goal.

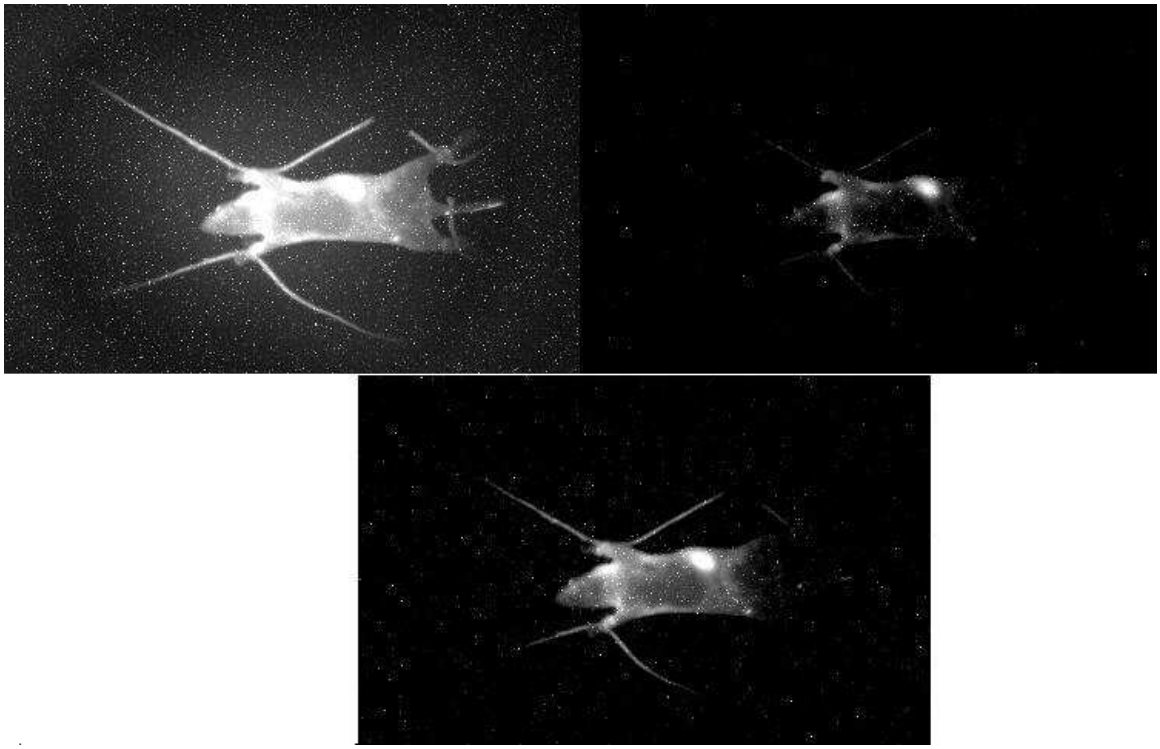

Figure 3.5: Top Left: Ventral area 50s exposure, Top right: Ventral area 50s exposure with contrast lowered, Bottem middle: Ventral are 150s exposure

3.3 Images Taken Seven Weeks After Injection

To sedate the mouse, it was given $0.17mL$ of Sodium Pentobarbital. However it is believed that not all was absorbed because the anesthetic only lasted for approximately 25 minutes which is far shorter than the other sessions, where the same amount was administered. In this trial fewer pictures were taken, partially because the mouse awakened.

In Figure 3.6, pictures were taken of the ventral portion of the mouse at fifty seconds exposure time. There is still a distinct accumulation of quantum dots in the upper lymphatic system. At this point it is unclear why the dots accumulated so much in the upper lymph nodes. There is a slight accumulation of dots in the spleen, and another undetermined portion of the gut. Clearly the dots have started to filter out, presumably through the urinary track. It was also unclear how long the quantum dots would have stayed in the upper lymphatic system. However, for accumulation purposes, we noted the absence of new

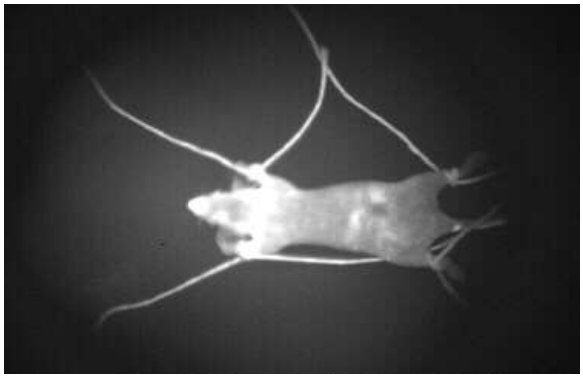

Figure 3.6: Ventral Picture after 7 Weeks at 50s exposure

accumulation sites, which was the purpose of this series of images.

