



# Fluorescent Imaging for Nano-Detection (FIND) of Cancer Cells

Kelly Zhang<sup>1\*</sup>, Jack Coakley<sup>2</sup>, Poonam Saraaf<sup>3</sup>, Bhaskara R. Jasti<sup>3</sup>, and Xiaoling Li<sup>3</sup>

**Student<sup>1</sup>, Teacher<sup>2</sup>:** The College Preparatory School, 6100 Broadway, Oakland, CA 94618

**Mentor<sup>3</sup>:** University of the Pacific, 3601 Pacific Avenue, Stockton, California 95211

\*Corresponding author: [kellyxzhang@gmail.com](mailto:kellyxzhang@gmail.com)

## Abstract

The purpose of this research is to introduce a biodegradable nanoparticle for imaging solid tumors to aid cancer surgery. Currently, surgeons operate blindly on tumor patients: many tumor cells escape surgery and spread. In this project, a nanoparticle system is explored for future use as an imaging agent for cancer surgery. A protocol for developing fluorescent nanoparticles composed of bovine serum albumin and fluorescein-isothiocyanate (FITC) is developed and optimized to reliably make nanoparticles under 200 nanometers. These nanoparticles glow green under a fluorescent microscope and their small size increases the chance of reaching the tumor in-vivo. In order to study the uptake of the nanoparticles by different types of cancer cells and demonstrate the general utility of this imaging method, the nanoparticles were incubated with a total of eight cancer cell lines and two non-cancer cell lines. The study reveals that many cancer cell lines have positive uptake of these particles, while non-cancer cell lines demonstrate significantly less uptake of the particles. A study of uptake competition showed that free albumin can compete for FITC-albumin nanoparticle in labeling tumor cells, indicating the possibility that the uptake is through albumin receptors. Differences in uptake levels at higher and lower temperatures also suggest that the uptake of albumin nanoparticles is by active transport. Lastly, a co-precipitation of receptor proteins suggests a difference in albumin receptor levels between cancer and non-cancer cells. Some protein bands were present in cancer cell lines, but not in non-cancer cell lines, which supports the hypothesis that these nanoparticles are tumor preferential. A fluorescent imaging system using such nanoparticles will allow significant improvement in cancer surgery by ensuring that all the cancer cells are removed, preventing remaining cells from spreading to other organs.

## Introduction

Currently, most cancer surgeons operate “blindly”, unable to determine whether they have completely removed all the patient’s tumor tissues or not. Unfortunately, most cancer patients die from the disease months or years after the initial surgery in which the primary tumor is removed. The relapse of cancer growth is often a result of an incomplete removal of residual cancer cells. This is because cancer cells and normal cells can’t be distinguished by naked eye without any aid of tumor imaging techniques<sup>1,2</sup>. It would be a revolutionary medical advancement if surgeons can distinguish cancer cells from normal cells during the operations. A dye system that specifically stains tumor cells would greatly aid the surgeons in completely removing all cancer cells. However,

to this date, such technology has not been available in the clinic.

This study explores a biodegradable agent that can specifically “paint” cancer cells with fluorescent color, and potentially aid surgeons in tumor removal operations. The tumor imaging agent has to have several properties: appropriate size, binding to tumor cells, preferentially staining tumor cells, and biodegradability.

The size of the particle is extremely important. It is shown that blood vessels in tumor tissues (tumor vasculature) are “leaky”, possessing pores that allow particles of several hundred nanometers to leak out into tumor tissues, a phenomenon known as the enhanced permeability and retention effect<sup>3,4</sup>. Reducing the size of a particle to a 100-200 nanometers enhances the chance that it will escape from the vascular system and accumulate in tumor tissues<sup>5,6</sup>. Particles that are smaller than 200 nanometers cannot be detected or phagocytized by macrophages, which reduces the chance of immune responses<sup>7</sup>. However, particles that are smaller than 100 nanometers tend to leak out nonspecifically and accumulate in the liver rather than the tumor<sup>8</sup>. Recent nanotechnology research shows that certain polymers can be used to make particles in the size of nanometer range, called nanoparticles. In this study, the first objective is to make nanoparticles that are stable and measures in nanometer range.

In addition to making particles of nanometer in size, the materials of the nanoparticle need to have some binding properties to the tumor cells. Tumors gain nutrients to sustain their rapid growth. Some nutrients attach to albumin, the most abundant serum proteins, to be transported across the endothelial membrane and then get engulfed by tumor cells<sup>9</sup>. Further, studies show that tumors cells are likely to express albumin binding receptor molecules on their surface<sup>10</sup>. This makes albumin a very appropriate protein to use for imaging if a fluorescent marker can be incorporated into the albumin nanoparticle which binds to tumor cells preferentially. In addition, albumin is a natural protein that can be degraded in patient’s body after injected, unlike alternative approaches such as using metal or synthetic polymers to make nanoparticles which are not biodegradable<sup>11</sup>. Therefore, albumin was selected as a biodegradable, tumor targeting material to make nanoparticles that carry fluorescent dyes.

