Biomedical Engineering Design

Biocast Measurement to Monitor Tumor Growth in Vivo

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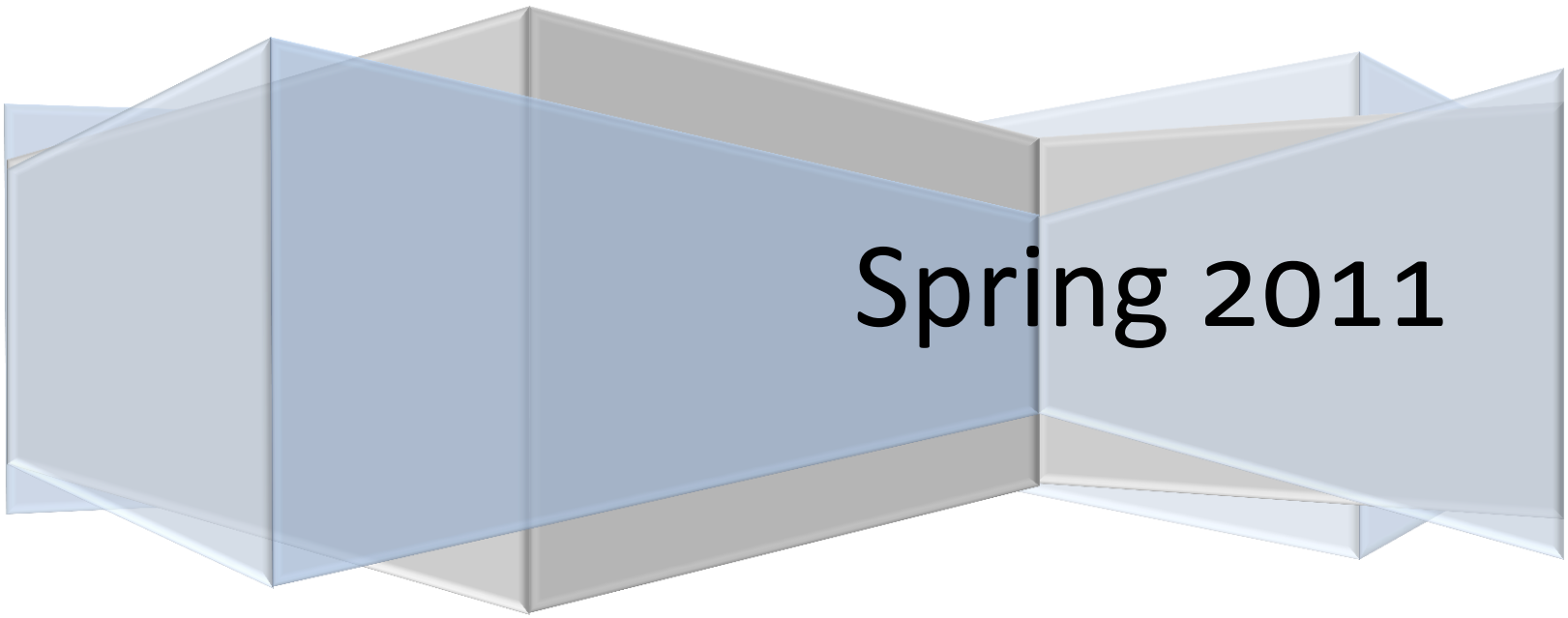

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Abstract

Our clients from the UW Department of Oncology are trying to assess various potential treatments for colorectal cancer as well as gain a better understanding of tumor behavior. They are doing so by monitoring tumor growth and recession in rats and mice with colorectal cancer by taking images of the tumors with an endoscope. However, the images obtained with the endoscope are distorted and do not have a frame of reference, making size comparisons between images difficult. Without accurate measurements of the change in tumor volume, our clients are limited in their research capabilities. We have devised a method to measure tumor volume by first creating a cast of the colon using alginate impression powder. Next we fill in the tumor impressions with dental stone, and by weighing the dental stone tumors and knowing the density we can find volume. Testing has shown that this method is accurate within our requirements and our clients will be adding it to their protocol.

Introduction

Background:

Colorectal cancer is the third leading type of cancer in both prevalence and mortality for both men and women. In order to assess the potential treatments for the cancer, our clients are trying to monitor tumor growth and recession over time. In order to increase the sample size they can study, an animal model with rats and mice is being used. The animals have a mutated Adenomatous Polyposis Coli gene in order to induce colorectal cancer [1].

The best way to measure the volume of these tumors would be with computed tomography (CT). However, CT is not an option for our clients. CT scans are expensive, and more importantly, the animals would need to be removed from the cleanroom in order to get a CT scan. Removing the animals from the cleanroom would introduce too many uncontrolled factors into the research. Because of this, our clients have to use an alternate method to image the tumors, colonoscopy. An endoscope is inserted into the colon of the rat or mouse and both images and video can be provided. An example image is shown in Figure 1. This procedure is done with the animal under anesthesia multiple times during the animals' lifespan; the end product being images and/or video of the tumors throughout the cancer's progression [1].

