Clinical confocal microlaparoscope for real-time in vivo optical biopsies

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Abstract. Successful treatment of cancer is highly dependent on the stage at which it is diagnosed. Early diagnosis, when the disease is still localized at its origin, results in very high cure rates—even for cancers that typically have poor prognosis. Biopsies are often used for diagnosis of disease. However, because biopsies are destructive, only a limited number can be taken. This leads to reduced sensitivity for detection due to sampling error. A real-time fluorescence confocal microlaparoscope has been developed that provides instant in vivo cellular images, comparable to those provided by histology, through a nondestructive procedure. The device includes an integrated contrast agent delivery mechanism and a computerized depth scan system. The instrument uses a fiber bundle to relay the image plane of a slit-scan confocal microlaparoscope into tissue. It has a 3-μm lateral resolution and a 25-μm axial resolution. Initial in vivo clinical testing using the device to image human ovaries has been done in 21 patients. Results indicate that the device can successfully image organs in vivo without complications. Results with excised tissue demonstrate that the instrument can resolve sufficient cellular detail to visualize the cellular changes associated with the onset of cancer. © 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3207139]

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1 Introduction

This paper describes the first clinical confocal microlaparoscope system. We describe the instrument and how it evolved during clinical testing. We present both in vivo and ex vivo results showing that the device has the ability to nondestructively resolve cellular detail and has the potential to diagnose cancer in situ.

Cancer frequently goes undetected until it reaches a late, difficult-to-treat, stage. Cancers that develop in epithelial cells (carcinomas) are the most common type of malignancy, and they account for 90% of all human cancers. Development of imaging devices that can more thoroughly interrogate epithelial surfaces for abnormalities will enable earlier detection of
cancer, resulting in significant improvement in patient survival.

The most effective treatments in terms of patient outcomes, quality of life, and cost are those that target cancer during its early development, before it has metastasized. Early diagnosis, when the disease is still localized at its origin, results in very high cure rates—even for cancers that typically have poor prognosis. Early detection provides the physician with more treatment options and the ability to use less invasive methods. Unfortunately, many cancers are not found until later stages due to inadequate detection methods or low-sensitivity diagnostic techniques.

Initial detection and diagnosis of cancer is frequently based on biopsies. Small samples of tissue are removed, and a pathologist makes a diagnosis using thin sections of stained and processed tissue. Since biopsies require removal of the tissue, only a limited number can be taken. Sampling error plays a significant role in achieving accurate early detection of cancer. Development of devices that can nondestructively interrogate epithelial surfaces for abnormalities would reduce sampling error.

Many groups are investigating new technologies to nondestructively image tissue at high resolution for the early diagnosis of cancer. Some of the promising methods are confocal microscopy,2–10 two-photon microscopy,11,12 spectroscopy,13–18 and optical coherence tomography (OCT).19–25

Conventional bright-field microscopy plays a central role in the diagnosis of disease from carefully prepared biopsy slides. Bright-field images of bulk tissue appear blurry due to simultaneous collection of out-of-focus planes. Improved imaging of thick samples can be achieved with confocal microscopy. Confocal images of bulk tissue are sharp because light from out-of-focus planes is rejected via a confocal aperture.

Since the confocal microscope alleviates the need for cutting tissue into thin sections, it has significant potential for imaging of tissue in situ. However, a standard confocal microscope is a large device that is not well suited for in vivo assessment of epithelial surfaces inside the human body. We have previously reported on technology developed to enable in vivo confocal imaging using a coherent fiber optic bundle.26–30 In this paper, we describe development of a confocal microlaparoscope.

A confocal microlaparoscope offers the potential for cellular imaging of a wide range of laparoscopically accessible organs. Almost every organ in the human body can be accessed using a laparoscope via a small incision.31 Organs that can be accessed in this manner include the stomach, intestines, pancreas, liver, esophagus, spleen, kidneys, peritoneal wall, bladder, gall bladder, lymph nodes, reproductive organs in females, and prostate in men. Laparoscopic surgery has significant advantages over open surgery. It minimizes the overall trauma to the skin and muscles, patient recovery time is shorter, and the infection rate is significantly reduced.