The conclusions drawn from the long term tracking of quantum dots was that they accumulated in the liver, bladder, and upper lymphatic system. It is thought that the reason why they accumulate in these regions is because the dots are getting caught in the mouse's natural filtration systems. Also there was a rapid decline in emission intensity over the first two weeks, but the decline was far slower between two weeks and seven weeks after injection.

3.4 Comparison To Commercial Setups

In this section we compare the images that we took over seven weeks with those taken with a commercial setup over a four week period. Inoue et al. imaged a nude albino mouse with PEG-coated quantum dots that had a re-emission wavelength of $705nm$ [5]. To capture the images, Inoue used a IVIS Imaging System 100, made by Xenogen. In the analysis of the images (Figure 3.7), it was noted that there was uptake in the liver, spleen, skeleton, and lymph nodes. This is in agreement with the analysis of our imaging, where a large up take in the spleen, liver, and reticuloendothelial (lymphatic) system was noticed as well. However no uptake in the skeletal system could be seen. This is most likely due to the bright emission of the upper lymphatic system. One sees, however, some uptake in the bone marrow, because quantum dots can be seen in the upper area of the skull. Both systems exhibited long term

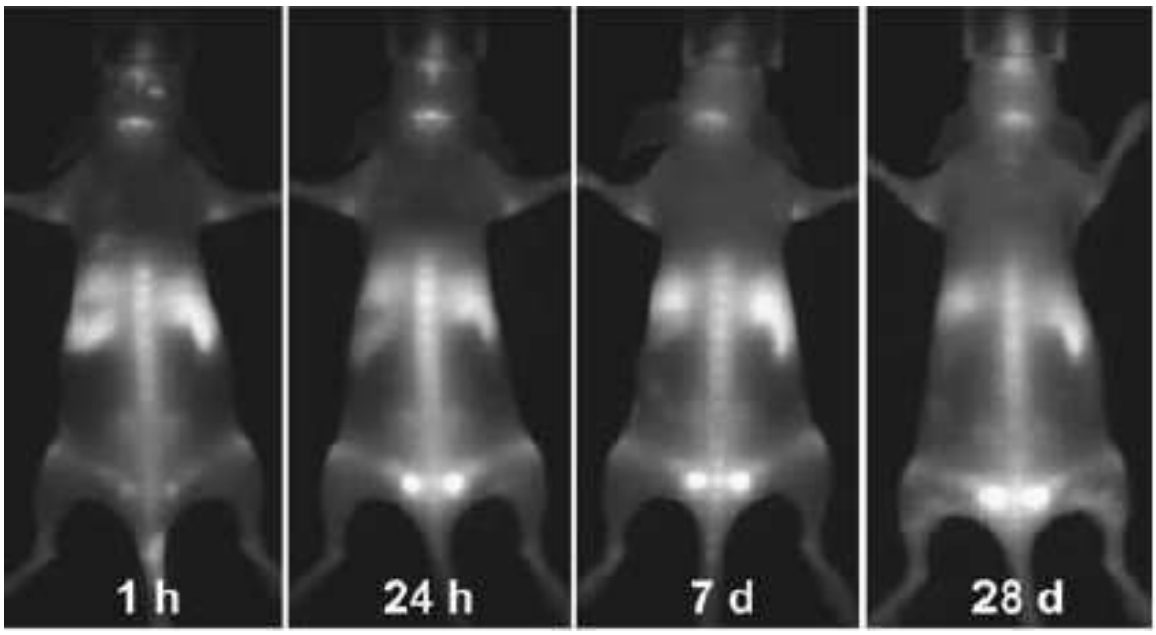

Figure 3.7: Pictures taken by Inoue et. Al

retention by the reticuloendothelial system.

