Clinical evaluation of a confocal microendoscope system for imaging the ovary

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ABSTRACT

We have developed a mobile confocal microendoscope system that provides live cellular imaging during surgery to aid in diagnosing microscopic abnormalities including cancer. We present initial clinical trial results using the device to image ovaries in-vivo using fluorescein and ex-vivo results using acridine orange. The imaging catheter has improved depth control and localized dye delivery mechanisms than previously presented. A manual control now provides a simple way for the surgeon to adjust and optimize imaging depth during the procedure while a tiny piezo valve in the imaging catheter controls the dye delivery.

Keywords: confocal, fluorescence, microscopy, clinical trials, microendoscope, imaging catheter, endoscope, laparoscope, ovarian cancer, fluorescein, acridine orange.

1. INTRODUCTION

Successful treatment of cancer is highly dependent on the stage at which diagnosis occurs. Early diagnosis, when the disease is still localized at its origin, results in very high cure rates—even for cancers that typical have poor prognosis. Unfortunately, many cancers are not found until later stages due to inadequate diagnostic techniques. Approximately 90% of cancers arise from the epithelial cells that cover organs.[1] Development of surgical devices that can better interrogate epithelial surfaces for abnormalities would enable earlier detection of cancer and significant gains in overall patient survival.

Diagnosis of cancer is often done by pathologists using thin sections of stained and processed biopsy tissue. Bright field microscopy has played a central role in the diagnosis of disease from carefully prepared biopsy slides. Confocal microscopy, a more recent innovation, is also being used with greater frequency because it can directly image bulk sections of tissue with high clarity. Bright field images of bulk tissue appear very blurry due to simultaneous collection of out of focus planes. However, confocal images of bulk tissue are sharp because the microscope only collects light from in focus planes; light from out of focus planes is rejected.

Since the confocal microscope alleviates the need for cutting tissue into thin sections, it has significant potential as an in-vivo imaging device that could supplant biopsies. However, a standard confocal microscope is a large device that is not especially suited for accessing the epithelial surface of most organs where biopsies are acquired. Realizing the potential of confocal imaging to ultimately supplant biopsies via in-vivo imaging, we worked on the initial technologies to enable in-vivo confocal imaging via coherent fiber optic bundles.[2] Since the initial work, our research group has continued developing technologies to allow live in-vivo human cellular imaging via confocal microendoscopy during surgery. In this paper we present our current state-of-the-art developments for in-vivo cellular imaging and initial clinical results as directly applied for detecting cancer of the human ovary.

The American Cancer Society estimates that over 230,000 new cases of ovarian cancer were diagnosed in 2007 while more than 141,000 women died from the disease worldwide.[3] For those diagnosed with the disease, only 30

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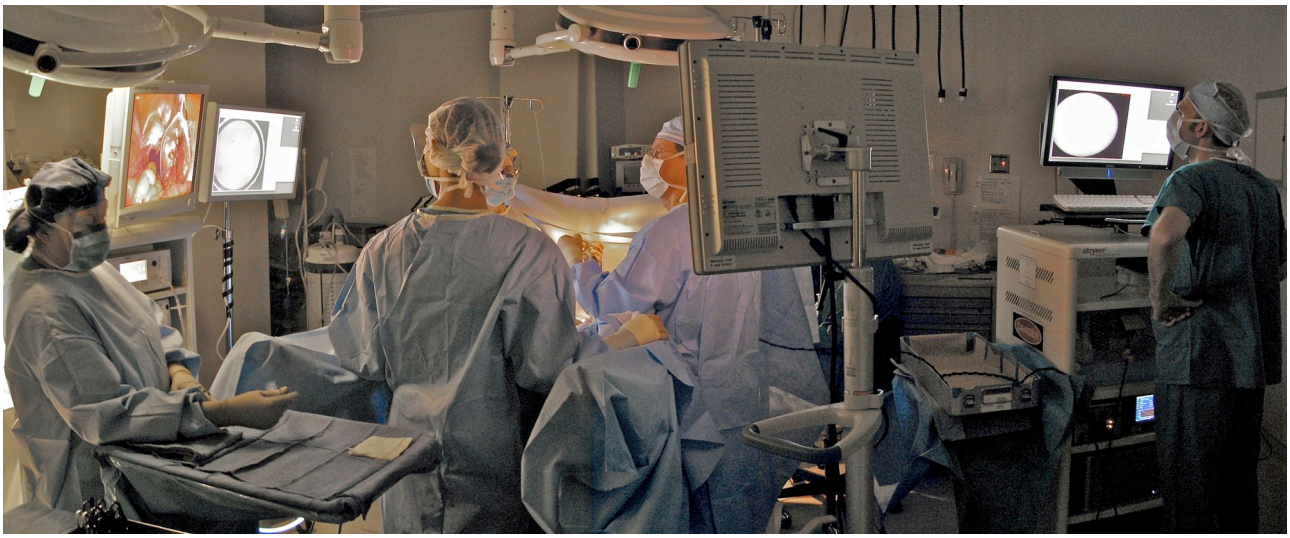