In the following sections, we discuss the four-year development of a mobile confocal microlaparoscope imaging system. To demonstrate the device’s potential, we present in vivo and ex vivo human imaging results.

2 Clinical Confocal Microlaparoscope System

We have developed a complete clinical confocal microlaparoscope system that can be wheeled into an operating room and set up to image within a few minutes. The system consists of a confocal microlaparoscope (hereafter referred to as a microlaparoscope) connected to a mobile cart housing an optical scan unit (OSU) and control systems. Once the system is running, the surgeon can interrogate regions of interest with the microlaparoscope; view live cellular images on the screen; and selectively record videos, depth scans, multispectral images, and still frames. Figure 1 shows the instrument in use in the surgical suite. The mobile cart is on the right, and the surgeon using the microlaparoscope is in the middle looking toward the left, where the gross anatomy is visible on a standard wide-field laparoscope screen. Directly adjacent to this
The microlaparoscope provides live images of excited fluorescence at 30 frames per second with an optical resolution of $3 \, \mu \text{m}$ laterally and $25 \, \mu \text{m}$ axially. The system frame rate is fundamentally limited by the acquisition rate of the CCD camera used for detection. Fluorescent contrast agents are delivered locally to the field of view using an integrated contrast agent delivery system. Using controls on the microlaparoscope, the surgeon can adjust focus from the nominal epithelial position down to an optical limit of $200 \, \mu \text{m}$ below the surface. Table 1 provides a detailed listing of the system’s specifications.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral resolution</td>
<td>$3 , \mu \text{m}$</td>
</tr>
<tr>
<td>Axial resolution</td>
<td>$25 , \mu \text{m}$</td>
</tr>
<tr>
<td>Collection elements (pixels)</td>
<td>30,000</td>
</tr>
<tr>
<td>Frame rate</td>
<td>30 fps</td>
</tr>
<tr>
<td>Excitation wavelength</td>
<td>488 nm</td>
</tr>
<tr>
<td>Spectral collection (150 spectral images)</td>
<td>6 s</td>
</tr>
<tr>
<td>Average spectral resolution</td>
<td>6 nm</td>
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<tr>
<td>Spectral collection</td>
<td>500 to 750 nm</td>
</tr>
<tr>
<td>Port compatibility</td>
<td>5 mm trocar</td>
</tr>
<tr>
<td>Dye delivery precision</td>
<td>50 nL</td>
</tr>
<tr>
<td>Numerical aperture in tissue</td>
<td>0.46</td>
</tr>
<tr>
<td>Micro-objective magnification</td>
<td>1.5</td>
</tr>
<tr>
<td>Chromatic correction</td>
<td>488 to 660 nm</td>
</tr>
<tr>
<td>Maximum imaging depth</td>
<td>200 $\mu \text{m}$</td>
</tr>
<tr>
<td>Field of view</td>
<td>450 $\mu \text{m}$</td>
</tr>
</tbody>
</table>

The microlaparoscope cable connects to the OSU. The secondary display (right) is placed next to the operative field for live in vivo imaging.

In the following sections, we describe the mobile cart, the associated systems that it houses, and the functions that it provides. Then we will discuss the evolution of the microlaparoscope, beginning with the early prototypes and ending with the state-of-the-art instrumentation currently being used in human clinical trials. Details concerning the integrated in-handle axial focus and contrast agent delivery systems are also presented.

2.1 Mobile Cart

The mobile cart houses the OSU and the control systems. The instrumentation fits on a standard endoscopy cart that has a 61 cm by 41 cm footprint. Figure 2 depicts the components on the mobile cart. The top shelf houses the sterile microlaparoscope and the surgical supplies that are used during the imaging procedure. The microlaparoscope cable connects to the OSU located on the second shelf. The third and fourth shelves house the laser, function generator, and computer. The bottom shelf houses a medical-grade isolation transformer. The operator display and controls for collecting data during surgery are located on a platform at standing height. A second mobile display is placed next to the operative field for viewing nearby the surgeon.

The system has been designed to streamline all operations during surgery. Once the system is plugged in and the safety interlocks engaged, the system starts, and all hardware is initialized. Hardware initialization includes the solid-state laser,
camera, dye delivery system, focus system, and function generator for scan mirror control. After the automatic initialization, the operator is presented with the software control interface for live imaging.