Fluorescent imaging is not harmful to the patients and surgeons, because its energy is low, unlike high energy imaging technologies such as X-ray and nuclear medicines<sup>12</sup>. Therefore, fluorescent imaging will be a good choice to be used in operation rooms. Fluorescein isothiocyanate (FITC) is a derivative of fluorescein used in wide variety of applications including labeling of proteins<sup>13</sup>. Its attachment to proteins makes it a suitable for integration into nanoparticles<sup>14</sup>. FITC has excitation and emission spectrum peak wavelengths of approximately 495 nm/521 nm, so under a fluorescent microscope, it glows bright green. Therefore,



FITC was chosen in this study because it is a widely used green fluorescent dye, and that its bright green color stands out from the reddish tissues during surgical operations.

It is important to know whether the cells uptake these albumin nanoparticles by receptor-mediated endocytosis or by non-receptor mediated endocytosis. In order to explore the mechanisms, the uptake of the fluorescent nanoparticles will be studied by incubating the fluorescent nanoparticles with tumor cell lines with or without competing albumin molecules. If non-fluorescent albumin molecules can compete with fluorescent nano-particles, it is likely that the uptake of nanoparticles are mediated by albumin receptors. To further investigate the nature of albumin uptake, the uptake of nanoparticles by tumor cell lines incubated at different temperatures will be compared. Temperature dependence of albumin intake may indicate an active transport mechanism that requires energy.

Finally, to investigate the existence of an albumin-binding protein (receptor) in tumor cells, a co-precipitation experiment will be conducted. Cancer and non-cancerous cell lines will be lysed, incubated with fluorescent albumin nanoparticles, precipitated by centrifugation, and washed to obtain the proteins that attach to the nanoparticles. The albumin receptor protein content of each cell line can be visualized through running a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The long term goal of this project is to further develop the fluorescent nanoparticle reagents into animal studies, and eventually into clinical studies. The final goal is to develop a tumor specific “fluorescent imaging for nano-detection (FIND)” system that surgeons can use to identify residual tumor cells during operations and be able to completely remove tumor tissues from a cancer patient.

## Materials and Methods

*Preparation of BSA Nanoparticles:* A desolvation technique was used to make bovine serum albumin (BSA) (Sigma Aldrich) nanoparticles. The method is based on a published protocol of making nanoparticles without fluorescent dyes. Several modifications were made after several trials to incorporate fluorescein isothiocyanate (FITC) (Sigma Aldrich). Briefly, 10 mg BSA was dissolved in 1.0 ml of purified water. In a separate beaker, 0.4 mg of FITC was dissolved in 8 ml of ethanol (Sigma Aldrich). The goal is to force BSA out of the water phase and form small aggregates (nanoparticles) that contain FITC. Under constant stirring, FITC in ethanol solution was drop-wise added to the 1% BSA solution. Subsequently, 25  $\mu$ l of 0.25% glutaraldehyde solution (Sigma Aldrich) were added. The purpose of the glutaraldehyde is to cross-link BSA molecules to each other such that the nanoparticles are stable. The aldehyde groups of this molecule will react with amine groups of BSA and “grab” BSA molecules into a more rigid matrix. After adding glutaraldehyde, the beaker was left stirring for about 4-5 hours, until the ethanol had evaporated (Zhang et al., 2004)<sup>6</sup>. The BSA nanoparticles were then collected to small Eppendorf tubes. The BSA nanoparticles were collected by 3 cycles of centrifugation at 15,000 rpm for 1 minute per cycle. The particles were suspended in purified water, centrifuged, and the supernatant was removed. *Determination of Size and Size Distribution of Nanoparticles:* The size of the nanoparticles was measured using an instrument called the Zeta Sizer that measures the size of particles in a fluid (such as a suspension) by observing the scattering of laser light from the particles and determining the diffusion speed. 100  $\mu$ l of each batch of BSA nanoparticles were suspended in 3 ml water in a cuvette and placed in the Zeta Sizer under the Standard Operating Procedure. Size measurements were performed from light scattered at 90 degree angle with the help of an auto-correlator. *Study of Cancer Cell Line Uptake:* In order to examine the uptake of nanoparticles by cells, HeLa cells (American Type Culture Collection) were seeded into a Lab-Tek 4-well chamber, with approximately 20,000 cells in each well. The number of cells was determined by a Z Series Coulter Counter that uses an electrical sensing zone method for counting cells. After incubating the cells at 37 degrees Celsius with Advanced Dulbecco’s Modified Eagle Medium (DMEM) (Invitrogen Corporation) overnight, the old medium was removed and the cells were washed with Hank’s Balanced Salt Solution (HBSS) (Invitrogen Corporation) twice. Then, 1 ml of 0.75mg/ml of fluorescent albumin nanoparticles (average size 180 nm) in serum-free DMEM was added to two of the wells. The other two wells containing HeLa cells were kept as negative controls, so the health of the cells with fluorescent nanoparticles could be compared to the health of the non-treated cells. The cells were subsequently incubated with the nanoparticles for 3 hours. Then, the cells were washed with HBSS two more times, and 1 ml of HBSS was added to each well to keep the cells hydrated during the imaging time. The cells were observed and pictures were taken by a Nikon TE 2000 Eclipse microscope. The variety of cancer cell lines was expanded to include Hep-G2 (human hepatocellular carcinoma, liver cancer), EA.hy 926 (a fusion of human lung carcinoma and human umbilical vein endothelial cells), MG-63 (human osteosarcoma, bone cancer), AGS (gastric adenocarcinoma, stomach cancer), A431 (human epithelial carcinoma), MDA-MB-231 (human gland carcinoma, breast cancer), and MCF-7 (human mammary carcinoma, breast cancer) (American Type Culture Collection). Each cell line was seeded into two wells of Lab-Tek 4-well chambers, with 20,000 cells in one well and 40,000 cells in the other. The cells were incubated and FITC-albumin nanoparticles with average size 190 nm were added with the same procedure as in the first study with HeLa cells (see above). Again, the cells were washed twice after a 3 hour incubation period with the nanoparticles. The cells were observed and pictures were taken by a Nikon TE 2000 Eclipse microscope. In order to determine the specificity of the fluorescent nanoparticles to cancer cells, the nanoparticles were incubated with the A431 cancer cell line, and two non-cancerous cell lines, Detroit-551 and HEK293. Each cell line was seeded into two wells of Lab-Tek 4-well chambers, with 20,000 cells in each well. The cells were incubated and FITC-albumin nanoparticles were added with the same procedure as in the first study with HeLa cells (see above). Again, the cells were washed twice after a 3 hour incubation period with the nanoparticles. The cells were observed and pictures were taken by a Nikon TE 2000 Eclipse microscope. In order to quantify the amount of nanoparticle uptake, ten random visual fields were chosen for each well under the microscope, and the number of fluorescent green cells was counted. Each frame was approximately 60% confluent, in order to keep the results consistent. The intensity of the green color under the microscope was observed and recorded. To determine the mechanism of nanoparticle uptake by the cells, a competition study



