System for Image-Guided Tumor Resection

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ABSTRACT

Two fluorescent phospholipid ether small molecules, CLR-1501 and CLR-1502, developed by Dr. Jamey Weichert's lab at the UW Madison School of Medicine and Public Health, are being proposed for use in tumor resection. The molecules accumulate preferentially in tumor cells; the fluorescence intensity ratio is 2.74:1 for glioblastoma tumor cells to normal brain cells for CLR-1501 [27]. This allows precise definition of tumor margins for accurate removal. As the extent of resection directly corresponds to patient outcome, the compounds will be a promising tool for the advancement of cancer surgery and prevention of recurrence. An imaging device capable of exciting the fluorescent molecules and displaying the feedback images is therefore needed to conduct clinical trials and later for use in conjunction with the molecules during tumor resection. The proposed device houses three small full CCD cameras with three LED lights, one emitting light in the 490-510 nm range (to excite CLR-1501), one emitting light in the 760-780 nm range (for CLR-1502), and one visible light LED for the standard reflected light image, inside a laparoscopic tube. The components will be integrated using a microcontroller connected to a computer running the open-source imaging software ImageJ. Control of the displayed video feeds will be hands-free.

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INTRODUCTION

Background

The development of biological compounds as markers has contributed to numerous advancements in the medical field and continues to be important for the study of various disease pathologies. Of particular interest for labeling and imaging cancerous masses in the body are fluorophores; much recent investigation has been conducted on how these fluorescent chemical compounds can aid in location and removal of tumors. Fluorophores are excited and emit light at specific wavelengths visible to the human eye, making them useful for tagging molecules of interest [1]. When the fluorophore is exposed to its corresponding wavelength of excitation, electrons within the compound absorb the light and enter an excited state. As the excited electrons are unstable, they quickly return to ground state. When this occurs, the electrons release energy in the form of light. This light is released at a higher wavelength than that used to excite the fluorophore. The difference in wavelengths allows for separation of emitted fluorescence from the excited light.

Fluorescence labeling utilizes fluorophores by attaching them to other molecules such as proteins, amino acids, and peptides [2]. There are two methods for labeling: direct and indirect. Indirect is more common as it does not require genetically modifying the desired molecule to produce fluorescence. Rather, the label is attached to an antibody that has been created specifically to attach itself to an antigen on the desired molecule. Indirect labels tend to be less expensive and less invasive. Since multiple labeled molecules can attach to a target molecule, the signal is amplified when using indirect labeling [3].

Immunofluorescence has traditionally been used to tag molecules of interest within a cell. When imaged using a confocal microscope, researchers are able to view and track the individual molecules movements throughout the cell [4]. Fluorophores can also be attached to molecules designed to be taken up by cells through endocytosis. Once these fluorophore-tagged molecules are

within the cell, the cell will fluoresce when imaged, allowing it to be distinguished from cells that have not taken up the tagged molecules. This is useful for the detection of cells which process the compounds different than surrounding cells. As cancerous cells exhibit abnormal behavior, compounds can be designed that will remain in cancerous cells after healthy cells have completed recycled it [5]. An alternative to fluorophores is the use of magnetic nanoparticles; however, they are primarily for noninvasive behavior tracking [6].

Dr. Jamey Weichart's lab has developed two fluorescent phospholipid ether small molecules, CLR-1501 and CLR-1502, which are intended for fluorescence imaging to aid in detection and removal of cancerous tissue. Phospholipid ether analogs have the ability to accumulate preferentially in tumors [7]. The use of tumor-specific fluorescent molecules allows a precise method for determining location and dimensions of tumors. These fluorophores were developed from CLR-1404, which is a radioiodinated alkyl phosphocholine analog that has shown significant tumor selectivity and prolonged retention. Because CLR-1404 does not localize in inflammatory lesions and has prolonged retention, CLR-1404 has the potential to be a superior alternative to fludeoxyglucose (FDG), which is a current radiopharmaceutical used in positron emission tomography (PET) imaging. CLR-1404 is currently going through clinical trials. CLR-1501 and CLR-1502 are created by incorporating fluorophores into the hydrophobic alkyl chain of CLR-1404 [8]. CLR-1501 is excited at 500 nm of light and emits at 520 nm, and CLR-1502 is excited at 772 nm and emits at 790 nm.

Endoscopes are medical imaging tools commonly employed in diagnostic and surgical procedures. They allow doctors to view the interior of a patient's body cavity by inserting a tube which houses optical fibers that feed a light source in and a visible image out. The endoscopic tube is inserted through a very small incision, typically less than 3 cm [9]. A traditional endoscope consists of either a rigid or flexible tube containing two separate optical fiber bundles. A visible light source

sends light down one optical fiber bundle while the light reflected within the body returns via the second optical fiber bundle. At the internal end of the endoscope a lens is used to transmit and collect light for a specific field of view, usually between 0 and 60 degrees. At the external end of the endoscope a CCD sensor attached to the second optical fiber bundle, then resolves this light into a digital image. Alternatively, the CCD sensor can be replaced with a lens that transmits the image to an eyepiece [10]. Laparoscopes, a type of endoscope, refer to those used in minimally-invasive procedures. Today's laparoscopes almost exclusively mount their CCD sensors at the external end of the endoscopic tube. However, technological advancements have allowed for smaller CCD sensors, making it possible to place the cameras directly at the internal end of the laparoscopic tube which could then simply house the electrical wires connecting the sensors to a computer [11].