2.1.1 Optical scan unit

Miniaturization of the OSU required a redesign of the previous bench-top system. By simplifying the optical layout and integrating the multispectral imaging segment into a common collection path, we were able to fit the whole scan system into a 50 cm by 30.5 cm housing. The housing also integrates the scan mirror power supply and control electronics, which were external in the previous system.

The components of the OSU are shown in Fig. 3. The top portion illustrates the standard grayscale mode of operation. A 488-nm laser is anamorphically shaped into a line and scanned onto the coherent fiber bundle in the microlaparoscope. The excited signal re-enters the system, is descanned, and is filtered through a confocal slit. The light is rescanned onto a two-dimensional 2-D CCD camera. In the spectral imaging mode (bottom inset), the image scan mirror is turned to its extreme position, redirecting the light through a dispersing prism.

Figure 3 Diagram of the OSU. The top portion illustrates the standard grayscale mode of operation. A 488-nm laser is anamorphically shaped into a line and scanned onto the coherent fiber bundle in the microlaparoscope. The excited signal re-enters the system, is descanned, and is filtered through a confocal slit. The light is rescanned onto a two-dimensional 2-D CCD camera. In the spectral imaging mode (bottom inset), the image scan mirror is turned to its extreme position, redirecting the light through a dispersing prism.

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Tissue fluorescence is collected back through the microlaparoscope and descanned by the object scan mirror. The dichroic filter passes the fluorescence signal, which is focused down onto a stationary confocal slit. The light exiting the slit is recollimated and rescanned using the image scan mirror. An emission filter removes residual excitation light. The beam is refocused back into a line that sweeps across the camera to build up a two-dimensional (2-D) image every thirtieth of a second.

In addition to 2-D grayscale imaging, the system can also collect multispectral data. This multispectral mode is activated in a fraction of a second via a software actuation button that deflects the image scan mirror to its extreme position (shown in the bottom of Fig. 3). During spectral collection, the image scan mirror is held stationary. The light passes through a prism dispersing the signal across the CCD. The CCD camera records spectral information in the direction perpendicular to the slit and one dimension of spatial data in the dimension parallel to the slit. The second spatial dimension of the image is collected over time by recording multiple frames as the object scan mirror is slowly stepped. The complete spectral data collection procedure executes in a few seconds. Once spectral collection is complete, the system reverts back to real-time grayscale operating mode.

2.1.2 Software control system

A software package that interfaces with the data collection devices and the system controls was written using Objective C 2.0 and Cocoa with automatic garbage collection on Mac OS X. Figure 4 provides a general overview of the software and hardware interface. The software links the surgical controls on the microlaparoscope to the control systems that manage contrast agent delivery, focus, and data acquisition. Parallel processing allows the software to run smoothly while live images are acquired, processed, and encoded at 30 frames per second. A custom image processing memory pool class enables efficient resource management under automatic garbage collection.
2.2 Confocal Microlaparoscopes

Use of the surgical microlaparoscope entails placing the distal end in contact with the tissue, locally delivering fluorescent contrast agents to the field of view, adjusting focus, and collecting the resultant fluorescent confocal image.

To create the surgical microlaparoscope, we had to develop (1) a reliable focus mechanism, (2) a localized contrast agent delivery system, and (3) an ergonomic sterilizable housing with surgical controls compatible with a 5-mm-diam trocar instrument port. The development process encompassed three generations of instruments, with the final version meeting all of the requirements. Table 2 summarizes the key properties of the three designs.

In all three designs, the microlaparoscope contains a 30,000-element fiber bundle connected via an subminiature version A (SMA) connector to the OSU. The OSU generates a line of laser illumination that sweeps across the proximal face of the fiber. The fiber bundle spatially relays the illumination pattern to the distal end of the fiber bundle, where a miniature 0.46 NA objective lens images the illumination line to the desired tissue depth. Image depth is controlled by adjusting the axial spacing between the fiber bundle and the miniature objective lens. Contrast agent is locally delivered to the tissue through the microlaparoscope. The excited fluorescent signal is collected by the miniature lens and relayed back through the fiber bundle into the OSU.