Figure 1. The confocal microendoscope system imaging the epithelial surface of an ovary during surgery. In this image the surgeon has located the left ovary using a wide field endoscope (left most display) and is inspecting the epithelial cells using the microendoscope via the second display from the left. The mobile instrument is on the far right.

to 45 percent will survive five years.[4, 5] The survival rate for ovarian cancer is poor because the disease frequently has no obvious signs until it has reached an advanced stage. Only 19 percent of all cases are diagnosed during the early localized stages of the disease; most of the population are diagnosed during the later stages when the treatment is expensive and generally unsuccessful.

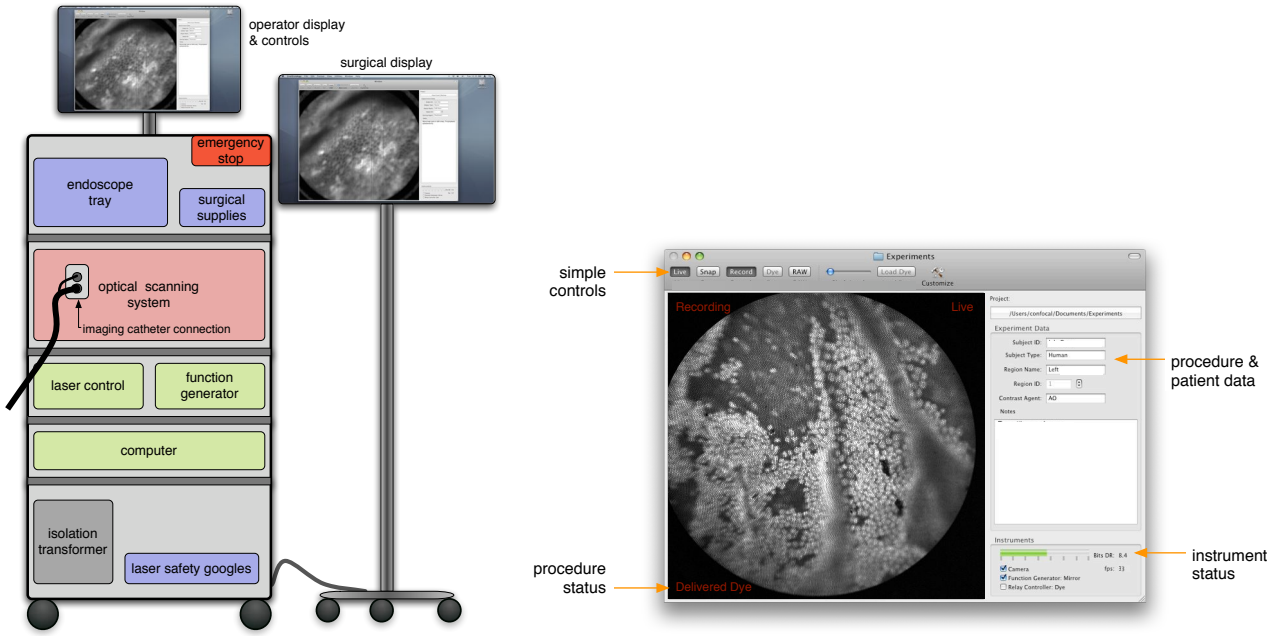
For the female population at large, most are at a low risk of acquiring ovarian cancer. It is estimated that approximately 1 in 58 newborn females (1.7 percent lifetime risk) will develop ovarian cancer in their lifetime.[6] However, the lifetime risk for the disease increases by two fold if the individual has one first or second-degree relative with the disease. With two first-degree relatives the risk increases by 25x (a 40 percent lifetime risk).[6–9]