Motivation

In the event that the entire tumor is accessible, the primary goal of cancer surgery is complete resection during the initial surgery, while minimizing the risk of surgery-related death. Incomplete excision of tumors is frequent, mainly due to insufficient methods used during intraoperative assessment of tumors. Current assessments are based on palpation and visual inspection and have proven to be inadequate [12]. Two studies showed that the intrahepatic recurrence rate is 35 to 60 percent [13, 14]. A meta-analysis on the local recurrence rates after surgery for rectal cancer found that in 52 percent of cases, local recurrence was reported [15]. It is evident that new visualization techniques are needed in order to better aid the surgeon during intraoperative assessment and removal of the tumors. Improved resection will reduce the high rate of recurrence.

Fluorescence image-guided surgery has undergone substantial growth recently and will be an integral tool in aiding surgeons during future intraoperative procedures [16, 17]. Several studies have shown that fluorescence imaging during operation is a safe and effective method in

accurately differentiating tumors from normal tissue [18], can increase sensitivity and specificity of diagnostic staging laparoscopy [19], and can also be useful in detection of nerves during surgical procedures [20].

Due to the relatively high rates of recurrence after curative surgery, and therefore the need for more accurate, brighter markers to define tumor margins, Dr. Weichert's lab has created two fluorescent analogs, CLR-1501 and CLR-1502, which accumulate in cancer cells. Another fluorescence tumor-labeling technique, developed by Dr. David Roberts and Dr. Keith Paulsen, utilizes the fluorescent cancer-specific compound 5-aminolevulinic acid (5-ALA) to highlight gliomas during surgery by illuminating the brain with blue fluorescent light to provide glowing images of the tumor [26]. As a natural biochemical precursor to hemoglobin, 5-ALA elicits production and accumulation of fluorescent porphyrins in epithelia cancerous tissue, and malignant glioma tissue [27]. Currently, 5-ALA does not fully visibly label all gliomas, creating a need for more efficient fluorescent tumor markers; CLR-1501 has shown to label the entire tumor while having a tendency for long term retention within the cell [28]. CLR-1501 not only provides a more accurate tumor border definition, but an increased fluorescence intensity ratio (cancer cell to normal cells) than 5-ALA, creating better visibility and therefore improving resection [28]. Additionally, CLR-1502 provides depth feedback due to its emission in the infrared range. The emission from an infrared fluorophore can be seen much deeper into the tissue, allowing for more precision during surgery, eliminating the need to remove a large margin of healthy tissue as a precaution. This will greatly increase the efficacy of resection and help prevent recurrence as well as the need for additional operations, saving countless lives and expenses to patients, the government, and the healthcare industry. With such advances in fluorescence-guided imaging, it is imperative to have a device capable of improving technique

and quality of images, in order to ensure safe and efficient removal of cancerous tissue. The client requires an imaging system capable of exciting fluorophores and producing high-quality, live images of the fluorescence emission during minimally invasive surgery. The device will be used for clinical trials in animals to help demonstrate the efficacy of the novel compounds. An endoscope-like device that houses the light source and imaging components at the internal end of the rigid endoscopic tubing was therefore proposed to provide a viable, cost-effective imaging tool.

Current Devices

Although similar devices are on the market, none of them work in conjunction with the specific combination of fluorophores designed by Dr. Weichert's team. A device called the Fluobeam, which emits near infrared light, is available from Fluoptics. It is available in two models with a laser light source producing excitation wavelengths of 680 nm or 780 nm, and a CCD sensor capable of detecting fluorescence-emitting light above 700 nm or above 800 nm, respectively. In both models a visible light image is produced using an array of visible light LEDs and captured using a black and white CCD camera. The device has a diameter of 7 cm, a length of 20 cm, and a weight of 600g [21]. The device is limited to use only during open surgery due to its large diameter. Additionally, it is currently only applicable to compounds that excite in the infrared range, so it would not be able to image CLR-1501 and CLR-1502 simultaneously. The Fluobeam is designed to be only used with infrared fluorophores because fluorescent light in the infrared spectrum can be removed from the returning light without removing any section of the visible light range.

Capsule endoscopy is a technique currently used to record images of the digestive tract. The capsule is approximately the size and shape of a pill and houses a tiny camera surrounded by

LEDs, a battery, and transmitter. The captured images are transmitted to sensors on the patient's torso then recorded digitally to device worn around the patient's wrist. About eight hours after the patient swallows the capsule, it has traveled through the patient's small intestine and the images gathered can be downloaded and examined. There are several endoscopy capsules that have been developed by different companies. Two available devices are the MiroCam (from SynMed Limited) and the EndoCapsule (from Olympus), which both have a diameter of 11mm, six white LED lights, and a high-resolution CCD housed at one end of the capsule [22, 23]. A trial found that video capsule endoscopy was superior to small bowel radiographs for evaluation of small bowel diseases; however, it was also stated that further improvements must be made to the device to allow for less difficulties in interpretation of the findings [24]. Another study comparing capsule endoscopy to colonoscopy for the detection of polyps and cancer found that the capsule endoscopy is a safe method for visualizing the colon but is significantly less sensitive in detection of polyps, adenomas, and colorectal cancer. Sensitivity of the capsule was also found to be highly dependent on colon cleanliness [25]. Though these studies suggest improvements must be made to the capsule endoscope, they show that the housing of optical components, such as LEDs and CCD cameras, in the internal end of an endoscope is potentially achievable. It is clearly shown that the electrical components of the device cause no harm to the patient and that small enough optical equipment is available for such a device.