2.2.1 First-generation design

The first-generation design—depicted in Fig. 6—utilized a sterilizable semirigid 5-mm-diam by 500-mm-long dual-lumen polycarbonate sleeve through which a nonsterile imaging catheter was inserted. This simple design allowed us to rapidly move to clinical testing by creating a sterile housing for our existing 3-mm flexible imaging catheter. The polycarbonate also integrated a small secondary lumen to deliver dye locally to the field of view. The front end of the polycarbonate sleeve was sealed with a 160-μm-thick glass window. A 500-μm-diam secondary lumen, used to deliver contrast

Table 2 Comparison of key properties for the three generations of microlaparoscopes.

<table>
<thead>
<tr>
<th></th>
<th>First Generation</th>
<th>Second Generation</th>
<th>Third Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSU connection</td>
<td>fixed</td>
<td>SMA</td>
<td>SMA</td>
</tr>
<tr>
<td>Cable length</td>
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<td>6 m</td>
<td>6 m</td>
</tr>
<tr>
<td>Focus type</td>
<td>motorized</td>
<td>manual</td>
<td>motorized</td>
</tr>
<tr>
<td>Focus control location</td>
<td>cart</td>
<td>handle</td>
<td>handle</td>
</tr>
<tr>
<td>Focus precision</td>
<td>20 μm</td>
<td>5 μm</td>
<td>0.5 μm</td>
</tr>
<tr>
<td>Dye control location</td>
<td>cart</td>
<td>handle</td>
<td>handle</td>
</tr>
<tr>
<td>Dye precision</td>
<td>3 μL</td>
<td>1 μL</td>
<td>50 nL</td>
</tr>
<tr>
<td>Axial position recording</td>
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<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>z-scan capability</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>
agent, ran parallel to the primary lumen. A 200-µm-diam hole in the window was placed over the secondary lumen, allowing contrast agent delivery to the tissue.

The rear end of the polycarbonate housing contained the couplings to mate the imaging catheter and the dye delivery line. In the operating room, the compression coupling of the sterilized polycarbonate housing was loosened, and the non-sterile imaging catheter was fed through the main 3.1-mm lumen until the miniature objective was in contact with the glass window. The fitting was tightened, and then a sterile plastic cover was slid back over the portion of the imaging catheter that was in the operative field. A Luer Lock fitting coupled the fluid lumen to a 0.58-mm-diam medical-grade Teflon tubing that ran back to the mobile cart.

The 3-mm flexible catheter consisted of a miniature objective lens connected to durable polyetheretherketone (PEEK) tubing. The 30,000-element fiber optic bundle ran through the lumen of the PEEK tubing. Originally, focus was accomplished by a manual micrometer at the proximal end that moved the fiber bundle relative to the PEEK housing. However, focus control was unreliable due to an inexact fit of the fiber bundle inside the PEEK tubing. This resulted in hysteresis in the proximal movement of the fiber bundle relative to the fixed lens. Various methods were studied to improve the focus system. Ultimately, the manual focus micrometer was replaced by a computerized stepper motor. A hysteresis correction algorithm was developed to position the distal fiber face with an accuracy better than the system’s axial resolution. Figure 7 illustrates the ability of the first-generation focus system to image the epithelial surface of tissue (mouse peritoneal wall) and then to controllably focus the underlying tissue layers in 25-µm increments.

Dye delivery was accomplished by loading a syringe with fluorescent contrast agent. The syringe was then inserted into a syringe pump located on the mobile cart. The syringe was connected to the secondary lumen of the polycarbonate housing through 0.58-mm-diam medical-grade Teflon tubing. Software control of the syringe pump enabled delivery of 1-to 3-µL droplets of contrast agent onto the tissue surface near the field of view of the miniature objective. Figure 8 shows a photograph of the distal end of the microlaparoscope with a droplet of dye coming out through the hole in the glass window.

The device worked well using the 1.2-m-long imaging catheter. However, it was determined that a longer length imaging catheter was needed in the operating room. With the longer length catheter, the focus motor and the syringe pump were located far from the distal microlaparoscope tip, and...
focus would drift during use. Delivery of small droplets of contrast agent was unreliable. The surgeon also noted that positioning the proximal tip accurately was difficult because the polycarbonate housing was not rigid enough.