was conducted, using different concentrations of BSA in the media to determine the mechanism of uptake by the cancer cells. 20,000 HeLa cells were seeded into each well of three Lab-tek 4-well chambers. The cells were incubated overnight and washed twice before adding the nanoparticles (average size 185 nm). For every 6 wells, there was one negative control with HeLa cells in serum-free DMEM, a positive control with HeLa cells in 1 ml of 0.75mg/ml of fluorescent albumin nanoparticles in serum-free DMEM, and four wells with varying amounts of BSA dissolved in the same serum-free DMEM media containing 0.75mg/ml of fluorescent albumin nanoparticles. The four concentrations of BSA in DMEM used were: 0.016 mg/ml, 0.8 mg/ml, 4 mg/ml, and 20 mg/ml. The experiment was in duplicated format, creating a total of 12 wells. The objective of the varying amounts of BSA was to observe whether the excess BSA in the media would compete with the BSA nanoparticles in binding to albumin receptors on cancer cells. The cells were incubated for 3 hours and then washed twice with HBSS. The cells were observed and pictures were taken by a Nikon TE 2000 Eclipse microscope. To quantify the results of the competition assays, five random visual fields were chosen for each well under the microscope, and the number of fluorescent green cells was counted. Each frame was approximately 60% confluent, in order to keep the results consistent. Because of the duplicated experiment, there were a total of ten counts for each concentration of BSA, and the results were averaged and graphed. Lastly, the nanoparticle uptake by cells under different temperatures was investigated. HeLa, A431, and Detroit 511 seeded into wells of Lab-Tek 4-well chambers, with 20,000 cells in each well. FITC-albumin nanoparticles were added with the same procedure as in the first study with HeLa cells (see above). This time, one well of each cell line was incubated at 37°C, while the other well of each cell line was incubated at 4°C. After 3 hours, the cells were washed twice with HBSS. The cells were observed and pictures were taken by a Nikon TE 2000 Eclipse microscope. The quantification procedure used to count the number of green cells was the same as the third study (see above).

**Co-precipitation of Cell Receptor Proteins:** In order to study the receptor protein levels of cancer and normal cells, a co-precipitation procedure was performed and the proteins were visualized on a protein gel<sup>16,17</sup>. A431, HeLa, and Detroit 511 cells were cultured collected into Eppendorf tubes. In order to lyse the cells, the tubes were centrifuged at 20,000 rpm for 1 minute in a cold room. The cells were re-suspended in 50 uL of freeze-thaw buffer (Invitrogen Corporation). The samples were frozen at -80°C and thawed by briefly shaking the samples in 37°C water. This freeze-thaw cycle was repeated two more times. 250 uL of Benzonase (Invitrogen Corporation) was added to digest cellular DNA. Next, 1 mL of a solution with nanoparticles of an average size of 210 nanometers was added to the cells. The goal was to use the nanoparticles to precipitate all albumin receptor proteins that would bind to the nanoparticles. The samples with nanoparticles were rocked slowly overnight in a cold room. The next morning, the samples were spun down and washed three times with 1xPBS Buffer (Invitrogen Corporation). The wash is important because the salt in PBS strips away non-albumin-specific proteins. In order to run the proteins through the gel, they needed to be denatured so the samples were boiled for 5-10 minutes. At this point, the samples are ready to run through an SDS-PAGE. To prepare the 12% acrylamide running gel, 10.2 ml of dH<sub>2</sub>O, 7.5 ml of 1.5 M Tris-HCl Buffer (Invitrogen Corporation), 0.15 ml of 20% sodium dodecyl sulfate (SDS) (Invitrogen Corporation), 12.0 ml of 30%/0.8% acrylamide/bis-acrylamide (Invitrogen Corporation), 0.15% ml of ammonium persulfate (Invitrogen Corporation), and 0.02 ml of TEMED (Invitrogen Corporation) were mixed and poured into a rectangular gel mold. Once the gel solidified, it was assembled into the SDS-PAGE apparatus. 90uL of A431, 160uL of HeLa, and 50uL D551 were measured out into droplets on wax paper. 20 uL of Coomassie Brilliant Blue dye (Invitrogen Corporation) was added to each sample, and the samples were loaded into the wells. 5 uL of BenchMark Protein Ladder (Invitrogen Corporation) was loaded into the left-most well. Then, the power was switched on and the gel ran for 45 minutes at 200 V. The gel was removed from the SDS-PAGE apparatus and placed in a rectangular container. Coomassie Blue staining solution (Invitrogen Corporation) was poured into the container and the gel is rocked gently for 2-3 hours to distribute the dye evenly over the gel. At end of the staining, the gel was washed 3 times with water, for 5 minutes per wash. The protein gel was then scanned onto a computer for further analysis.