The major difference between the images taken here and those of Inoue et. al, was the detail. The images in Figure 3.8 are a little blurry and and the lymph nodes run together somewhat. It was not possible to match the clarity of organs obtained by Inoue et al. This could interfere with quantitative analysis of pictures. Although there were differences, they were not significant enough to warrant concern. The images are very similar and show uptake in similar regions.

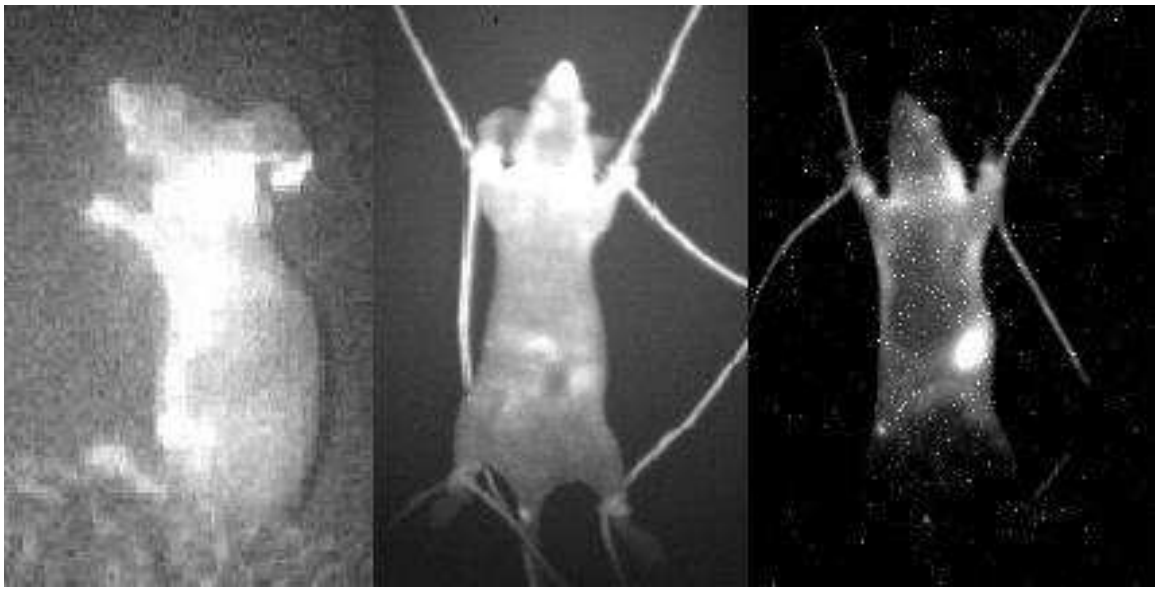

Figure 3.8: Our pictures: (Left) 1h, (Middle) 13d, (Right) 49d

Chapter 4

Imaging of MMTV Mice

4.1 Imaging Using Qtracker Dots

4.1.1 Motivation

The focus of this trial has been to compare images of a normal "hairy" mouse and that same mouse after begin shaved. Up to this point, in this lab, all experimentation done has been with nude albino mice. There are a number of motivations for trying to image a hairy mouse. The first is to fully study the properties of quantum dots including the dot's interactions with hair. Secondly, nude albino mice are expensive and generally not available with tumors. Thus, when imaging for a specific tumor, haired mice will be used. Skin does not significantly distort the quality of images, but the effect of hair has not fully been studied. For example, light intensity emitted through the hair could be significantly decreased. Thus we sought to provide a control for site-specific imaging for non-nude mice, which was done by comparison with shaven mice.

To study mice with breast cancer, RGD-peptide conjugated dots has been used. The mice used are carriers of Mouse Mammary Tumor Virus (MMTV). Thus an MMTV mouse was injected with $800nm$ Qtracker quantum dots, imaged with hair, then shaved and imaged again.

Figure 4.1: Hairy MMTV Mouse with Quantum Dots

4.1.2 Results

During this particular trial, a 27.2g mouse (Figure 4.1) was injected with $0.23mL$ of Sodium Pentobarbital. Then $0.40\mu L$ of Qtracker $800nm$ Dots, stock solution, was brought to $100\mu L$ with a saline solution. In this injection, a total of about $70\mu L$ of solution was injected intravenously into the tail vain. Several images were then taken. During the imaging a few things were noticed. A small amount of dots were seen through the hair but the quantum dots that were lodged in the tail dominated the images. Since the light coming from the dots in the tail was overwhelming the contrast of the camera, images were then taken with the tail covered to reduce the excess light from those dots. It was noted that the hair did block a large amount of light emitted from the quantum dots.