### 2.2.2 Second-generation design

A second-generation device was developed to address the issues concerning device rigidity, focus drift, and reliability of contrast agent delivery across a 6-m-long connection between the microlaparoscope and the OSU. The second design abandoned the use of the existing flexible imaging catheter. To address the reliability issues of focus and dye delivery in a 6-m-long device, the controls for focus and dye delivery were located close to the distal tip. To address the rigidity issue, a rigid instrument was made using a stainless steel tube with an inner diameter matching the fiber bundle’s outer diameter. Figure 9 shows a diagram of the second-generation microlaparoscope.

The miniature objective attached to the distal end of the 3-mm-diam rigid stainless steel tube. The fiber bundle ran through the 1-mm inside diameter of the rigid tube. To adjust focus, the fiber bundle moved axially relative to the fixed lens position. The tube and fiber bundle were held in place by a round handle containing a manual focus mechanism. Rotation of the handle knob resulted in precise movement of the proximal fiber face relative to the rigid tube. To deliver contrast agents, a 21-gauge hypodermic tube ran parallel to the 3-mm rigid tube. Both tubes were sealed inside medical-grade fluorinated ethylene-propylene (FEP) heat-shrink tubing, resulting in a final 5-mm outer diameter. At the distal tip, the hypodermic tube made a 90° bend, dispensing contrast agents at the edge of the imaging field. At the back end of the handle, the hypodermic tubing passed into a computer-controlled piezo valve immediately downstream from a pressurized syringe. A 6-m-long flexible cable ran from the handle to the OSU. The cable contained the fiber bundle and piezo valve electrical wires. The proximal end of the protective housing broke out into optical and electrical connectors. A standard SMA connector coupled the fiber bundle to the OSU.

Compared to the first-generation system, the second-generation system was much easier to use because the whole microlaparoscope could be sterilized and then quickly connected to the OSU in the operating room. In contrast, the first-generation system required careful placement of the sterilized polycarbonate housing over the nonsterile imaging catheter permanently connected to the OSU. The rigid stainless steel tubing solved the rigidity issues, enabling the surgeon to precisely position the device. A press of a button at the operator console enabled rapid delivery of a small droplet of contrast agent, as shown in Fig. 10. Focus was extremely reliable and never drifted. In fact, during typical use, the surgeon would set the focus at the epithelial surface and image multiple sites without needing to refocus.

Although the second-generation device addressed the primary issues encountered with the first-generation instrument, there were additional improvements that were important to make a successful clinical tool. Even though the manual focus system was reliable, a computerized system was desired to enable depth scans. Second, to allow the device to be controlled more efficiently by the surgeon, the microlaparoscope needed to integrate basic controls for focus, dye delivery, and data recording into the handle. The dye delivery system also needed some refinement. In the second-generation device, to ensure repeatable delivery of small contrast agent droplets, the pressure in the syringe had to be well regulated at a relatively high 1.76 kg/cm². This pressure would sometimes cause contrast agent to squirt out of the distal tip rather than pool up in a localized droplet.

### 2.2.3 Third-generation design

To implement these improvements, a third-generation device was developed. The new microlaparoscope, shown in Fig. 11, has an ergonomic handle, control buttons, a computerized focus system, and a refined contrast agent delivery system. Control of focus and dye delivery are consistent and reliable since
the control systems were miniaturized and located in the handle only 35 cm from the distal end of the device. A non-pressurized dye delivery system removed the problem encountered with dye ejection.

Mechanically, the third-generation microlaparoscope followed the same design principles as the second-generation device. Figure 12 illustrates the mechanical aspects of the device. The fiber bundle couples to the focus motor inside the handle. A 1-cm³ syringe with contrast agent is placed into a spring-loaded receiver at the front end of the handle. A second miniature motor in the handle acts as a plunger for the syringe. A spring forces the syringe to mate with a Luer Lock connection that guides the fluid down a 21-gauge hypodermic tube.