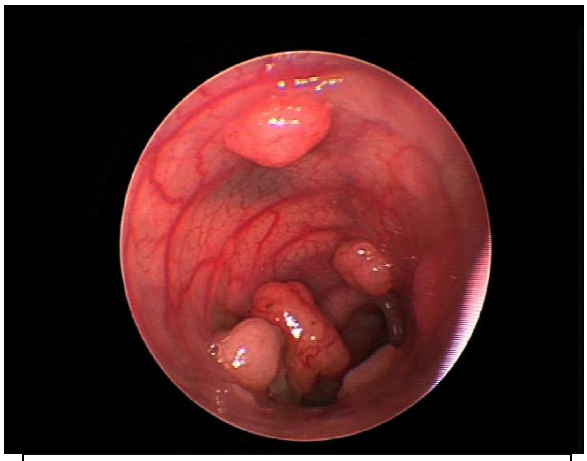

Figure 1. Image obtained from endoscope within a rat colon. Multiple tumors are shown.

Problem statement/Problem Overview:

As stated previously, our clients currently

use an optical endoscope, which is capable of capturing video and still images, to monitor the tumor growth. While this device lends itself to provide qualitative observations of a tumor's change over the course of treatment, there is currently no way for our clients to gather any direct quantitative data regarding the changing size of the tumor. In order for our clients to effectively measure potential treatments for cancer, it is important for them to be able to quantitatively measure a tumor's growth or regression in response to treatments. As such, the main goal of our project is to design a device or a technique that would allow for the researchers to accurately measure the volume of tumors, *in vivo*. Additionally, measured values such as the tumor's diameter, and cross-sectional area are desired in order to aid in the analysis of tumor change with respect to treatment.

The benefit of using the optical endoscope, besides gathering a visual image of the tumor, is that the endoscope has a working channel. This is a channel that runs the length of the endoscope and can essentially allow any tool small enough to fit through the working channel to come into contact with the tumor site, which opens up a wealth of possible approaches to measuring the tumor without any direct change into the basic methodology of the endoscope use.

Problem Motivation:

Beyond the basic need for our clients to be able to monitor tumor growth or recession over the course of treatment, this project has the potential to change a much larger field of research. By developing an effective method to measure tumor volume and dimensions *in vivo*, applications of our design could readily be applied to a clinical environment and be used to assist in the measurement of human colon tumors. The desire for a way to monitor tumor size is not only restricted to studies related to colon cancer, but would also be existent in studies of areas such as the gastrointestinal tract. As such, our motivation for this project is to develop a method of accurately measuring tumor size *in vivo* over the course of an experiment with the goal of not only assisting our clients, but also possibly creating a device that has the potential to assist in other fields of cancer research.

Client requirements:

The main stipulation of our project is that or measurement technique or device must not harm the tumor or the surrounding tissue of the animal. It would be undesirable if the measurement technique interfered with the experiment. From these measurements it would be ideal to get an absolute volume of the tumor during each measurement. However, our clients would be satisfied with a technique that could measure relative changes in tumor volume; this comes with the thought that the absolute volume can be measured at the end point, and then from the relative volumes, absolute volumes at the different stages can be measured. Our measurements must not allow more than 10 to 15 % error. Measurements of specific tumors might be monitored upward of six times throughout an experiment, so our device must be able to be used often. In addition, our technique would need to minimize experimenter error by placing the burden of acquiring accurate results on the device and not

the experimenter. Our design must also be able to fit within our clients' specific protocol for their lab. Finally, our project was provided with a budget of $1000 to complete this project.

Current Devices:

Endoscopes are currently used to visually assess the size of tumors throughout their development. The Storz 7219BA endoscope is used for rat test subjects and the Storz 1232AA endoscope is used for mouse test subjects. The endoscope used for rat testing has a 30 degree tilt angle while the endoscope used for mouse testing has a 0 degree tilt angle. Figure 2 shows the effect lens tilt angles have on the field of view of an endoscope.

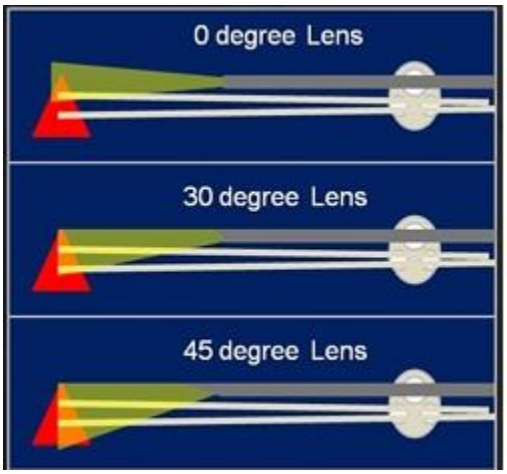

Figure 2: Different lens angles for endoscopes
Source: http://www.karlstorz.com/cps/rde/xchg/karlstorz-en/hs.xsl/146.htm

The lens for each endoscope creates a fish-eye distortion. The effect of fish-eye distortion can be seen in figure 3. Notice how the fish-eye lens distorts in image, causing straight lines to become curved and the relative size of objects to be altered. The distortion of size and shape created by a fish-eye lens makes it impossible for the client to determine the true size of any tumors viewed with the endoscope.