Devices capable of capturing both visible light and fluorescent images have been developed. One such device is a dual-mode laparoscope that provides nearly simultaneous visible light and high-brightness fluorescence imaging of nerves using a single camera [20]. This shows that it is possible to excite a fluorophore and capture visible light and fluorescent images using a single endoscope. This device shares significant functionality with the laparoscope that

was developed for this project. However, this device utilizes a laser and optical fibers with the housing of the optical components at the external end of the endoscopic tube.

DESIGN CRITERIA

The client requires a system to image fluorophore-containing cancerous tissue during minimally invasive resection procedures. The phospholipid ether small molecules CLR-1501 and CLR-1502 will be injected into the patient a few days prior to their resection surgery. These molecules were designed specifically to accumulate within cancerous cells. CLR-1501 and CLR-1502 contain fluorophores that can be excited by 500 nm (green) and 772 nm (infrared) light, respectively. This creates the need for light sources which produce light at these wavelengths. A visible image needs to be captured as well so a visible light source is also needed. Emission from the green fluorophores, near-IR fluorophores, and the reflected visible light images must all be captured by high-resolution cameras with each camera capturing at 30 frames per second. This high frame is necessary to be consistent with current surgical imaging quality. The device needs to display 4 separate, high quality, real-time video feeds of the inside of the abdominal cavity during surgery, one showing CLR-1501's fluorescent emission at 520 nm, one showing CLR 1502's fluorescent emission at 790 nm, a composite of the fluorescent colors, which shows the depth of cancerous mass, and a visible light image as a reference guide for the surgeon. The internal end of the device must remain clean while within the body to allow a clear image at all times. It is also necessary that the device be easy to sterilize between use in different surgeries so that it can be reused. As the device will be inserted into the human body it must not pose any health or safety risks to patient or user. Additionally, the design should be lightweight, portable, and able to interface with an image display system that is controlled, hands-free, by the user. The cameras and illumination sources should be housed in a surgical-grade stainless steel tube that fits the above requirements and is less than 2 cm in diameter.

The product must be durable, so that it can withstand repetitive use without the need for frequent maintenance. Since the device will be used with live patients, it must be reliable as failure could result in loss of life or serious damage. Additionally, and perhaps most importantly, an accurate, consistent, and

sharp image will be vital to the efficacy of the surgery. Because the device will be used for minimally invasive procedures, the surgeon's success will be highly dependent on the reliability of the imaging system. The client also recommended that the design be similar to current devices, as it is difficult to introduce new technology into the market when a viable option is already common. An exact budget was not given, as the clients were unsure of the components necessary and specific costs, although a potential estimate of a few thousand dollars was discussed. The goal was to develop a relatively inexpensive prototype that is as efficient as current laparoscopic devices, and has the ability to collect images for 3 separate light spectrums based on compatibility with the compounds developed in Dr. Weichert's lab.

OVERVIEW OF THE DESIGN PROCESS

DESIGN PROCESS

Background research was necessary to fully understand the surgical procedures during which the device would be used. To facilitate this, the team met with the client to gain a comprehensive understanding of the background of Dr. Weichert's compounds and the goals of the project. As this product is intended to be commercially produced, work on this project began with an in-depth market survey. The customer base for the product was estimated at over 4000 distinct hospitals that would be able to perform cancer surgery. Based on the projected cost of the product versus the benefit it delivers, it was deemed that high sales of this product were extremely viable. It was at this time that a specific design began to be outlined. Research was conducted and the team met with experts in optical imaging and fluorescence microscopy to discuss the feasibility of various designs. A design matrix was created to select a final design, with aspects such as image quality and ease of use most heavily weighted. After discussing the final design with the client, some major changes were made to ensure client satisfaction. Following this, a broad survey of parts available was conducted to assess the options. Then, specific parts were researched and chosen as components for the final design. In the future, parts will be ordered and construction and testing will begin, which is outlined in detail later in this paper.

DESIGN ALTERNATIVES

The system needs to image fluorophores at a macroscopic level; however, fluorescent imaging is most commonly employed at a microscopic level. Due to this disparity, primary research was focused on common microscopic imaging techniques, such as confocal microscopy, to determine the light and optic requirements needed for the fluorescent imaging. These requirements were then transferred to an imaging system at a macroscopic level. Simultaneous imaging of two fluorescent sources as well as visible light imaging is almost unheard of due to the difficulties associated with either separating three images from one capture, or designing a high speed system capable of switching capture mode quick enough to produce three separate quality video feeds. As mentioned earlier in the paper, infrared fluorescence can been imaged simultaneously with a visible light as they are not in the same spectrum

Many minimally invasive surgeries currently implement the use of a visible light laparoscope to give the surgeon a good view of his work. As current medical professionals are trained to use these visible light endoscopes, it's important that our laparoscope mimic current laparoscopes ergonomics and user interface. It was also important that the endoscopic tube of the laparoscope matches the dimensions of current devices so that incision size and placement could be maintained providing an easy transition for surgeons.

The laparoscope design is quite complex, as this is a novel product that has never been produced by an imaging company. Today's laparoscopes are simply used to take visible light images, which do not require any filters or excitation light, because they only gather reflected visible light. Alternatively, this system needs to capture a visible light image while simultaneously exciting fluorophores at two separate wavelengths and receiving both emission wavelengths separately. Due to the desired frame rate, a single camera cannot be used. This is because when the fluorescent image is captured, a specific filter needs to be in place over the camera lens. This filter would be different for each fluorophore and no filter can be present during visible imaging. It was deemed unfeasible to create a system that would change this filter fast enough to produce three separate video streams at acceptable frame rates, as the fastest available filter switches at 23 milliseconds, or approximately 43 Hz, and the client desired a frame rate of 30 Hz for each

image, necessitating a filter that could switch at 90 Hz.