## Results

In order to optimize the desolvation protocol to achieve nanoparticles of smaller size, the optimal volume of ethanol was determined by varying the ethanol volumes keeping other conditions constant. A Zeta-sizer was used to determine the size of the particles. The Zeta-sizer produced a size distribution chart, as shown in Figure 1, and the average sizes of the particles were calculated by the instrument.

A sample from each batch of nanoparticles made in each trial was measured. Figure 2 shows the size of each of the samples from each trial for the different amounts of ethanol used in the procedure: 4 ml, 6 ml, 8 ml, 10 ml, and 12 ml. As shown, the smallest average size was 166.2 nanometers, when 8 ml of ethanol was used.

To further optimize the procedure, the concentration of bovine serum albumin was varied, using 0.25%, 0.5%, 1%, 2%, and 3% of BSA, while keeping all other variables constant. Nanoparticles were made with each concentration two times. Figure 3 shows the size of each of the samples from each trial for the different concentrations of bovine serum albumin in water: 0.25%, .5%, 1%, 2%, and 3%. As

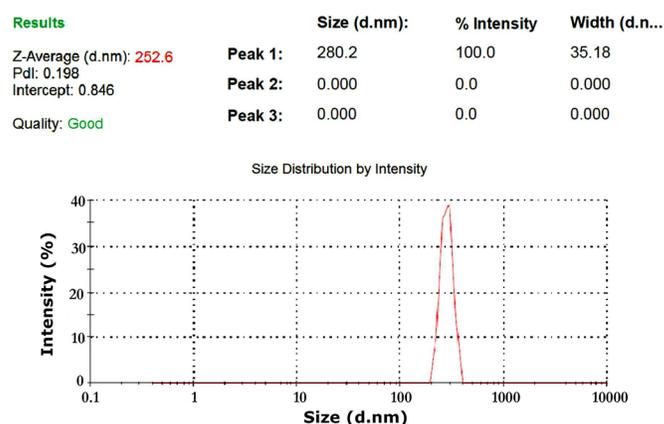
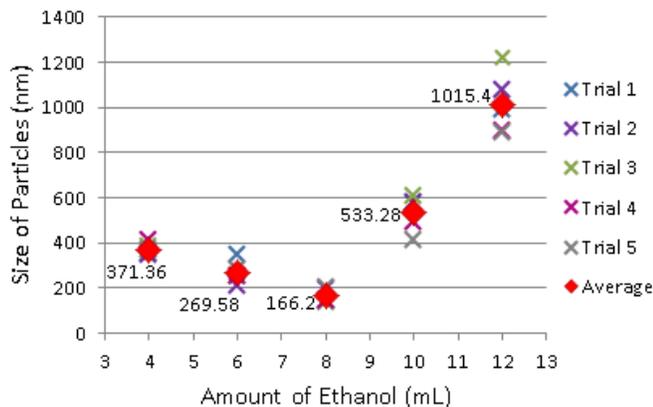
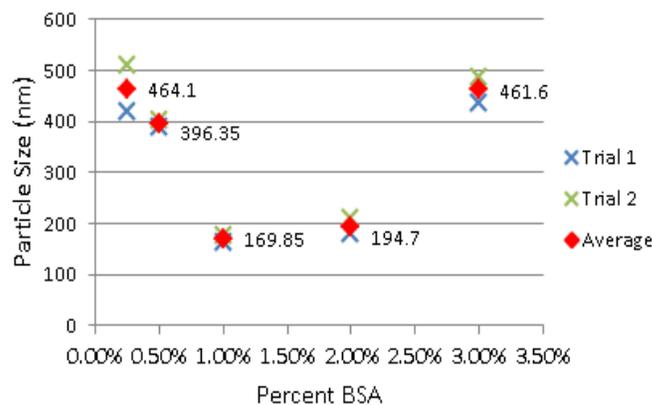


Figure 1. Particle size of albumin nanoparticles measured by zeta-sizer.