The ventral area of the mouse was then shaved and more images were taken (Figure 4.2) . In this trial, when the exposure time was set to $50s$ the picture went completely black. This is an anomaly since that was never observed it before using this setup. Thus the pictures were taken with an exposure time of $49s$ or less. In figure 4.3, the exposure time was set to $49s$, which gave the best resolution.

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There was a massive uptake in the abdomen, and also in the region of the heart. It also appears there were dots accumulating in some lower glands. There was excellent definition and clarity that is on par with images taken using a nude-albino mouse.

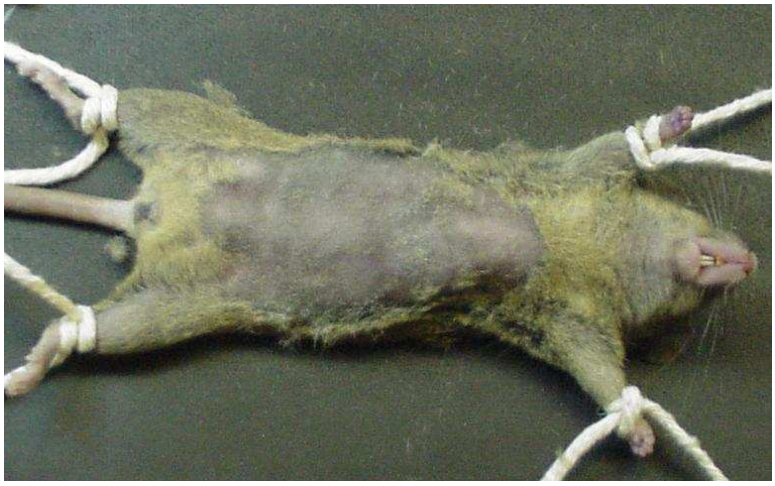

Figure 4.2: Shaved MMTV Mouse with Quantum Dots

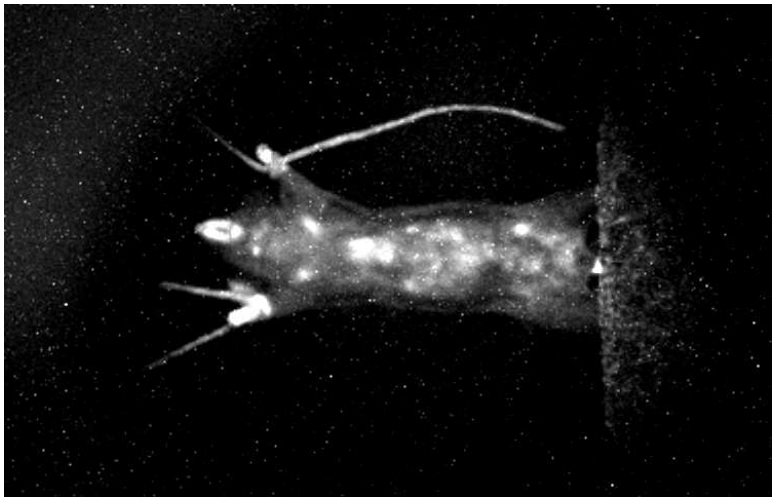

Figure 4.3: 49s exposure of shaved mouse with quantum dots

Also noted during imaging, is that there were two spots that correlated to locations where white hair had not been fully shaven off. The region that was thought to be the heart was a lighter pigment skin, so it was considered that lighter hair/pigment regions could be reflecting the emitted light. To study this another mouse was anesthetized, consistent in weight and of the MMTV strain. No quantum dots were administered but the mouse was shaved and imaged. We determined that there was no need to take pictures prior to shaving the animal, because the previous trial dictated that one must shave the mice in such experiments. Ideally when such pictures were taken, since the excitation light is $465nm$, no