Figure 3: Top-image taken using a fisheye lens; Bottom-Image taken using a non-distorting lens

Source: http://en.wikipedia.org/wiki/File:Panotools5618.jpg

Competing Methods:

In a paper titled *Three dimensional measurement endoscope system with rulers*, Nakatani, et al. describe a method of fish-eye distortion correction and 3D visualization of tumors. Such a method could provide researchers with the ability to create 3D representations of tumors and use software to determine the volume of these 3D shapes. Nakatani, et al. attached four lasers to the end of an endoscope (Figure 4)

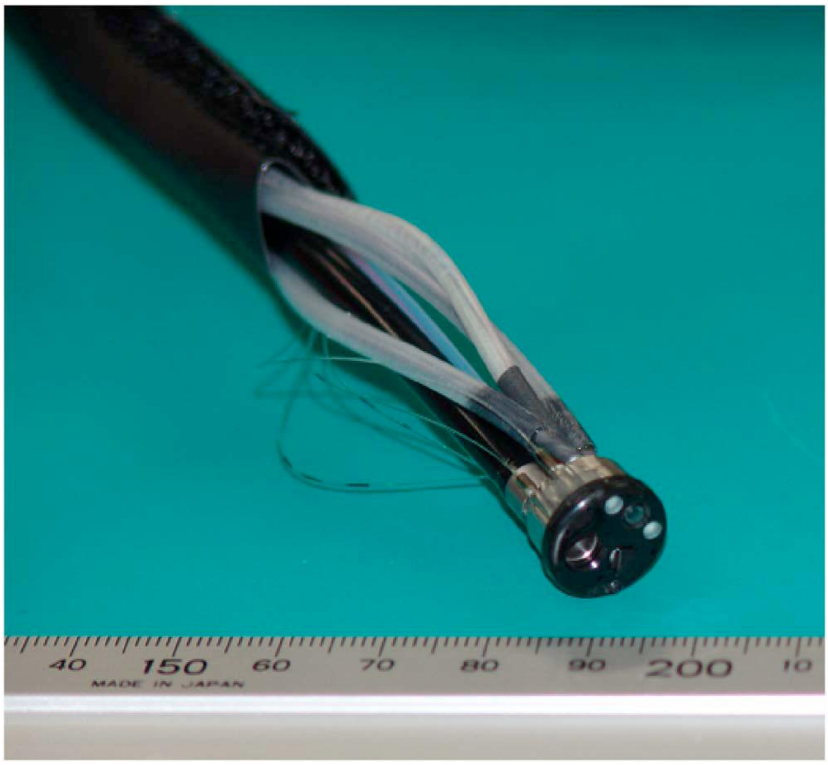

Figure :4 Distal end of an endoscope altered by the addition of four lasers (Nakatani, et al.)

Using contrast detection software, Nakatani et al. were able to locate each of the four laser points in the endoscope's field of view. The location of the four laser points relative to one another was used to determine the curvature of the surface onto which the four lasers projected. By determining the curvature of tumors, Nakatani et al. were able to create 3D representations of these tumors. We decided not to attempt to recreate this approach due to the cost constraint of $1000 on the project and the time constraint of one semester. Due to the extensive testing needed to configure such a system we determined it would take far too long to implement this approach.

Potential Designs

3D Image Creation:

One design uses a series of pictures taken by the endoscope to stitch together a 3D image of the tumor. This approach requires first that the endoscope fish-eye distortion be corrected. We have contacted Storz, the company that manufactures our client's endoscopes, and found that they do not currently have any software available to correct fish-eye distortion. If our team were to use this approach we would need to develop fisheye correction software on our own. *Videoendoscopic Distortion Correction and Its Application to Virtual Guidance of Endoscopy* by Helferty et al. outlines a set of algorithms that can be used to create this type of software for an endoscope. This process would require testing to ensure that the correction variables for the client's endoscopes are correct.

To create a 3D representation of the tumors using 2D images, the exact positioning of the endoscope would need to be known for each of the 2D images taken. This could be achieved using a moving stage with a micrometer on each dial (figure 5).

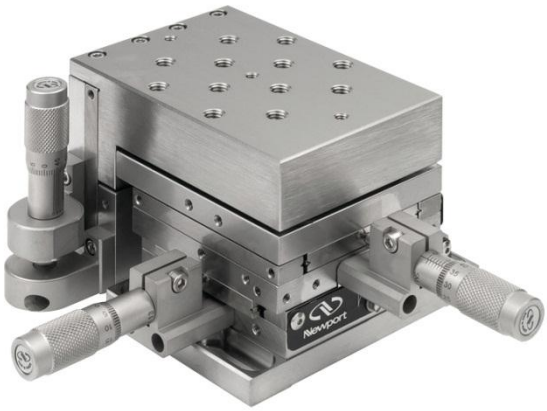

Figure 5: A moving stage with micrometers on each dial.

Source: http://www.newport.com/store/product.aspx?id=140089&lang=1033

The path taken by the endoscope in this method is shown in figure 6. The endoscope would first take images moving down the depth of the tumor (Y direction), then along the length of the tumor (X direction). Each time an image is taken the position of the moving stage would be recorded.