For the subgroups of women at increased risk, few options exist to allow early detection of the disease. The delicate epithelial surface of the ovary is not amenable for biopsy. The NIH 1994 consensus stated that there is no single acceptable screening test for ovarian cancer and no evidence that combining the available screening tests—CA125, transvaginal ultrasound, and pelvic exam—has an acceptable sensitivity and specificity.[10] Ovarian cancer is thought to metastasize early in the course of the disease and many experts believe that a different biology occurs during Stage I cancer as compared to the later stages. In Stage I, it is thought that the cancer often metastasizes before a lesion in the ovary becomes grossly visible. Without a viable method for early detection of the disease, most women at high risk are provided the option of prophylactic oophorectomy. Preemptive oophorectomies have the negative side effects of sterility and loss of natural hormone production. Hormone replacement is typically not an option for this subgroup since they are also at an increased risk for breast cancer and hormone replacement can further increase this risk.

In the following sections we discuss a confocal microendoscope system that is generally applicable to in-vivo imaging of epithelial cells. The technology is applied to the subgroup of women at high risk for ovarian cancer who have no suitable methods to detect cancer in its early stages. Our mobile device provides the surgeon with real-time in-vivo confocal images. The long term objective is to allow the physician to determine if the ovary is healthy and circumvent unnecessary prophylactic oophorectomy.

2. SURGICAL SYSTEM FOR LIVE CELLULAR IMAGING

Our surgical confocal microendoscope system consists of an imaging catheter connected to a mobile cart. Figure 1 shows the system in use during clinical trials. The mobile cart is on the right. The left most display shows the wide field endoscope images. The second display from the left shows the live microendoscope images.



(a) System hardware.

(b) System software.

Figure 2. Mobile confocal microendoscope system.

2.1. Mobile system

We have developed a mobile system that can be easily moved into an operating room when live cellular imaging is needed. All the system components are housed on a mobile endoscopy cart. Figure 2a depicts the system's components. The top shelf houses the sterile imaging catheter and other necessary surgical supplies. The second shelf contains the optical scanning system—discussed in the following paragraphs—which directly connects to the imaging catheter. The third and fourth shelves house the laser, function generator, and computer. The bottom shelf houses the medical grade isolation transformer and laser safety goggles. The operator display and the controls for collecting data during surgery are located on a platform at standing height.

Also visible in Figure 2a is the surgical display. This display presents the surgeon with live cellular images during the procedure. It generally sits next to the wide field endoscope display as seen in Figures 1 and 6.

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

Figure 2b shows the software control system. It provides a simple interface for viewing and collecting live images during the surgical procedure. The software has controls to: (1) start live acquisition, (2) save the current frame, (3) record video, (4) delivery dye, (5) load dye, and (6) adjust histogram optimization. In addition to the basic controls, the system also records procedure and patient information, which is archived with the images. Basic diagnostic information such as image dynamic range and frame rate are also visible. During operation the surgeon can easily see real-time imagery in the main window along with data acquisition and dye delivery status.

At the core of the confocal microendoscope system is the optical scanning system. The components and layout are shown in Figure 3. The left side of the figure shows how the system operates in the standard live