**Figure 2. Particle size of albumin nanoparticles depends on ethanol concentration.**

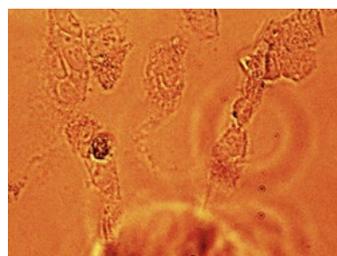


**Figure 3. Particle size of albumin nanoparticles depends on BSA concentration.**

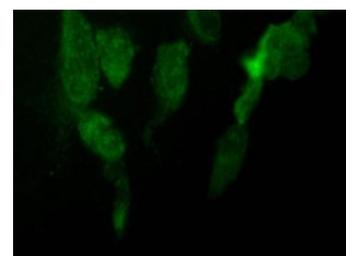
shown, the smallest average size was 169.85 nanometers, in which 1%, or 10 mg in 1 ml in water, of BSA was used.

After optimizing the procedure for making fluorescent nanoparticles, the first study was to test whether such nanoparticles can be uptaken by tumor cells and stain the cells green. In this study, Hela cells were incubated with fluorescent nanoparticles generated from the optimized procedure, and subsequently observed under a fluorescent microscope. Under the normal microscope channel, the actual cells can be observed as healthy growing cells (see Figure 4). Under the FITC channel, the cells glowed bright green (Figure 5), indicating a successful uptake of the nanoparticles by Hela cells within three hours. In contrast, the negative controls in which no nanoparticles were added showed no fluorescence (data not shown).

In the second study, different cancer cell lines were used to demonstrate the general utility of this method. Seven more cancer cell lines were incubated with the same amount of fluorescent nanoparticles. The cells were observed under the fluorescent microscope and pictures were taken in a similar fashion. While all of the cells had a positive uptake of the fluorescent nanoparticles, some cell lines had a stronger uptake than others. The “strength” of a cell line’s uptake was observed using two factors: 1) the number of observed glowing cells in proportion to the number of observed total cells and 2) the intensity of the green fluorescence visualized under the microscope. Figure 6 shows three pictures of the strongest positive cell line, A431, human epithelial carcinoma cells. Figure 6a shows the cells viewed by the normal microscope. Figure 6b reveals the same cells under the FITC channel. Then, using an image calculating program, the first two images were overlaid into Figure 6c that shows exact overlay of cell images and the fluorescent green images, indicating that fluorescent dyes are uptaken into the cells and render the cells green fluorescent under excitation. For A431 cells, almost every cell was stained green with clear cell borders, indicating a very effective uptake of fluorescent nanoparticles.

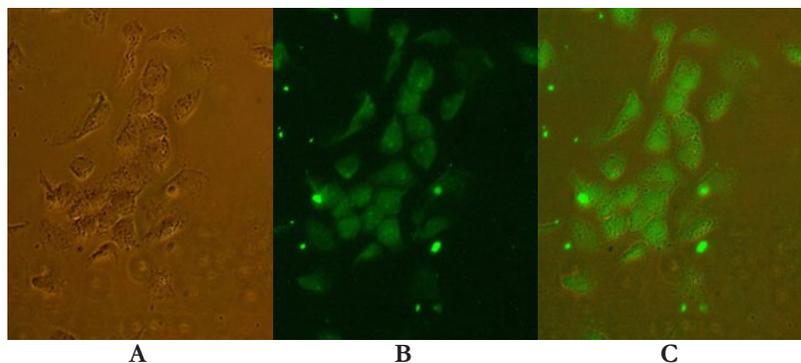


**Figure 4. Hela cells, normal channel.**



**Figure 5. Hela cells, fluorescent channel.**

Figure 7 shows the overlaying pictures for the remaining 6 cell lines. Both HEP-G2 (liver cancer) and MCF-7 (breast cancer) were strong positives, with a large number of intensely glowing green cells. Most AGS (stomach cancer) cells were also fluorescent, but their fluorescence was less intense and the cell borders occasionally unclear. MDA-MB-231 (breast cancer), EA.hy 926 (lung cancer fused with endothelial), and MG-63 (bone cancer) were observed as weak positives. This result demonstrated that different cancer cell lines can be labeled with green fluorescence when incubated with FITC-containing albumin nanoparticles.



**Figure 6. Uptake of nanoparticles by cancer cell lines. (A) Cells viewed by the normal microscope. (B) Cells under the FITC channel. (C) Overlay of (A) and (B).**

Lastly, the uptake of nanoparticles by cancer cells and non-cancer cells was compared. In Figure 8, representative pictures of A431, Detroit 551, and HEK293 cells under the white microscope lamp are juxtaposed with the pictures of the cells under the FITC channel, all at 400 milliseconds of exposure time.

In the cancer cell line A431, almost every cell in the microscopic view was green at a 400 ms exposure, while non-cancer cell Detroit



551 had no green fluorescent cells. Transformed cell line HEK293 had extremely few fluorescent cells, but there were some slightly green cells visible, as shown in the picture.

To investigate whether the albumin nanoparticle uptake is mediated by cell receptors, a competition study was performed. As shown in Figure 9, it was found that the higher concentration of free BSA in the media, the fewer cells were stained green, indicating less uptake of FITC-albumin nanoparticles. This result suggests that the cellular uptake of albumin nanoparticles is likely receptor mediated, because the free BSA in the media can effectively compete with the fluorescent-albumin nanoparticles for uptake.

In a separate study, cancerous and non-cancerous cells were incubated at two different temperatures – 37 and 4 degrees Celsius to compare the amount of nanoparticle uptake at the two temperatures. Figure 10 shows the change in the number of green cells. HeLa cells exhibited a 71.4% decrease in fluorescing cells when incubated at 4 degrees, while A431 had a 56.4% decrease and Detroit 551 showed a 47.8% decrease. In contrast, non-cancer cells Detroit 551 showed much lower uptake of albumin nanoparticles.