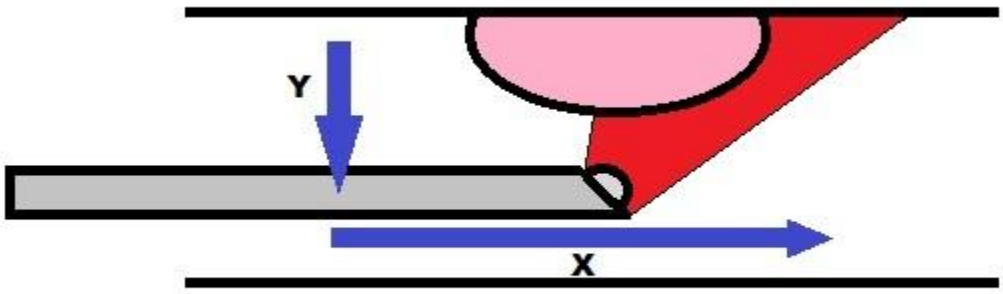

Figure 6: The path taken by an endoscope in the 3D image method

The reconstruction of the 2D images into a 3D image could be accomplished using ImageJ software. ImageJ is an open source imaging software. Independent developers have created plugins to the program that would allow us to stitch together a series of 2D images into

a 3D representation. Once a 3D representation of the tumor is created, the volume of the tumor could be determined using ImageJ software.

Alginate Cast:

Our next design idea is to make an alginate cast of the tumors that we can analyze for volume outside of the rats. Alginate (Figure 7) or alginic acid is an anionic polysaccharide found in the cell walls of brown algae. When treated with Ca^{2+} ions and D-glucono-d-lactone (GDL) homogeneous, strong and biocompatible gels can be formulated with controlled gelation rates proportional to the concentration of Ca^{2+} ions. These gels can gel *in situ* in 30 seconds to a minute [4]. It is these properties that make alginate a good candidate for a gel cast of the tumors.

To see the procedure to form this cast see appendix Alginate Cast. First an angioplasty balloon will be threaded into the colon from the *anus* so that the bulb of the balloon marks the end of the area of interest to the researchers. This balloon, along with the tube leading to it will be inflated to create a temporary blockage in the colon. At this point the alginate will be prepared and injected into the colon from the *anus*. This gel will then fill the colon completely and stop at the blockage. After 30 seconds to a minute the alginate will have gelled. At this point the angioplasty balloon will be deflated along with the tube leading to it. This will free up space down the central axis of the gel cast allowing the gel to press less tightly against the walls of the colon. The gel will then be gripped with a pair of tweezers from the entry way to the colon and removed, with the intention of not harming or removing any of the tumors.

This gel will be a cast of the negative volume of all of the tumors. These tumor voids can be filled with soft clay to exactly match the shape of the tumor molds. Once these pseudo tumors have been formed the gel can be stored or discarded. The clay tumors can then be individually placed in a graduated cylinder filled with water to assess water displacement and therefore tumor volume.

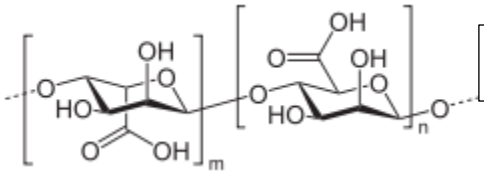

Figure 7: Chemical Structure of Alginic Acid

Physical Estimation:

In this design, direct physical measurements of specific parts of the tumor are utilized in order to estimate the tumor's dimensions and its overall volume. This is accomplished by modeling the tumor as an ellipsoid. Under this model, the tumor volume would be provided by the following equation:

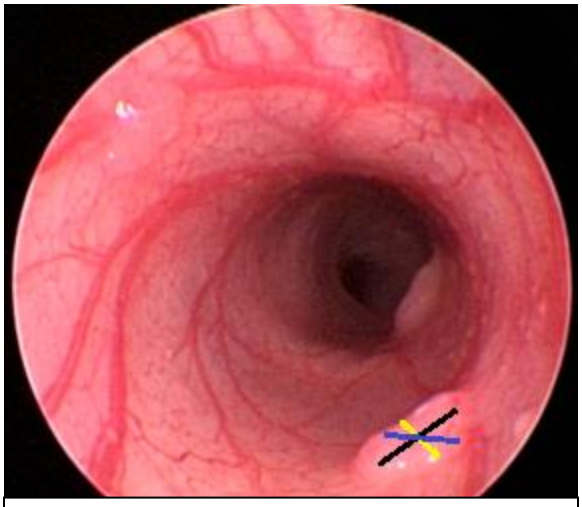

Figure 8: Orientation of the respective tumor radii needed to model the equation.

$$\frac{4}{3}\pi abc.$$

Here, a, b, and c represent the largest radius of the tumor in the x, y, and z directions with respect to the tumor. This is illustrated in Figure 8**.** In this figure, a radius value of the tumor would be measured coming orthogonal to the colon wall, a denoted by the yellow line. Another radius value would be measured going parallel to the wall, as denoted by the black line. A final radius measurement would be measured going from the anterior to the posterior regions of the tumor, as denoted by the blue line. Ideally, these measurements would be able to be recorded by a small caliper-like device that is fitted through the working channel and is controlled externally by the experimenter.