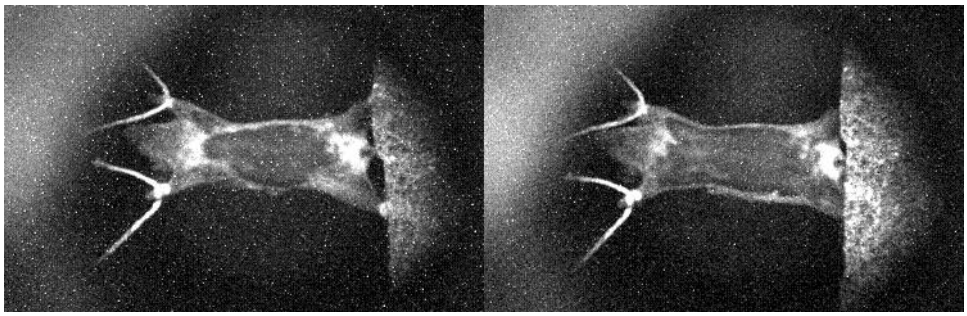

Figure 4.4: Left:First time shaven; Right: More Hair Shaved

image should have transmitted through the $800nm$ bandpass filter. However clear images of the mouse were seen (Figure 4.4), as the lighter regions of hair did reflect some light. The lighter pigmented regions, such as the chest, did not reflect light. This confirmed that, in figure 4.3, it was probably the heart or lungs in which the quantum dots were accumulating. The mouse was then shaven more fully and additional images were taken, to study this effect. It was noted that the amount of reflection was considerably reduced (figure **??**. .

In conclusion, we note that if the mouse is shaved completely, there should be very little reflection. Also, the light emitted from the dots will most likely dominate the picture just as the light from the tail overwhelms the images when it is not covered. Thus the small amount of light from any reflection should be masked. The most significant result from this trial is that a hairy mouse can be used for imaging as long as it is shaven. A few things remain as open questions. It is still not known why seeing the exposure time above 50s resulted in a picture that went completely black (in both shaven and unshaven). The second is to determine how to manipulate the exposure time to get the best clarity.

4.2 Imaging with RGD conjugated dots

As discussed in Section 1.1, ligands can be used to functionalize quantum dots. The purpose of conjugating biomolecules onto the quantum dots is to obtain site specific imaging. It could be of considerable value to image an overexposure of integrin $\alpha_v\beta_3$ which is a sign of tumor angiogenisis. Thiolated RGD-peptide binds to $\alpha_v\beta_3$, which would potentially enable

Figure 4.5: The MMTV mouse with multiple tumors

imaging of tumors. The thiol group is composed of a sulpher and hydrogen atom (-SH). The negatively charged thiol will electrostatically bind to the positively charged shell of the quantum dots which is why the RGD-peptide must be thiolated.

To conjugate biomolecules to a dot's surface one uses an exchange reaction, where quantum dots are mixed with thiolated biomolecules and incubated until an equilibrium is reached with the biomolecules on the dot's surface[3]. There are, however, complications with this process. Relative to the Qdot's size, the peptides which are to be conjugated are small. It has been described as "trying to glue a piece of fuzz onto a bowling ball".

4.2.1 MMTV Mouse With RGD-peptide Conjugated Dots

In this trial a MMTV carrying mouse, with multiple tumors, was imaged. Figure 4.5 depicts the mouse shaved, on the side where the tumors are most prevalent. It was anesthetized with $0.23mL$ of Sodium Pentobarbital, and later was given $.07\mu L$ more to keep it sedated.