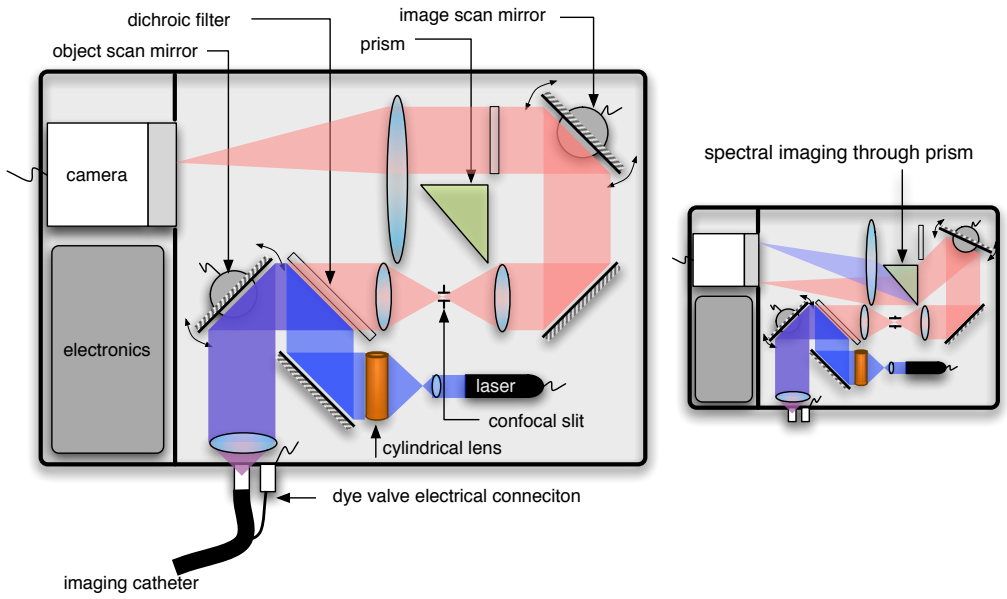


Figure 3. Diagram of the optical scanning system. The left side illustrates the standard mode of operation. A 488 nm laser is anamorphically shaped into a line and scanned onto the coherent fiber bundle in the imaging catheter. The excited signal re-enters the system, is descanned and filtered through a confocal slit. Then the light is rescanned onto a two dimensional detector. The spectral imaging mode is illustrated on the right. In this configuration, the image scan mirror is turned to its extreme position redirecting the light through a dispersing prism.

Table 1. System specifications.

lateral resolution	2 μm	laparoscope compatibility	5 mm trocar
axial resolution	25 μm	endoscope compatibility	4.2 mm port
frame rate	30 fps	minimum delivered dye volume	0.1 μL
excitation wavelength	488 nm	micro-objective magnification	1.5x
spectral collection (150 spectral images)	6 sec	chromatic correction	488-750 nm
average spectral resolution	6 nm	maximum imaging depth	200 μm
spectral collection	500-750 nm	field of view	450 μm

imaging mode. In this mode a 488 nm solid state laser beam is expanded and anamorphically shaped into a line via a cylindrical lens. This laser line is then reflected into the image path by a dichroic filter and scanned across the coherent fiber bundle face at the proximal end of the imaging catheter's connector.

Tissue fluorescence is collected by the imaging catheter, collimated back into the optical scanning system, and de-scanned using the object scan mirror. The dichroic filter passes the fluorescence signal, which is focused down to a stationary line. A slit serves as the confocal aperture. The light exiting the slit is then re-collimated and rescanned using the image scan mirror. A final filter removes residual excitation. Finally the beam is refocused back into a line that sweeps across the camera to collect a two-dimensional image every $1/30^{\text{th}}$ of a second.

In addition to live two-dimensional imaging, the system can also collect multi-spectral data.[11,12] This *multi-spectral* mode is instantly achieved via a software button that deflects the object scan mirror to its extreme position as shown in the right side of Figure 3. In this configuration, the light passes through a prism and the dispersed signal is collected by the camera. Since one spatial dimension on the camera is used for spectral collection, the second image spatial dimension is collected over time. The complete spectral data collection procedure executes in a few seconds. Once spectral collection is complete the system reverts back to its grayscale operating mode. Table 1 summarizes the key system specifications.

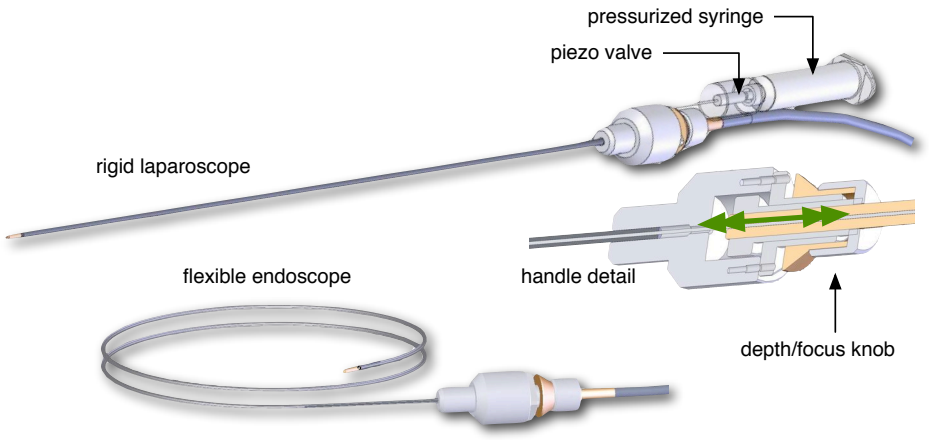


Figure 4. The confocal imaging catheters. A laparoscopic version is shown on top with an integrated dye delivery system that uses a piezo valve and pressurized syringe. The lower left of the figure shows the flexible endoscope version of the device. Both devices have the same handle that uses a depth/focus knob to translate the coherent fiber bundle routed through the center of the device (handle detail shown to the right).