We were able to locate a paper which used a similar approach to measure tumor volumes. One factor they needed to add in order to get accurate measurements was to multiply the ellipsoid volume equation by a constant, which was determined experimentally in order to account for the irregular shape of tumors [5]. With a calibrated equation the researchers were able to acquire statistically accurate readings within a p-value of .001 for their calculations of the tumor volume and the actual tumor volume [5]. The need for the experimental constant in order to compensate for the size would make this design less than idea, as we would have to sacrifice many animals in order to obtain a corrected equation. In addition, this method may prove difficult in measuring the radii of small, flat tumors, placing more on an emphasis on the operator's dexterity in acquiring the needed values than the actual method itself. Additionally, this method assumes that the measured radii of the tumor are in fact the largest values. This assumption may not hold true, as the determination of where to measure would lie with the experimenter, leaving this design open to human error. Even greater possibilities for error would result if more than one individual were to acquire the data as there would be inconsistencies with the points of measurement between the researchers.

Final Design Selection/Design Matrix

Table 1: Design Matrix

	Ease of Procedure	Time Requirements	Cost	Estimated Accuracy	Adherence to Protocol	Repeatability	Potential Damage to Tumor	Applicability to Different Tumor Shapes	Resolution	Total
Maximum Points	10	10	5	15	5	15	15	10	15	100
Alginate Cast	8	8	2	12	1	12	6	8	15	72
3D Image	4	3	5	9	5	8	15	8	10	67
Physical Measurement	5	5	1	7	5	6	12	7	5	53

Our design matrix determined that the alginate cast idea is our best option. The alginate cast benefitted from being simple and easy to perform repeatedly. It also has the potential to be the most accurate. The cast would give an actual representation of the entire tumor unlike the other two ideas. The physical measurement idea would only have actual information for the dimensions measured, and the 3D Image idea would have no information

for the back side of the tumor where the endoscope cannot reach. The 3D Image idea suffered from being a tedious procedure that even with precise measurements would be hard to repeat exactly due to peristalsis causing the colon to move. The physical measurement idea's main flaws were that it would be very difficult for us to fabricate a device both small and precise enough to be used. It also would require a high degree of dexterity by the user.

However, the alginate cast idea still has some potential flaws. The most likely flaw is that removing the cast could also remove tumors, especially for larger tumors which would provide more resistance against the cast. Removing a tumor would be very bad for our client's research, as they would be unable to monitor the further progression of that tumor. Because of this, we have decided not to eliminate the 3D image idea yet. 3D image analysis was determined to be our second best choice, and it also complements the alginate cast very well. The alginate cast may be unusable for larger tumors, but modeling larger tumors in 3D is actually easier since more spatial information could be inferred from the endoscope images. The alginate cast could also provide the information on the back side of the tumors which would otherwise be lacking in the 3D image idea, increasing the accuracy of the approximation. Even if the alginate cast accurately gives volume change, 3D imaging could still be useful since the 3D models could be easily stored, which may be useful for our clients.

Note: While pursing the alginate cast design no potential setbacks were encountered. Thus we decided to discard the 3D imaging technique as it would be an unnecessary additive as deemed by our clients.

Final Design/Procedure

The final design is called Biocast Tumor Volume Determination and it involves injecting the colon of the rat or mouse with dental alginate to make a mold of the colon and any tumors inside. This mold can be removed and each tumor indent analyzed for volume by making dental stone tumor molds that can be weighed to calculate volume. The steps to the procedure are as follows:

1. Anesthetize the rat or mouse
2. Perform an initial inspect of the colon of the animal with a Storz 7219BA endoscope. See Appendix
3. Remove the endoscope from the colon of the animal and slide the silicon injection tube with leur lock attachment over the bare endoscope rod like a sheath. The side of the tube with the leur lock attachment should slide on first and the view of the endoscope should be unhindered once the tube has been put fully over the endoscope rod.
4. Insert the endoscope with injection tube sheath to desired injection depth into the colon of the animal. Use the video feed from the endoscope to navigate around sensitive tissue such as an tumors that might be blocking the path. See Appendix A.

5. Once at desired injection depth remove endoscope from the animal, leaving the injection tube in place with leur lock attachment protruding from the animal. See Appendix B.
6. Mix 12g dental alginate with 36mL D.I. water in a bowl.
7. Quickly poor this smooth mixture into the back of a 60cc syringe without hammer. Insert the hammer and quickly turn upside down. Push hammer up and into the syringe to remove air trapped in the column. See Appendix C.
8. Screw the loaded syringe onto the leur lock attachment of the injection tube and begin to slowly inject dental alginate into the colon of the animal. Slowly remove the injection tube as the colon fills with dental alginate. Remove completely once filled. See Appendix D.
9. Allow to set for 3 to 6 minutes or until sturdy. Remove mold with forceps, endoscope biopsy tool, or by the procedure used to remove fecal pellets from an animal before a procedure. See Appendix E, F and G.
10. Once the mold is removed mix 10 grams dental stone with 4 mL of water. Fill tumor indent of interest with dental stone slury. Allow to sit over 10 minutes or until hard. See Appendix H.
11. Remove dental stone tumor representations and weigh. See Appendix I.
12. Calculate volume of tumor of interest using the following equation: volume =(mass)/(density). The empirically found density of dental stone is 2188.88g/L