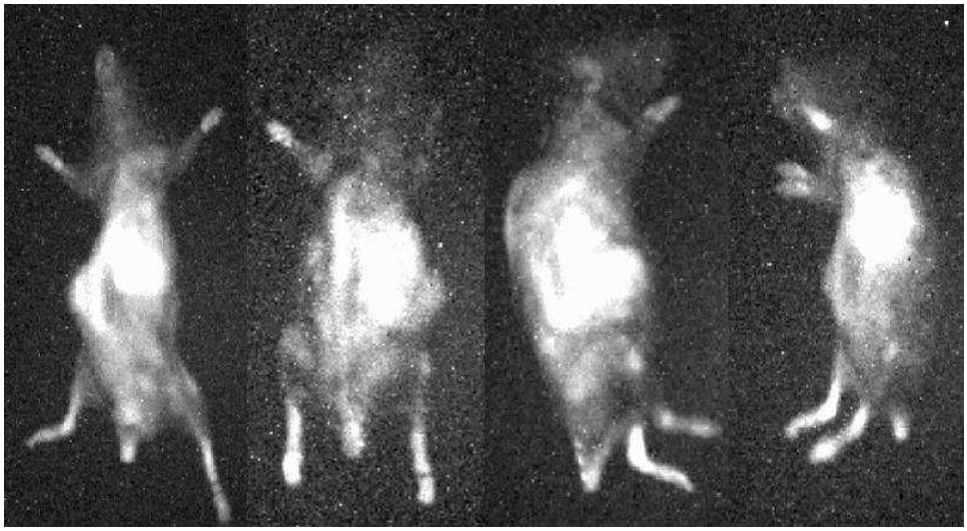

Figure 4.6: Left to Right: Ventral, Dorsal, Lateral Tumor Side, Lateral No Tumor. $50s$ exposure

Multiple images were taken prior to shaving the mouse and then also after shaving the mouse before any quantum dots were administered. This was to provide a subtraction image, so any that reflection off the skin could be taken into account when imaging with the quantum dots.

Then $100\mu L$, of $800nm$ quantum dots were administered to the mouse. Invitrogen preformed a custom synthesis, in which they conjugated cyclo (Arg-Gly-Asp-Tyr-Lys), c(RGDyK) peptide to the quantum dots. The peptide was also thiolated by Invitrogen. It was supplied in a 1:20 dilution with a PBS, pH 7.2, buffer. The conjugate was not further diluted before injection.

The mouse's ventral, dorsal, and both lateral areas were imaged at an exposure time of $50s$, see Figure 4.6. Almost immediately there was uptake in the various areas of the tumor. However, there were other areas such as the heart, feet, and liver that showed uptake. This is not surprising since the heart had to distribute the quantum dots to the areas of interest and also it is expected that the liver would show quantum dots. It is not immediately clear why the feet were bright.

It was intended that this mouse would be imaged further at 6 hours post injection, however, at hour 2 the mouse died. It is thought that the mouse went into shock due to the

Figure 4.7: Left to Right: Spleen, Heart, Tumor, Mammary Glands (Not Pictured:Liver).

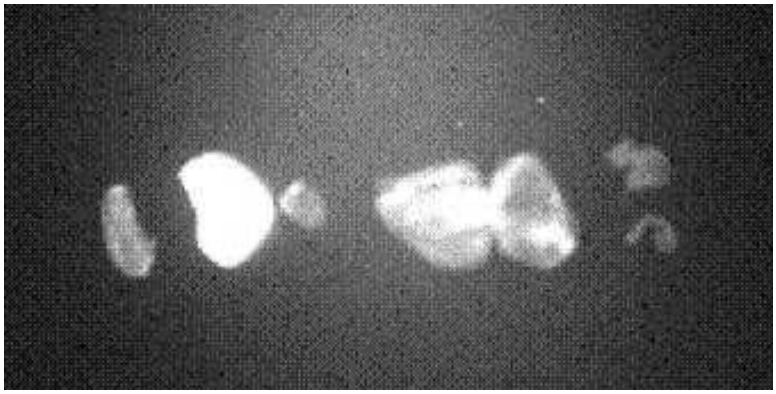

Figure 4.8: Left to Right: Spleen, Liver, Heart, Tumor, Mammary Glands.

amount of anesthesia that was administered ($0.3\mu L$) total. The mouse was dissected and the tumor, spleen, heart, liver, and two mammary glands were retrieved, see Figure 4.7. There was no tumor growth in the mammary glands however there was a large tumor on it's back, The glands and then the tumor were imaged ex-vivo.

There was some uptake in the spleen, heart, and mammaries, however the largest uptake was in the liver, Figure 4.8. This was expected from the previous trials using the Qtracker dots. The tumor also showed large uptake, Figure 4.9. The tumor will be imaged under a microscope to gather information about uptake at a cellular level.