Co-precipitation of albumin nanoparticles with of cancer and non-cancer cell lyates reveals the presence of cancer cell specific proteins, as shown in Figure 11. The protein gel in Figure 11 shows the co-precipitated proteins from cancer cell line A431, HeLa, and non-cancer cell Detroit-551. The column on the left is the protein ladder, with known protein sizes in kilo-Daltons (kDa). Several bands, such as the ones slightly above 66.4 kDa and around 48-55 kDa, are shared by all three cell lines. However, A431 and HeLa cancer cells share fairly thick bands around 33-35 kDa that D551 does not have. This result indicates the possible existence of cancer cell specific albumin binding proteins or receptors.

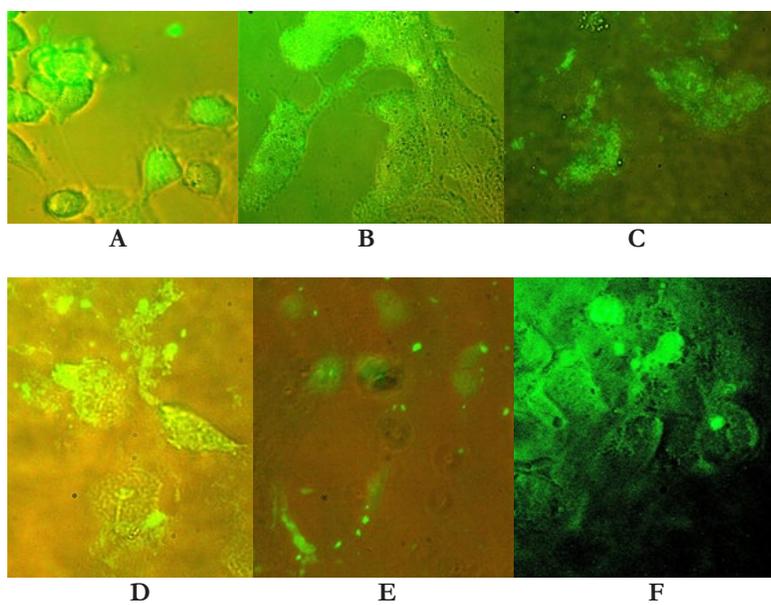
## Discussion

In the search for the most effective method to make fluorescent nanoparticles below 200 nanometers, two experimental variables were tested: the amount of ethanol used during the procedure, and the initial concentration of albumin. An optimized procedure was developed. The average size of the particles under these conditions were around 160-170 nm, within target size of 100-200 nm. Particles of this size have the highest chance of escaping from the blood vessel circulation into the tumor tissue, making it effective for targeting cancer cells. This work provides an economic method to generate biodegradable fluorescent albumin nanoparticles which bind and label tumor cells.

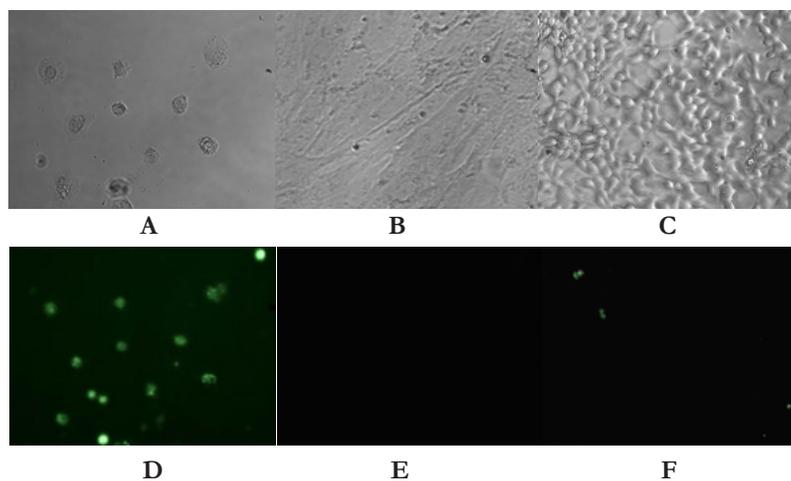
Although previous studies by others suggested that albumin can be used to make nanoparticles as drug carriers for tumor cells, few studies have been done to utilize FITC and albumin to form a biodegradable green fluorescent nanoparticle for tumor imaging. The work reported here established a reliable procedure to produce such reagent, which paved the way to a clinically important agent for surgical tumor imaging.

The study of cellular uptake of the fluorescent nanoparticles reveals that many cancer cell lines have active uptake of these particles. This is extremely important because it indicates that the fluorescent albumin nanoparticles can be used for imaging different types of cancers. A431, HeLa, HEP-G2, and MCF-7 were more effectively labeled among the tested cancer cell lines. However, the other cell lines AGS, MDA-MB-231, EA.hy 926, and MG-63 showed weaker but positive uptake. In addition, non-cancer cell lines, Detroit-551 and HEK293 demonstrated almost no uptake of the nanoparticles, which supports the hypothesis that these nanoparticles can be preferential to tumors.