Testing

Our first testing was concerned simply with the gel. The alginate gel was first tested with flexible straws by inserting the gel with a syringe and allowing it to solidify. After 2-3 minutes, the gel could easily be removed and contained a reverse impression of the flexible portion of the straw. From this successful impression molding, we then moved to experimenting with fixed colons containing tumors provided to us by our clients. We were afforded two separated colons: one containing small to medium sized tumors, and another containing larger tumors. Experimenting with the colon containing smaller tumors we inserted the gel into the colon using a syringe and allowed it to solidify. This technique was again successful in creating an impression mold of the tumors. However, we found that it was difficult to remove the gel from the colon, requiring us to make an incision at the *anus* to remove the gel. The gel impressions of the smaller tumors was performed twice more, allowing us to compare different trials of the gel impression. Under visual inspection, the gels seemed very similar; each tumor impression in the gel resembled its respective counterpart in the other gels. Upon experimentation with the colon containing the larger tumors, we ran into some problems. While filling the colon with a syringe, we accidentally filled the colon too much with

gel, causing the membrane to burst. This caused the gel to place a large amount of pressure on the fixed tumors, creating a mechanical hold, which inadvertently caused the gel to rip off some of the larger tumors. However two bits of information stopped us from abandoning the gel procedure since it ripped off the tumors: the tumors were fixed so their properties were different than that of living tissue, and we filled the colon far further than we needed. Upon conferring our observations and testing with our clients we decided to move onto testing on a recently dead rat to see how our technique may be affected by tissue that was closer to being alive.

Upon experimenting with rats that were recently deceased both with and without a heating pad, we found that the colon cancer tumors were not in any danger of being pulled off by the gel. There was also a less apparent risk of filling the colon with too much gel. That is, with the give and elasticity afforded by the partially alive tissues, the gel was able to seep out of the *anus* once the colon was full. With gel molding test of recently alive animals, we performed multiple trials of gel filling and removal on two rats and a mouse. Each time we compared the respective gel impressions that were removed and found the tumor impressions between the trials for each animal to be qualitatively similar.

After we were able to reliably attain gel molds of the tumors, our next test was to see if we could produce dental stones of similar size and mass from a single tumor impression. By creating two stone molds and measuring their masses, we recorded a difference of 0.78% between the two values. With this data, we were confident that we could repeatedly produce similar stone molds from tumor impressions.

Going off of this, we found it was much harder to actually measure the stones' volume than simply using a water displacement method since the stones were so small and due to their size had a tendency to dissolve in water. To work around this, we found the density of a larger piece of dental stone, which was 2188g/L. Using this density, we could determine a stone's volume simply by knowing its mass.

Our final test was to compare the volumes of the dental stone impressions to the actual tumor volume. Considering the stone mold volume measurements and tumor volumes from two different rats, we found that the stone volume differed from the actual tumor volume by only 10%. So while this method may not be able to provide absolute measurements of tumor volume, with a 10% error, relative changes in tumor growth can be measured.

Ethical Considerations

Since our design will be used *in vivo*, it is important that we consider the ethics of such a procedure. We made sure to use a known biocompatible material for this reason. Alginate impression powder is commonly used by dentists to make impressions of their patient's teeth

and has been approved by the FDA for intraoral use. Since our procedure is done within the colons of rodents, this approval doesn't guarantee that the powder is safe for our uses. Additionally, alginate comes from a natural source, algae, and is commonly used as a food additive. It is also known that alginate does not interfere with mammalian cells through receptor or protein reactions, causing minimal cell adhesion [6,7]. Another potential ethical concern is pulling off tumors. Besides being detrimental to our clients' research, this could cause pain and excessive bleeding in the animal.

In our testing on recently alive animals, we didn't find any potential ethical problems. The alginate impression powder had no adverse material effects on the animal. We also didn't notice the impression pulling off any tumors until after many trials and the animal had started to decay. Even if tumors are removed, the bleeding can be stopped by cauterization which is what our clients do when bleeding is a problem when they take biopsies. While we didn't find any problems, it will be important to continue to monitor for them while our clients are waiting for approval to the amended protocol.

The other materials we use are also chosen to minimize any adverse effects. The tube inserted into the rat is silicone, and our clients will order high purity silicone for actual implementation. The only other material in contact with the animal is deionized water which is what is mixed with the alginate powder.

Future Work/Conclusions

The next step in developing this method involves testing on live animals. In order for this to occur the experimental protocol must be amended to include our method and this amended protocol must be approved. The researchers in the Dove lab think that it will not be difficult to obtain approval because dental alginate is a biologically inert substance and has already received approval from the FDA for use in the human mouth. Once approval has been obtained, we will work with the Dove lab to perform an acceptable number of trials on living test animals to ensure that our method repeatedly provides an accurate measurement of tumor volume. To assess the accuracy of our method, the Dove lab would like to compare volume measurements obtained using dental alginate with those obtained by CT scanning and direct volume measurement of tumors removed from recently sacrificed test animals. Once sufficient evidence has been obtained to show the accuracy of our method, we will help the Dove lab publish an article on this method.

The method of determining tumor volume in vivo presented in this paper is a novel method that has not been attempted by other researchers. For this reason we are exploring the possibility of obtaining a patent for this process. We have made a disclosure to WARF and will meet with them during the week of May 1st to discuss the candidacy of our method for a

patent. If WARF decides this process is a good candidate for a patent we will work with them to complete the patent process.