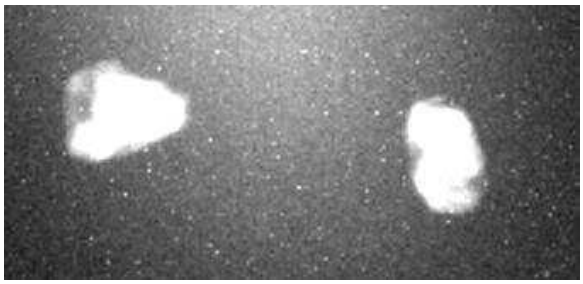

Figure 4.9: Tumor

4.2.2 Comparison to Commercial Setups

Cai et al. outlined a process for conjugation and imaging with RGD-peptide [2]. They imaged a nude-albino mouse with a human glioblastoma tumors. To capture images they used a Maestro in vivo imaging system. In Figure 4.10, one can see uptake in the tumor,

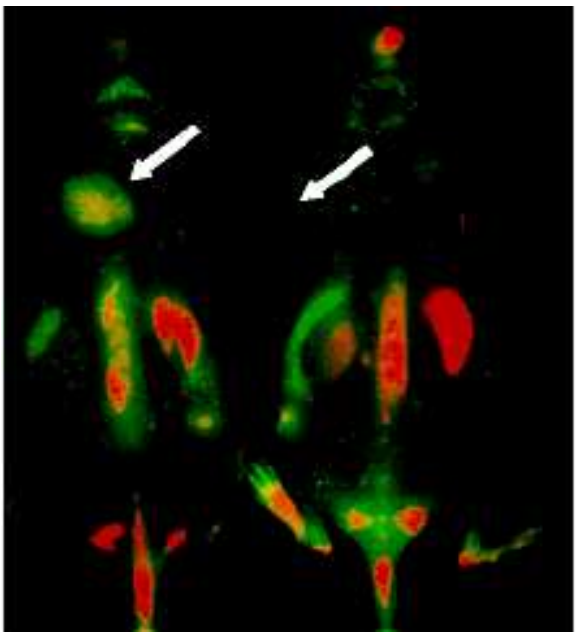

Figure 4.10: Image of Tumored Mouse by Cai [2]

above the left shoulder, the liver, and the feet, which is consistent with our imaging. The multi-color photographs are products of digital modification, which is enabled by the imaging system. The images taken here showed poorer resolution, however these were comparable for purposes of in-vivo imaging. We conclude that the imaging of the tumor, in-vivo, provided

similar results.

Chapter 5

Conclusion

5.1 Results of Experiments

The goal of this study has been to carry out a stringent test of the setup previously assembled by Anne Guzzi and Kevin Smith. The camera was adjusted for the best resolution and imaged three dilutions of quantum dots: undiluted, 10 : 1, 100 : 1. A mouse has been imaged with $800nm$ non-targeted quantum dots. It was determined that a shaved hairy mouse produces results comparable to a nude-albino mouse. This allows for hairy mice to be used when imaging tumors, instead of being constrained to imaging only nude-albino mice.

We were also able to successfully image a MMTV mouse with RGD-conjugated dots. The results were similar to the results of Cai et al. In combination with the images obtained from the Qtracker dots, and the RGD-conjugated dots, we conclude that our images are comparable to those obtained by commercial setups for our imaging purposes.

5.2 Further Research in Quantum Dots

The next phase of the research will be to obtain a successful color image of a mouse, using the SBIG-ST6 camera. This could lead to multi-color imaging of mice, using a low cost setup. Once color imagining is achieved, a multi-color image should be possible. It would also be valuable to be able to conjugate ligands, in house, to quantum dots so to make different dots bind to different locations.

An interest is to use quantum dots to further investigate angiogenesis. An over expression in VEGF has been shown in cases of Breast Cancer and Rheumatoid Arthritis. Thus, a ligand that binds to an over expression of VEGF can be an early detector of these two diseases. Integrin $\alpha_v\beta_3$ can regulate the progression of VEGF in tumor cells. Integrins are cell adhesion molecules which are expressed on endothelial cells and survive angiogenesis [1]. The $\alpha_v\beta_3$ integrin binds to arginine-glycine-aspartic-acid (RGD) [1] which can be manipulated for our imaging purposes. Unfortunately, due to the large size of quantum dots, one can only image the vasculature, but not the cells themselves.

Another experiment of interest would be to better understand the conjugation process. Currently this lab is not set up to do a two step reaction to thiolate and then conjugate the material. Chen outlines a procedure for conjugation [1], which would lower the cost of such experiments. Nonetheless Chen et. al used an RGD-peptide that binds to integrin to image tumors in mice [1]. A future goal would be to conjugate quantum dots to RGD peptide and thus use that to seek out integrin so as to provide early detection of these ailments.

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