Understanding the mechanisms of tumor cell uptake



**Figure 7. Uptake of nanoparticles by different cancer cell lines.** (A) HEP-G2 Strong Positive (B) MCF-7 Strong Positive (C) AGS Medium Positive (D) MDA-MB-231 Weak Positive (E) EA.hy 926 Weak Positive (F) MG-63 Very Weak Positive



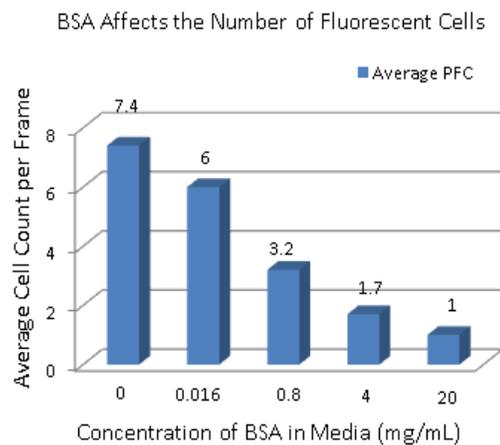
**Figure 8. Uptake of nanoparticles by cancer cells and non-cancer cells.** (A) A431 – White Light (B) Detroit 551 – White Light (C) HEK293 – White Light (D) A431 – FITC Channel (E) Detroit 551 – FITC Channel (F) HEK293 – FITC Channel.



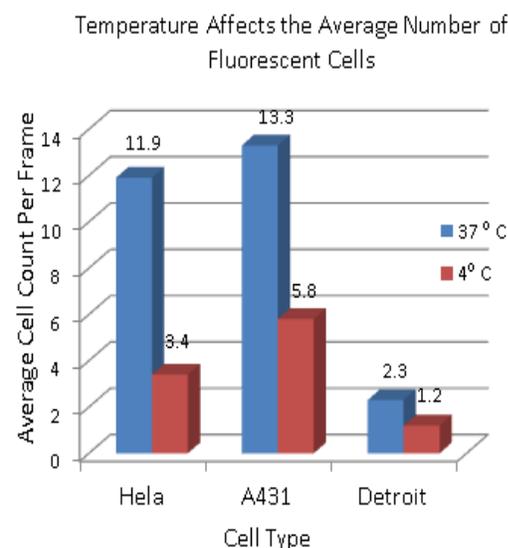
of albumin nanoparticles is important. If fluorescent nanoparticles are taken up through albumin receptors, then the FITC-albumin system may have a tumor preferential uptake determined by higher levels of receptor proteins on the cells. The competition study supports such a hypothesis. Since free albumin can compete for FITC-albumin nanoparticle in labeling tumor cells, it is very likely that the uptake is through albumin receptors. The co-precipitation result also supported the existence of cancer specific albumin binding protein. If that is the case, we expect different tumor types to have different albumin receptor levels. This is also supported by the variation of fluorescent staining to different tumor cell lines in this study. Future surgical screening may include the testing of albumin receptor levels of patients' tumor tissues by pathology and help to decide whether this imaging agent should be used. This type of personalized medicine will allow the surgeons to select the best imaging method to image individual patient.

The presence of albumin receptor proteins may also explain how albumin nanoparticles will be tumor preferential. Cancer cells may over-express albumin receptor proteins in comparison to normal cells, a theory which is supported by the cancer vs. non-cancer cell studies. The receptor proteins of cancer and non-cancer cells were also studied by a co-precipitation experiment. The cancer cell lines, HeLa and A431, possessed a protein band around 33-35 kDa that was not present on Detroit-551 cell line, indicating a difference in the protein receptor level of cancer and non-cancer cells. Further studies should be performed to reinforce this hypothesis by repeating the co-precipitation experiment with other types of cancer and non-cancer cells. In the future, the proteins may be analyzed by isolating the cancer-specific proteins and determining their amino acid sequences.

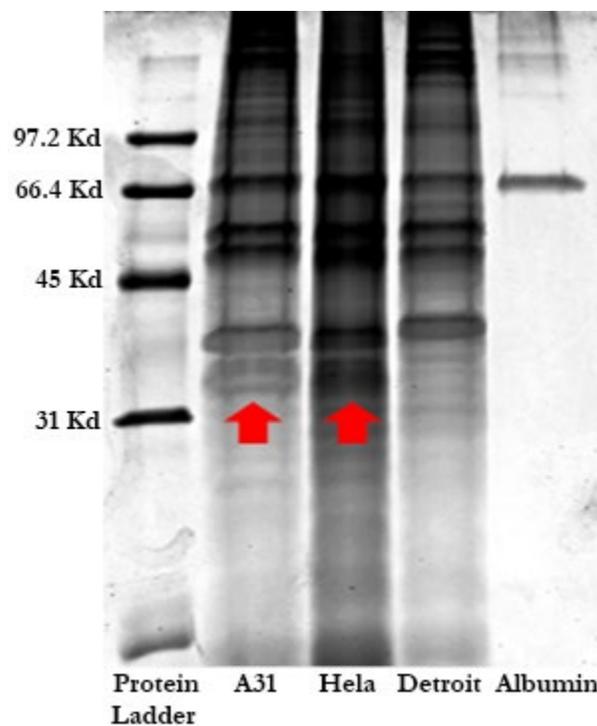
In summary, a fluorescent imaging system will allow significant improvement in cancer surgery by ensuring that all the cancer cells are removed, and therefore prevent remaining cells from spreading to other organs. When such a system is developed and proven to be effective in clinical trials, it is not hard to imagine that future cancer surgeons will use fluorescent imaging systems to guide their operations. A fluorescent camera can be positioned above the operation area and taking real time images of the tissues under surgery. Green cells on computer monitor or through surgeons' fluorescent viewing eye-glasses will highlight cancer cells that need to be removed. Surgeons will no longer rely on their naked eyes to find tumor tissues. This study explored a potential methodology to make such an imaging system using FITC-albumin nanoparticles. Such a system can be expanded to include other tumor targeting molecules such as tumor specific antibodies. In short, Fluorescent Imaging for Nano-Detection (FIND) of cancer cells is a necessity of future tumor surgery, and this preliminary study provides initial data to this potentially revolutionary development.