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[5] Mai, K. et al. 2003. A Simple Technique for Calculation of Volume of Prostatic Adenocarcinomas in Radical Prostatectomy Specimens. In Pathology. 599 – 604

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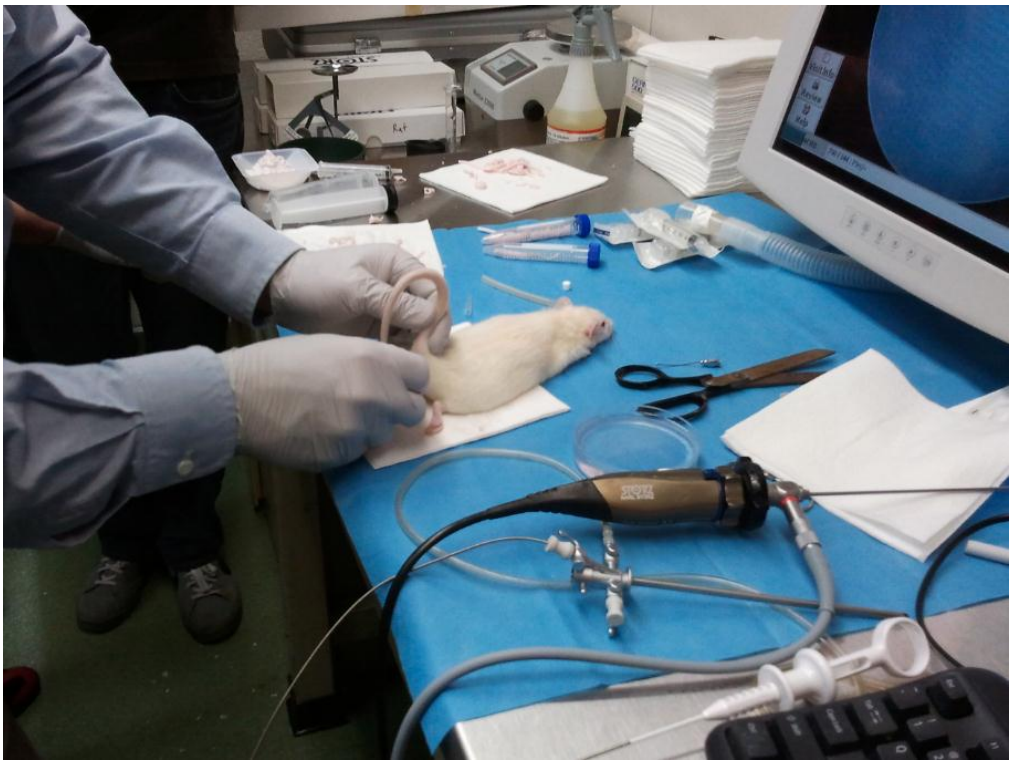
[7] Rowley, J. A., et al. *Biomaterials* 20, 45-53., (1999).

Appendix

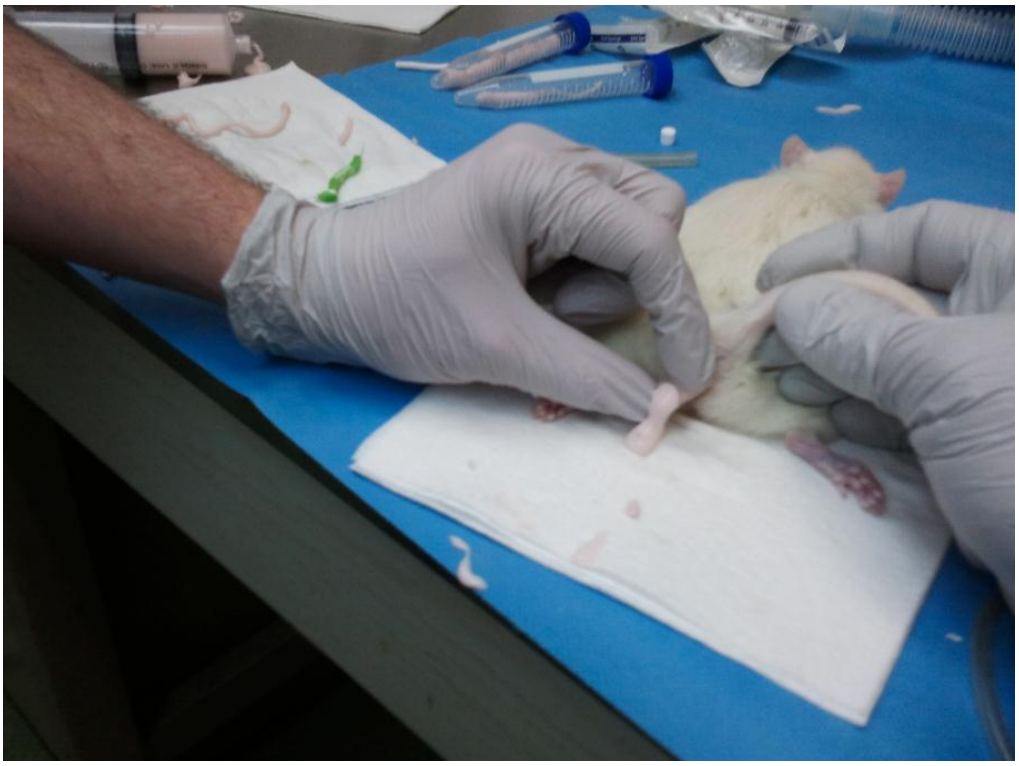
A. Initial colon navigation with silicon tube sheath.