Once it had been confirmed that three separate cameras were necessary, research into commercially available fluorescent imaging cameras was conducted. As a live video feed was necessary, cameras with full CCD's needed to be used. Traditionally fluorescence imaging is conducted using a scanning camera [29] which is less expensive than a full CCD camera; however, the frame rate produced by these cameras is much too slow. Finally, two of the cameras needed to be designed to capture light in the visible spectrum while the third camera needed to capture light in the infrared spectrum. The light received by the cameras capturing the fluorescent images needed to be filtered using a high pass optical filter. This filter prevents the reflected excitation light from entering the CCD sensor and only allows the higher wavelength light emitted from the fluorophores to enter the CCD sensor. This is necessary because if the excitation light is reflected back to the sensors it will render the image useless, as the excitation light is much stronger than the light emitted from the fluorophore. The high pass filters for the green and infrared capturing CCD sensors should have a cut off at approximately 510 nanometers and 780 nanometers, respectively.

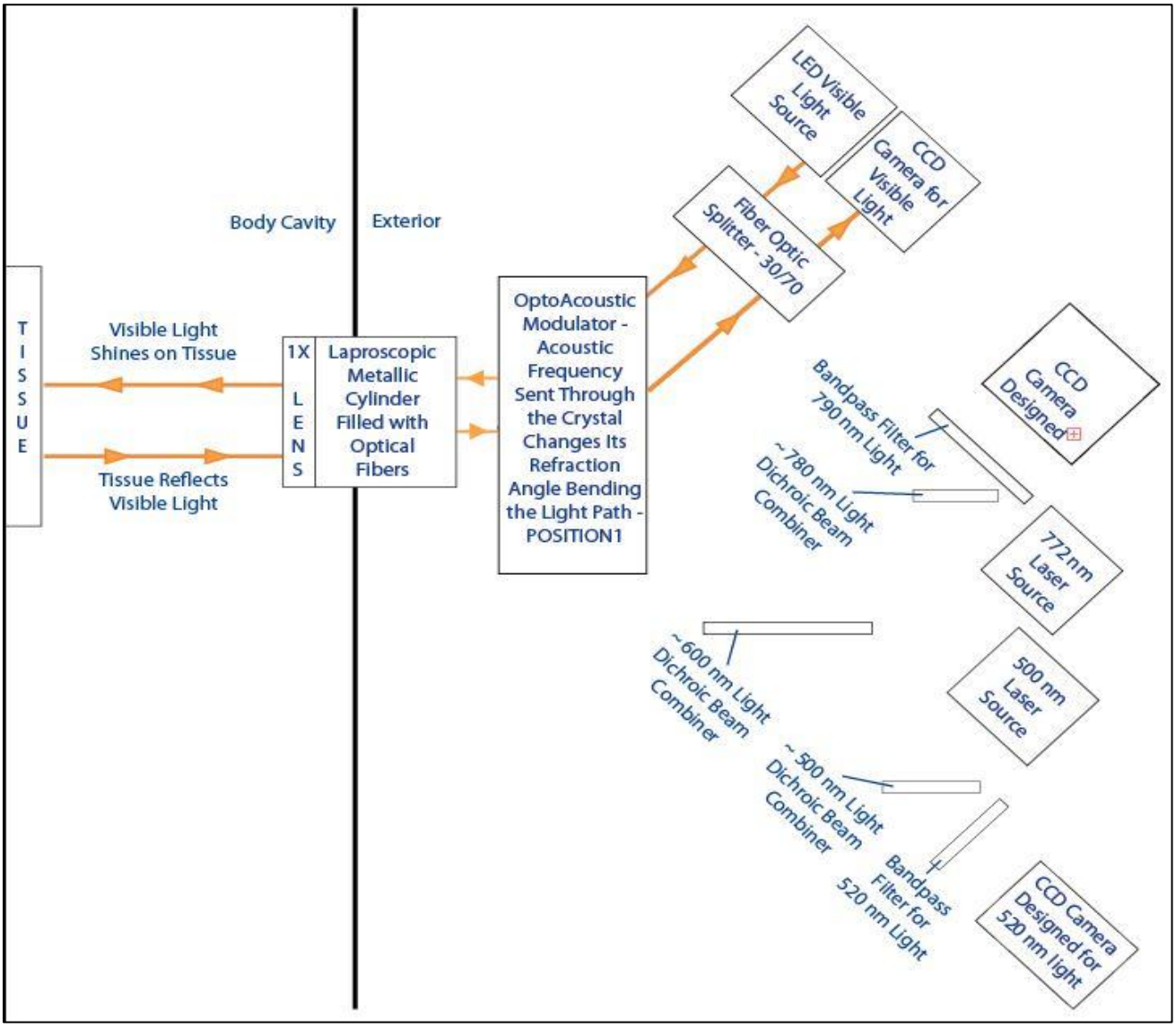
To facilitate the necessary light needed for all three imaging techniques, three separate light sources were necessary, as the only single light source that could produce all necessary wavelengths is an extremely expensive white laser. The light necessary for this design could be produced by lasers or LEDs. One light source was needed to produce the visible light necessary to capture a reflected visible light image. Another light source was needed to excite the fluorophore in the green light spectrum and another was needed to excite the fluorophore in the infrared spectrum. The fluorescent light sources needed to have low pass optical filters to ensure that all the excitation light sent into the body cavity was at a lower wavelength than the light emitted by the fluorophores. Without these filters, excitation light would be produced at the same wavelength as the fluorophores and would then be reflected into the CCD sensors as it would travel through the high pass filters without issue. This reflection would be much stronger and would greatly overpower the light emitted by the fluorophores, effectively rendering them useless. The

low pass filters for the green and near-IR needed to have a cut off at approximately 510 nanometers and 780 nanometers, respectively.