A customized tip helps to channel the dye and minimize tissue abrasion. The new tip is a 20-mm PEEK housing mated to the FEP outer tubing. A tiny 150-μm lumen inside the PEEK couples the hypodermic fluid line routing the dye to an exit port at the edge of the system’s field of view. In the second-generation design, the hypodermic tubing was bent around the tip of the miniature objective and had the potential to abrade the tissue surface. The new smooth tip helps to protect the tissue surface and allows the fluid exit port to be placed closer to the edge of the imaging field of view.

The handle contains four ergonomically positioned controls. Two upper thumb controls on the back allow the surgeon to adjust focus and acquire a depth scan. The trigger button saves still frames with a short press and acquires video with a long press. The side button delivers a predefined amount of contrast agent to the field of view.

The electrical wires and the fiber bundle are routed through the handle into a flexible 6-m protective cable. The rear end of the cable breaks out into an electrical connector and an SMA connector. Both connections couple to receptacles on the OSU. The electrical connection routes through the electronics of the OSU and exits as a single USB cable connecting to the computer which link the buttons and motors to the control software. The SMA connector couples the fiber bundle to the OSU.

The third-generation microlaparoscope represents a viable surgical tool. Its rigid probe enables precise positioning control. The in-handle computerized focus system enables axial focus and depth scans with accurate 1-μm positioning. The contrast agent system delivers volumes with precision down to 50 nL. Last, the whole device has an ergonomic design that is comfortable for the surgeon to use and provides controls for all the tasks that the surgeon needs to perform.

3 Results
As previously mentioned, laparoscopic techniques can be used to access most organs in the body. To demonstrate the
clinical viability of the confocal microlaparoscope system, we present results from two organs imaged using the device. The first set of results are in vivo and ex vivo images of the epithelial surface of ovarian tissue. Since there is no reliable way to detect ovarian cancer in its early stages, the microlaparoscope could be used to detect ovarian cancer in high-risk women. The second set of results are ex vivo images of human esophagus. Although laparoscopic esophageal surgery is a recent innovation, it is now second only to biliary tract surgery in the frequency of minimally invasive procedures performed in everyday surgical practice. The microlaparoscope’s ability to visualize the cellular boundaries of tumors could improve the success rates in laparoscopic staging of carcinoma of the esophagus and laparoscopic esophagectomy.

3.1 Ovarian Images

Currently, the microlaparoscope is being evaluated in a clinical trial to image the epithelial surface of the ovary at the University of Arizona’s Medical Center in Tucson. The device was granted “nonsignificant risk” status by the University of Arizona’s Institutional Review Board and has been approved for use in humans using a protocol that includes topical application of contrast agents. To date, 21 patients have been imaged in vivo.

The current protocol entails imaging human ovaries in vivo before oophorectomy or hysterectomy. The imaging protocol begins with the surgeon locating the ovary and isolating it in an endobag with the ovary still connected to the blood supply (see Fig. 13). Once the ovary is isolated, the surgeon brings the tip of the microlaparoscope into contact with the epithelial surface. Then the surgeon delivers the contrast agent, and live imaging begins. The endobag protects the patient from inadvertent exposure to contrast agent. After the microscopic imaging is done, the surgical procedure is completed as normal, and the ovaries are removed. The removed ovary is typically imaged again ex vivo using the microlaparoscope with additional contrast agents. Biopsies are also taken for correlated pathology.

For the initial in vivo clinical studies, fluorescein sodium was selected because of its existing track record of safe use in humans. (All patients participating in the clinical trials were consented and imaged in accordance with human subjects protocols approved by the Institutional Review Board of the University of Arizona.) Although fluorescein provides limited diagnostic contrast when applied to the surface of the ovary, it is a safe contrast agent and allowed us to test the safety and basic functionality of the microlaparoscope system in vivo.

Figure 14 shows nine examples of in vivo images obtained with the microlaparoscope system. The images demonstrate that the device functions as designed. The microlaparoscope can deliver controlled volumes of dye to the image site and then display real-time cellular-level images to the surgeon. The focus mechanism works well. After an initial adjustment of the focus, the instrument can be moved to various sites on the ovary while still maintaining good focus on the epithelial surface.