**Figure 9. Uptake of albumin nanoparticles decreases with increasing concentrations of BSA.**



**Figure 10. Uptake of albumin nanoparticles is temperature dependent.**



**Figure 11. Co-precipitation of albumin receptors from cancer cells.** Arrows indicate protein bands that A431 and HeLa cancer cell lines express that the Detroit noncancerous cell line does not express.



## References

1. Frangioni, J (2008). New Technologies for Human Cancer Imaging. *Journal of Clinical Oncology*. American Society of Clinical Oncology 26: 4012-402.
2. Keereweer, S (2010). Optical Image-guided Surgery—Where Do We Stand? *Molecular Imaging Biology* 13: 199-207.
3. Maeda, H (2011). Enhanced Permeability and Retention Effect in Relation to Tumor Targeting. *Drug Delivery in Oncology: From Basic Research to Cancer Therapy* (eds F. Kratz, P. Senter and H. Steinhagen), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
4. Jun, YJ, Kim JI, Jun MJ, Sohn YS (2005). Selective Tumor targeting by enhanced permeability and retention effect. Synthesis and antitumor activity of polyphosphazene-platinum (II) conjugates. *J Inorganic Biochemistry* 99 (8): 1593-601.
5. Muller B, H Leuenberger, T Kissel (1996). Albumin nanospheres as carriers for passive drug targeting: an optimized manufacturing technique. *Pharmaceutical Research* 13(1): 32-37.
6. Zhang, L, S Hou, S Mao, D Wei, X Song, Y Lu (2004). Uptake of folate-conjugated albumin nanoparticles to the SKOV3 cells. *International Journal of Pharmaceutics* 287: 155-162.
7. Anhorn, Marion G., S Wagner, J Kreuter, K Langer, H von Brisen (2008). Specific Targeting of HER2 Overexpressing Breast Cancer Cells with Doxorubicin-Loaded Trastuzumab-Modified Human Serum Albumin Nanoparticles. *Bioconjugate Chem*, 19: 2321-2331.
8. Dreis, S., R Rothweiler, M Michaelis, J Cinatl Jr., J Kreuter, K Langer (2007). Preparation, characterization and maintenance of drug efficacy of doxorubicin-laded human serum albumin (HSA) nanoparticles. *International Journal of Pharmaceutics* 341: 207-214.
9. Brigger, Irene, Nubernet, Catherine, Couvreur Patrick (2002). Nanoparticles in cancer therapy and diagnosis. *Advance Drug Delivery Reviews* 54 (5): 631-651.
10. Wang J, H Ueno, T Masuko, and Y Hashimoto (1994). Binding of Serum Albumin on Tumor Cells and Characterization of the Albumin Binding Protein. *J Biochem*: 898-903.
11. Rahimnejad M., M Jahanshahi, GD Najafpour (2006). Production of biological nanoparticles from bovine serum albumin for drug delivery. *African Journal of Biotechnology* Vol. 5 (20): 1918-1923.
12. Alander, Jarmo T (2012). A Review of Indocyanine Green Fluorescent Imaging in Surgery. Hindawi Publishing Corporation. *International Journal of Biomedical Imaging* 2012: 1-26.
13. Santra, Swadeshmukul, Yang, Heesun, Dutta, Debamitra (2004). TAT conjugated, FITC doped silica nanoparticles for bioimaging applications. *Chem Commun* 2004: 2810-2811.
14. Huang, Min. Ma, Zengshuan, Ma, Khor, Eugene (2002). Uptake of FITC-Chitosan Nanoparticles by A549 Cells. *Pharmaceutical Research* 19 (10): 1488-1494.
15. Langer, K., S Balthasar, V Vogel, N Dinauer, H von Briesen, D Shubert (2003). Optimization of the preparation process for human serum albumin (HSA) nanoparticles. *International Journal of Pharmaceutics* 257: 169-180.
16. Golemis E. (2002). Protein-protein Interactions : A molecular cloning manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. ix, 682.
17. Phizicky E. M. and Fields S. (1995). Protein-protein interactions: Methods for detection and analysis. *Microbiology Review*. 59, 94-123.

## Acknowledgements

I would like to thank my father, Tony Zhang, for supervising and mentoring me while I conducted my experiments, and my mother, Michelle Sun, for her encouragement during my research. Finally, I would like to acknowledge Dr. Song Li at the University of California Berkeley for introducing me to the world of scientific research during my internship and fostering my passion for science.