After contacting many commercial camera suppliers, no cameras small enough to be housed within the tip of a laparoscopic tube were found. Three separate light sources needed to be housed within the tip of the tube as well. Because this seemed highly unlikely, exploration into using a traditional laparoscopic tube full of optical fibers began. This design has a lot of attractive aspects. A laparoscope housing only optical fibers could be made much sleeker than one that needed to house a large amount of camera equipment. Also, no electrical components would ever enter the body, providing another level of safety. Finally, all the cameras and filters could be housed in an external location and would simply be connected to the device through an optical fiber cable, preventing the need for bulky cameras mounted on the end of the tool, similar to current laparoscopes. Another advantage of housing the cameras and filters at an external location was that extremely sensitive cameras and accurate filters could be used. As camera sensitivity increases with size, the external location allows for large cameras and filters. These cameras and filters could also be cooled in the external location to reduce noise during imaging.

It was important to maximize light transmission through the laparoscopic tube in both directions. As more excitation light reaches the fluorophores, they will emit a greater amount of photons. And the more photons that eventually reach the camera, the better the image produced. With this in mind, an initial design was developed that used the same optical fiber bundle for transmission and reception of light all three necessary image captures, allowing for the full diameter of the laparoscopic tube to be used for all light transfers. The optical fiber from the laparoscopic tube would be connected to an optoacoustic switch that would oscillate at 60 Hz between two different light paths. These optoacoustic switches have been employed in microscopic imaging during which both visible and fluorescent images needed to be captured [30].

FIGURE 1.



One light path would be used to capture the visible image (Figure 1) while the other would capture both the fluorescent images at the same time simultaneously (Figure 2). This would allow for 30 frames per second for each video feed. Figure 1 shows the optoacoustic switch in a position to capture a visible light image. The optoacoustic switch is a glass prism through which an acoustic signal is sent through, depending on the frequency of the acoustic signal sent through the glass, it will transmit light at a different angle. Since the frequency of the acoustic signal can be switched extremely fast, it can be used to switch the light path between imaging systems. While in the visible light position a visible light source is sent down the optical fiber bundle using a coupler. A visible light camera then receives a visible light

image back through the same optical fiber bundle as the other device coupled. As we are just imaging reflected light, no filters are needed.

FIGURE 2.