After in vivo imaging with fluorescein, the surgeon removes the ovaries. The ovaries are reimaged ex vivo using acridine orange (AO). Compared to fluorescein, AO provides superior diagnostic contrast. Example images with AO, shown in Fig. 15, demonstrate the excellent cellular-level contrast achievable with the instrument. The epithelial surface of a healthy ovary is characterized by a homogeneous distribution of bright nuclei, as seen in Fig. 15(a). The epithelial surface cells of the ovary are delicate, and partial denuding can occur, exposing the underlying stroma [Fig. 15(b)]. Below the epithelial surface, healthy stroma also exhibits a characteristically homogenous structure albeit with a different nuclear size distribution and shape [Fig. 15(c)]. In the case of ovarian cancer, the tissue structure is visibly different [Fig. 15(d)]. The epithelial surface is irregular, and the high degree of heterogeneity in the size and spatial distribution of nuclei is indicative of ovarian cancer.
We have previously shown\(^1\) that the microlaparoscope system can easily differentiate between normal epithelium and ovarian cancer using automated algorithms. It also appears that the microlaparoscope system may be able to visualize cellular changes that happen prior to the onset of cancer. Less distinct tissue changes such as tissue sclerosis may also be detectable [Figs. 15(e) and 15(f)].

With the success of our initial testing with fluorescein, we have now begun imaging \textit{in vivo} with AO. (Approval from the FDA to use AO in this context has been granted under IND 102603.) Figure 16 shows our preliminary results. These images depict the same homogeneous structure of nuclei as seen in Figs. 15(a) and 15(b). Denuding of the delicate epithelium is also visible in some of the images. By optimizing the concentration of AO and giving surgeons more time to practice using the device, we believe we will achieve results comparable to the images obtained \textit{ex vivo}.

### 3.2 Esophagus Images

We have conducted \textit{ex vivo} imaging of esophagus tissue biopsies from more than 30 patients. Figure 17(a) shows an example of normal squamous epithelium. Figure 17(b) shows epithelial tissue that closely resembles the intestine, with columnar-appearance mucosa and intestinal metaplasia, a condition known as Barrett’s esophagus. Finally, Fig. 17(c) illustrates a tumor in the esophagus.

These images illustrate the device’s ability to resolve the cellular details of tissue in the esophagus. Nuclear distributions are easily characterized, and morphological tissue changes can be readily discerned. In the case of laparoscopic esophagectomy, the microlaparoscope would allow the surgeon to optically biopsy suspect tissues to potentially find locations containing cancer.

### 4 Conclusions and Future Work

A mobile confocal microlaparoscope system capable of performing live optical biopsies \textit{in vivo} has been developed. The system is currently being evaluated in clinical trials to assess its safety and efficacy for detecting ovarian cancer. Results show that the instrument is safe and can successfully image ovaries \textit{in vivo}.

The \textit{in vivo} and \textit{ex vivo} results indicate that the microlaparoscope can resolve sufficient cellular detail to detect the transformation of normal epithelium and visualize cellular changes that happen with the onset of cancer. As we continue testing, we hope to show that the device can obtain the same high-quality images \textit{in vivo} as have been obtained \textit{ex vivo}.

Because the microlaparoscope can nondestructively provide real-time cellular images \textit{in vivo}, it has the potential to significantly improve the rate of early cancer detection in laparoscopically accessible organs. Today, early detection of cancer is highly dependent on the selection of biopsy sites. Since biopsies are destructive, only a few are typically taken. If cancer is in its early stages, it will often be small, reducing the probability that it will be biopsied. Moreover, it takes time to
get the biopsies processed and evaluated. However, because the microendoscope nondestructively acquires optical biopsies instantly, a much larger region of tissue can be interrogated at the cellular level. Additionally, if the surgeon finds a small cancer, the microendoscope could be used to locate the cancer boundaries allowing for immediate resection. Thus, the device might improve the probability of early cancer detection and help the surgeon fully resect the tumor during the same procedure.

In the future, we will continue to investigate the wide range of potential applications for the confocal microendoscope system. Since the microendoscope is based on an inherently flexible fiber bundle, we plan on making a device with a flexible probe. We have previously shown a flexible imaging catheter and plan to apply the technology developed for the microendoscope to a new confocal microendoscope for interrogating the gastrointestinal tract.

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