B. Removal of endoscope, leaving injection tube in place.

C. Loading of 60cc syringe with alginate from the back.

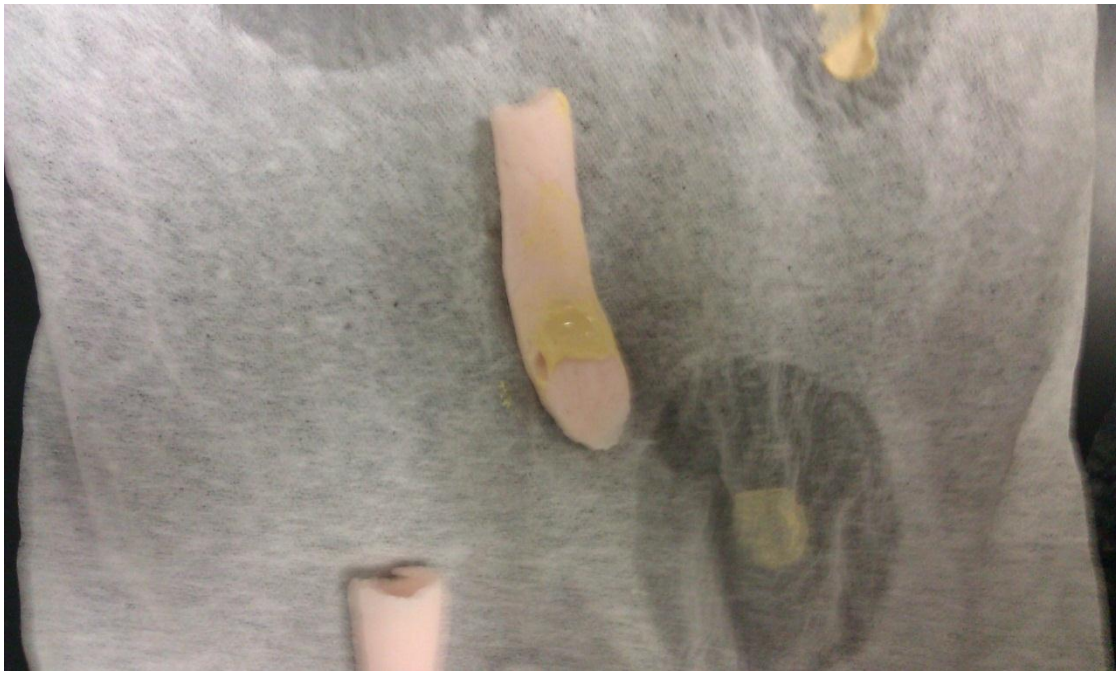
D. Injection of dental alginate.



E. Allowing filled colon alginate mold to solidify

F. Removal of alginate colon mold

G. Fully removed alginate cast with tumor indents. Large: Rat. Small: Mouse.



H. Filling tumor indents with dental stone.

I. Dental stone tumor molds ready to be analyzed for volume.

P*roduct Design Specifications:*

Function:

Our goal is to devise a method for measuring the size (ideally volume) of tumors by looking at pictures of tumors within a rat's colon taken with an endoscope. This must be done without harming the animal or destroying the tumor. This will allow our clients to better research colon cancer with an animal model.

Client Requirements:

J.

- Find volume of tumor
- Can't harm the animal
- Can't harm the tumor
- Budget of $1000 to build
- Less than $100 per measurement
- Animals can remain in clean room
- Clients have to be able to use any software necessary in our design

Design Requirements:

1, Physical and Operational Characteristics

a. Performance Requirements:
    - Usable up to ten times per day

b. Safety:
    - Biologically compatible materials
    - No danger of parts breaking off
    - No sharp edges ideally

c. Accuracy and Reliability:
    - Measure volume consistently with under 5% error

d. Life in Service
    - Must be operational for daily use for 5 years

e. Shelf Life:

- The shelf life would be the same as the shelf life of the endoscope and the computer

f. Operating Environment:

- Within a rat or mouse colon in an oncology lab

g. Ergonomics:

- No more challenging or uncomfortable than using an endoscope

h. Size:

- Any device would have to fit in a 2.2-2.5 mm (5 french) working channel
- Programming should be limited to be a reasonable file size for a personal laptop

i. Weight

- Light weight enough for one person to hold and maneuver comfortably

j. Materials

- Aluminum shaft used as a cover for the scope probe

k. Aesthetics, Appearance, and Finish:

- Programming would need a simple enough GUI for our clients to be able to use without much programming background

2**. Production Characteristics**

a. Quantity: 1 deliverable

b. Target Product Cost: Less than $1000

3. Miscellaneous

a. Standards and Specifications: Adhere to any standards for animal testing

b. Customer/Patient related concerns: Cannot harm rat or user either by shock or physical damage

c. Competition: Nothing currently available that deals exactly with this problem. Some things deal with facets of the problem but nothing covers it all.