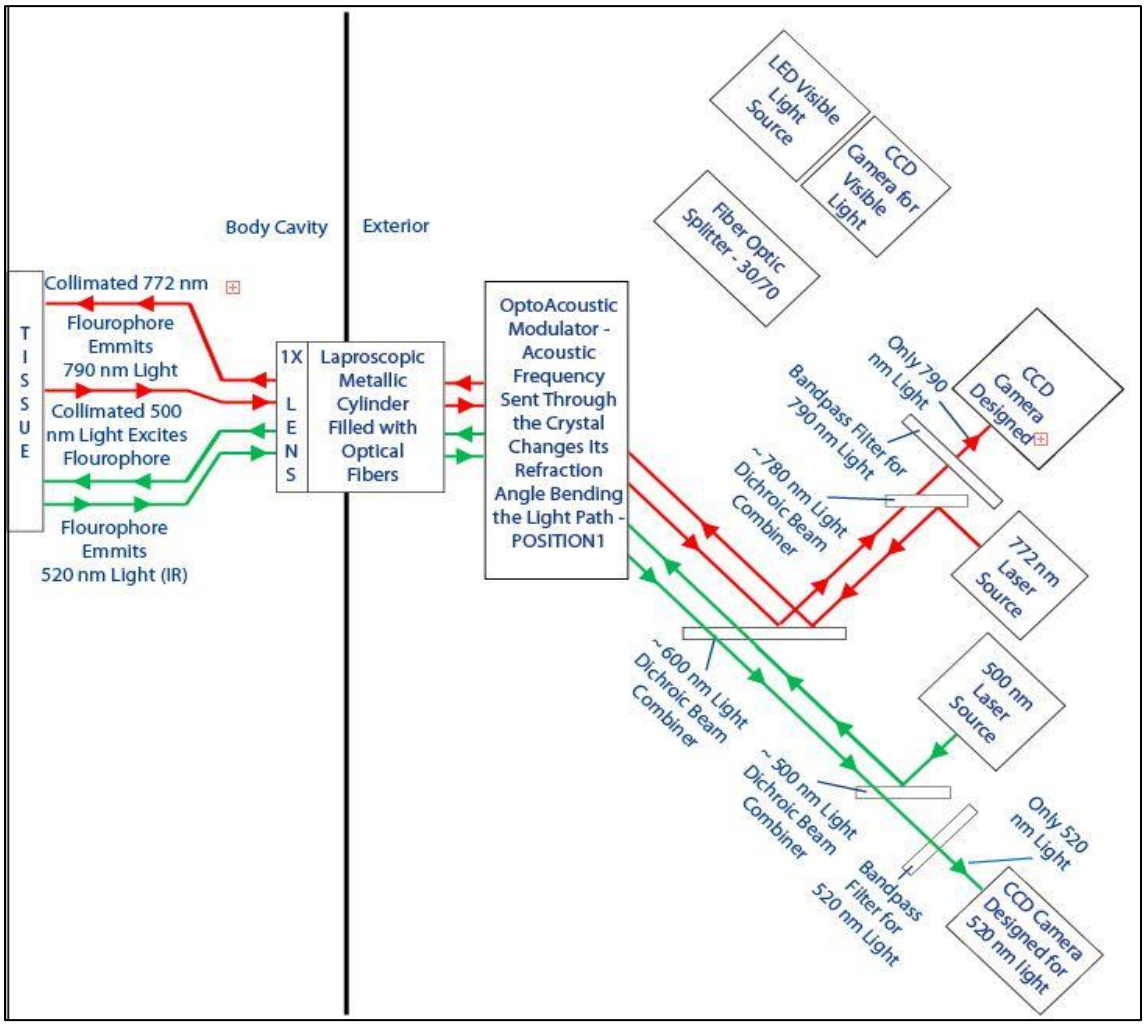


Figure 2 shows the optoacoustic switch in the position necessary to capture the fluorescent images. Dichroics are used to combine and split the necessary wavelengths of light. Dichroics are glass pieces that reflect light below a certain wavelength and transmit light beneath it. Depending on their orientation, they can be used to split or combine beams of light. Beam combining dichroics combing both the 500 nm and 772 nm excitation wavelengths and transmit them down into the body cavity to excite the fluorophores. The returning light is then sent through a beam splitter and the 520 nm and 790 nm emitted wavelengths are sent to their respective cameras.

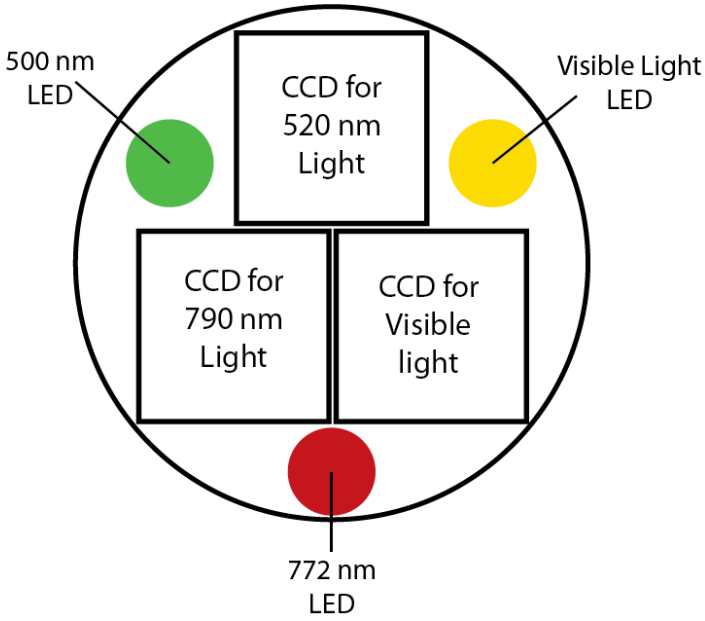
Although this design was very promising, after further research problems began to surface. A

large issue was found when it was determined that all light could not be sent and received down the same optical fiber bundles. Theoretically, light paths do not interfere with each other. However, in practice, light being sent down neighboring optical fibers in a bundle have a tendency to interfere with one another. This interference would be present in the final image and would be unacceptable. This problem could be solved by having each light transmitted through its own optical fiber and having each image being received through its own optical fiber. This solution also eliminates the need for an optoacoustic switch as each imaging system uses a separate light path. However, this solution greatly reduces the transmission of light through the laparoscope. This limitation of light transfer would greatly reduce the area that could be imaged at one time within the body cavity. Initial estimates for a theoretical area were only a few millimeters squared which would not be practical for surgical procedures.

FINAL DESIGN

To eliminate the need for optical fibers and the problems they present, a design in which the cameras and light sources were housed at the internal tip of the laparoscopic tube was again pursued. The components will be housed in a surgical-grade stainless steel tube, no more than 1.5 cm in diameter. As stated earlier, cameras and filters small enough for this application are not commercially available; therefore custom cameras need to be created. Each camera will consist of a custom ordered CCD panel with sufficient pixel density, custom cut and ground lenses to match the size of the CCD, and custom built high pass optical filters to filter the light incident on the CCD panel. The light sources would be a visible spectrum LED, a green LED with a custom low pass filter, and a near-infrared LED with a custom low pass optical filter. These components will be arranged as shown in Figure 3.

FIGURE 3.



The quality of a CCD is measured in megapixels, which is simply a count of the number of pixels on the CCD panel. Pixels correlate to capacitors on the CCD panel itself. Modern manufacturing techniques allow for these capacitors to be small enough so that each pixel corresponds to approximately 1.5 micrometer squared on the CCD panel. Theoretically the CCD panels could be 9 millimeters squared, which would allow for a sensitivity of 6 megapixels. However, in practice it is much more likely that the sensitivity will be in the 1-4 megapixel range.

The custom lens will determine the field of view of the camera; the larger the field of view, the lower the resolution of the image. This is because the pixel count is set, so when a larger image is represented by the same number of pixel as a comparable smaller image, it has much less detail to it. Future testing will decide what an appropriate field of view will be based on testing of the image quality produced.

Another limiting factor for this field of view is how large a section of tissue can be excited properly by our light sources. The visible light image will be captured first, followed by both fluorescent images. To achieve a frame rate of 30 frames per second these captures much alternate at 60 Hz. This would mean that the CCD sensors must be capable of 60 frames per second as they must only require an exposure time of 1/60th of a second. Light switching can be performed instantaneously and theoretically will not slow down the capture rate. Again, this rate is hypothetical, and in application frame rate will most likely be in the range of 20-30 frames per second. This is acceptable as modern high definition video

feeds only transmit at 24 frames per second. Another benefit of a lowered frame rate is a longer exposure time. A longer exposure time allows for more light to reach the CCD resulting in a better final image. With modern imaging software a mechanical shutter is not necessary.

The CCD sensors and LED lights will connect via electrical cables to an external microcontroller that is ultimately connected to a desktop computer. An application programming interface would allow the user to control the microcontroller with traditional imaging software. The pseudocode in Figure 4 shows how the imaging software would control the camera system. Open-source imaging software such as ImageJ could be used to separate the frames and assemble them into the 4 necessary individual video feeds.

FIGURE 4.

Image Capture Pseudocode

```
While (Capturing Images){
        Visible Light On;
        Capture Visible Image;
        Refresh Screen 1 to Visible Image;
        Visible Light Off;
        IR Light On;
        Capture IR Image;
        Refresh Screen 2 to IR Image;
        IR Light Off;
        Green Light On;
        Capture Green Image;
        Refresh Screen 3 to Green Image;
        Green Light Off;
        Refresh Screen 4 to Overlay of Green and IR Images;
}
```

ERGONOMICS

The device should be compact and easy to maneuver, so as to minimize fatigue and repetitive strain on the user, as well as interference with user mobility. A research study by the Department of Medicine at University of California, San Francisco showed that up to 90% of physicians who regularly perform endoscopic surgeries experience work-related injuries due to overuse and prolonged awkward postures [31]. To address this, the final design will be small, light, and streamlined so that it can be easily manipulated from multiple angles and able to interface with a hands-free support device, since there will be little need to move the camera during the surgery. There will be a single cable of ample length to allow a large range of motion and distance from the image display. Another consideration was the hands-free image display, which would allow the surgeon to view the procedure and change which color spectrum is displayed on command. This would enable accurate excision of the cancerous mass while minimizing distraction or need to perform multiple functions at once. Additionally, since the system will interface multiple hardware and software components, and it is assumed that not all users will have a background in electronics and computer science, it is important that the device and the operating software be simple and user-friendly. Some of these issues will be addressed in designing the image display and software components of the project in future work.

ETHICAL CONSIDERATIONS

An important aspect to consider in the design of any medical device is the ethical concerns created by the production or use of the technology. To ensure safety to patients and surgeons, the device will operate using only micro-currents running through insulated wires housed entirely within the device, with no electrical components exposed. All components of the design will be secure and durable so there is no risk of failure during or between procedures. Additionally, the laparoscope will be easy to sterilize after each use and made of surgical grade stainless steel, with no potential for patient or user exposure to bioreactive or harmful components. The device will comply with all professional and regulatory standards in regards to ethics to ensure a safe, effective design that can eventually be marketed for use in human

subjects. A protocol outlining the proper use and maintenance of the device will be developed, as well as a testing protocol containing guidelines for proper ethical procedure in animal testing, compliant with Institutional Animal Care and Use Committees (IACUC) provisions, for the clinical trials. Criteria listed on the UW Research Animal Resources Center website (http://www.rarc.wisc.edu/) will be followed to develop the animal testing protocol. When the device reaches the stages of human testing, a protocol consistent with standards of the Institutional Review Board for human subjects research will be written. All steps of the design process, product development, testing, and clinical trial and patient use will adhere to the FDA regulations as outlined in the 510K Medical Device Development procedure.

Present tumor excision uses X-ray and other imaging techniques to locate the tumor, after which the surgeon cuts out the cancer some of the surrounding tissue to ensure complete removal of the harmful mass. Due to the ability of CLR-1502 and CLR-1501 molecules to accumulate in high concentrations in cancer cells, it is thought that directly imaging and removing the fluorescing tissue will eliminate cancer cells in the body more effectively and completely than current methods. One possible issue is that due to lack of imaging device sensitivity to fluorophores or low visibility of fluorophores in far-outlying cancer cells, the procedure may not be as accurate as assumed, and the surgeon may inadvertently miss some of the cancerous tissue. While this already happens frequently in current surgeries, the expected success of the device may lead to less caution in follow-up practices or a false sense of security for both the patient and doctor. Post-operative recurrence can have devastating effects, especially if it is not detected immediately. Studies on abdominal cancer show that the majority of deaths of gastric cancer patients are attributed to peritoneal recurrence [32]. It is estimated that 22% of gastric cancer patients who undergo tumor removal surgery experience recurrence [33]. Of those who develop peritoneal recurrence after curative surgery, the average survival rate is about 6 months [34]. The hope is that the molecules developed by Dr. Weichert, along with an effective imaging system, will greatly reduce recurrence rates like this. However, since it is such a newly developed technology, it still needs to undergo extensive testing and clinical trials, and close monitoring for return of cancer after use as a marketed product.

Another consideration is that the device must be cost-effective so that it is widely available. Too often, new healthcare technology is not practical because it cannot help a large range of people; it is so expensive that only the wealthy or privileged have access to it. In order to maximize the benefit, this technology not only needs to be an improvement in terms of procedural efficiency and precision, but also address society's growing need for the most effective treatments to be commonly available. To achieve this, the device must be inexpensive and easy to manufacture on a large scale.

FUTURE WORK

Photon Budget Analysis

Before fabrication of the device commences, a thorough photon budget analysis should be conducted to test the proof of concept and ensure the correct optical components are selected. The following is a brief procedure on how a photon budget analysis may be conducted based off a similar analysis for fluorescence microscopy [35]. The first step is to get an estimate of the power of the light source that will be needed for sufficient excitation. This can be done experimenting on a set voxel of the specimen. Next, the number of fluorescence molecules per voxel should be determined by multiplying fluorescent molecule concentration by Avogadro's number then by the volume of the voxel. Assuming the number of photons emitted by each molecule before photodestruction ends is known, the average number of photons per recording can be determined. The quantum yield (the ratio of emitted photons to absorbed photons) must be taken into account, considering not all photons that enter the volume will be admitted by the volume, due to factors such as reabsorption. An absorption coefficient for one voxel path length can be found using the known molar extinction coefficient and the depth of one voxel. Now that the number of necessary excitation photons is known, the transmission efficiencies of the filters and lens need to be taken into account. In order to determine these numbers the pixel size, magnification, numerical aperture of the lens and excitation wavelength must be known. At this point, only the number of excitation photons per second required for illumination of entire field of view can be found, and consequently the required power of the light source can be found.

After determination of the required power for the light source, the signal to noise ratio at the detector should be taken into account. The numerical aperture is needed to determine the number of photons that can be captured by the lens. Possible noise sources that should be accounted for include photon noise, dark current noise, readout noise, and quantization noise. This calculation will determine the minimum power of the light source necessary to illuminate the fluorescent tissue and the sensitivity of the CCD needed to provide a high-quality image throughout the surgical procedure.

Fabrication

The next step in the design process is the fabrication of a prototype. Components will be ordered based on the requirements outlined by the photon calculation. In addition, a program must be written that will execute the pseudocode outlined earlier. ImageJ would preferably be used to control the microcontroller that controls the laparoscope's internal components. Once the device has been assembled, testing of efficacy can begin. Standards for testing the efficiency of the device must be set, so that illumination effectiveness and image quality can be quantified. Fabrication will continue in a reiterative process, as testing indicates necessary changes to the device, until the device consistently produces high-quality images at the proper intensity. Once there is confidence in the functionality of the internal components, the system will be ready for packaging in the stainless steel laparoscopic tube. The device will then undergo further testing under a standardized protocol before it can be made available for clinical trials.

Testing and Additional Future Work

Before conducting any tests on the prototype, a standardized testing protocol will be written. The protocol will be followed to ensure that the evaluation is thorough and indiscriminate In addition, the effectiveness of the light sources should be assessed, if the light sources are unable to excite fluorophores in a large enough area a stronger light source, such as a laser light source, may be considered. The photon calculations allow for an estimation from which the original components for the prototype can be selected; however, the testing will provide a more concise measure of device efficacy and may lead to

changes in the final design. Appropriate statistical analysis will be performed on the results to compare the luminescence efficiency to modern imaging methods. In addition, the optical system program will be tested to insure the lights respond as needed during use. After the modifications have been made to the design following initial testing, testing will repeat according to the protocol. If additional tests or alterations to the tests are needed, the protocol will be edited appropriately.

Upon completion of fabrication and standard testing of the final product, a user manual will be written. At this point, the final product can be used for clinical trials in animals with protocols in place to ensure proper use and ethical conduction of trials is followed. A protocol for clinical trials which adheres to FDA 510K and IACUC standards will be developed. As this product is intended to be sold on the open market, it will be important to pursue a patent on the design to prevent the competition from using any design features for their own marketable product. The U.S. Patent and Trademark Office guidelines will be followed.

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APPENDIX

Appendix A: Product Design Specifications

Our client, Professor Thomas "Rock" Mackie, is in need of an endoscopic camera that will be used during the surgical removal of cancerous tumor cells. Recent work in Jamey Weichert's UW lab has provided two fluorescent phospholipid ether small molecules, CLR-1501 and CLR-1502. These compounds are fluorescent versions of CLR-1404, a current radiolabeled cancer drug under clinical trial. A typical cancer patient would be injected with CLR-1501 and CLR-1502 two or more days before surgery. These compounds build up in tumor cells allowing the surgeon to efficiently and wholly remove any cancerous material. The surgeon must be able to view these cells during their excision, to accomplish this they must be excited with a specific wavelengths of light so that they fluoresce. Our team will be creating an endoscopic camera with an LED assembly and self-cleaning mechanism to allow the surgeon to effectively view these cells.

The LED light assembly will sequentially emit light at 500 nm (excitation wavelength for CLR-1501), 772 nm (excitation wavelength for CLR-1502), and a visible white light. The switching of the LED assembly will be timed to the frame rate of the camera, allowing the camera to capture three different images every three frames, one at 520 nm (emission spectrum for CLR-1501), 790 nm (emission spectrum for CLR-1502), and the third capturing all visible light. These three frames will then be separated into three different live video feeds, with each feed having a frame rate one third that of the camera and displaying its corresponding light spectrum image. The images will be displayed on a hands free monitor system. The endoscopic camera must also be able to clean the camera lens well within the patient's body. Finally the camera and its lighting/cleaning assembly must all fit through a two cm incision in the patient.

Equipment and funding will be provided to us by Thomas Mackie. Our team will also have access to the rapid prototyping equipment at MIR. Finally Jamey Weichert will supply fluorescent compounds and existing minimally invasive surgical cameras and lighting systems that are currently available.

Appendix B: Semester Timeline

Tasks	September				October				November					Dec	
	7	14	21	28	5	12	19	26	2	9	16	23	30	7	14
Product R&D															
Background Info	X	X	X	X	X										
Design Alternatives						X	X		X	X	X				
Final Design							X	X				X	X		
Materials							X	X					X	X	
Construction															
Testing															
Presentations															
Mid-Semester							X								
Final															X
Deliverables															
Progress Reports	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mid-Semester Report								X							
Final Report													X	X	X
Meetings															
Team Meetings	X					X	X	X	X				X	X	X
Advisor Meetings	X	X	X	X			X	X	X		X			X	X
Client Meetings		X	X		X	X	X		X				X		
Website	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X