2.2. Endoscopes

The imaging catheter comes in two varieties, a rigid laparoscope 5 mm in diameter and a flexible endoscope 4.2 mm in diameter; both provide the same functionality. This compact instrument contains the micro-objective, coherent fiber optic bundle, dye delivery system, and depth/focus mechanism. In use, the imaging catheter feels like a wide field endoscope except that imaging is done with the probe in contact with the tissue.

Figure 4 depicts the two varieties of the imaging catheter. A twenty foot flexible housing connects to the optical scanning system and protects the coherent fiber optic bundle and electrical connections. The distal tip of the instrument contains a micro-objective[13] lens that images tissue onto the coherent fiber bundle. The micro-objective connects to a housing that contains the fiber bundle. Running parallel to the housing is a 21.5 gauge channel that delivers controlled volumes of fluorescent dyes onto the tissue in the imaging catheter's field of view. The outer surface of the lens and the fiber housing (excluding the face of the lens) are sealed inside a medical grade teflon sheath. The imaging catheter is designed for reuse; it can be quickly disconnected from the optical scanning system and sterilized using Ethylene Oxide.

We have investigated a variety of depth/focus mechanisms[11, 13, 14] and now present a new method that has proven to be more reliable and easier to use during surgery. Rotation of the depth/focus knob on the handle causes the fiber optic bundle to translate along the optical axis. Translation of the fiber via the depth/focus knob allows the surgeon to select the desired imaging plane in the tissue. Since the system is designed for contact imaging on the epithelial surface of organs, once optimal focus has been obtained refocus is not necessary. The surgeon can simply move across the tissue and change sites while maintaining focus since contacting the tissue will bring the epithelial cells into focus. The surgeon can use the depth/focus knob to image planes below the surface for further interrogation of abnormalities.

We have developed a localized dye delivery system that minimizes patient exposure to fluorescent contrast agents and provides a method to mark imaged tissue sites. At the tip of the imaging catheter, a 21.5 gauge channel conforms around the face of the micro-objective to deliver dye directly to the field of view. The system is capable of delivering very small dye quantities on demand, down to 0.1 μL. Once the operator pushes the dye delivery button, a piezo valve located behind the imaging catheter handle (shown in Figure 4) opens for a few milliseconds. A pressurized syringe behind the valve containing a fluorescent contrast agent supplies dye

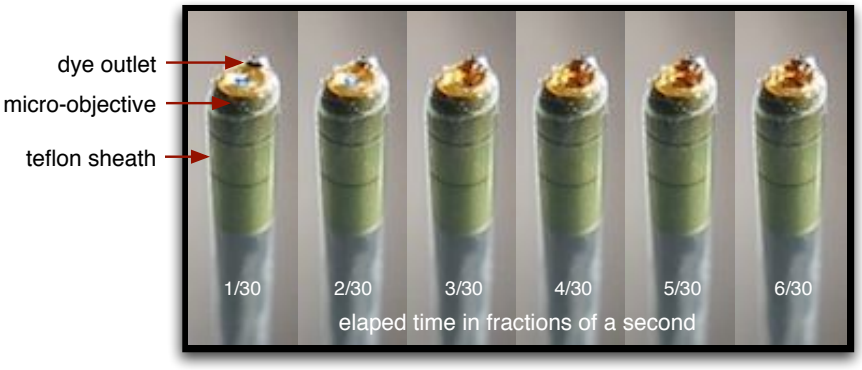


Figure 5. Demonstration of the imaging catheter's ability to locally deliver small volumes of dye to the field of view. In this sequence of images (at $1/30^{\text{th}}$) second intervals the operator presses the dye delivery button and the dye is delivered to the distal tip via actuation of the piezo valve near the imaging catheter's handle. The delivered volume in this example is approximately 1 μL.

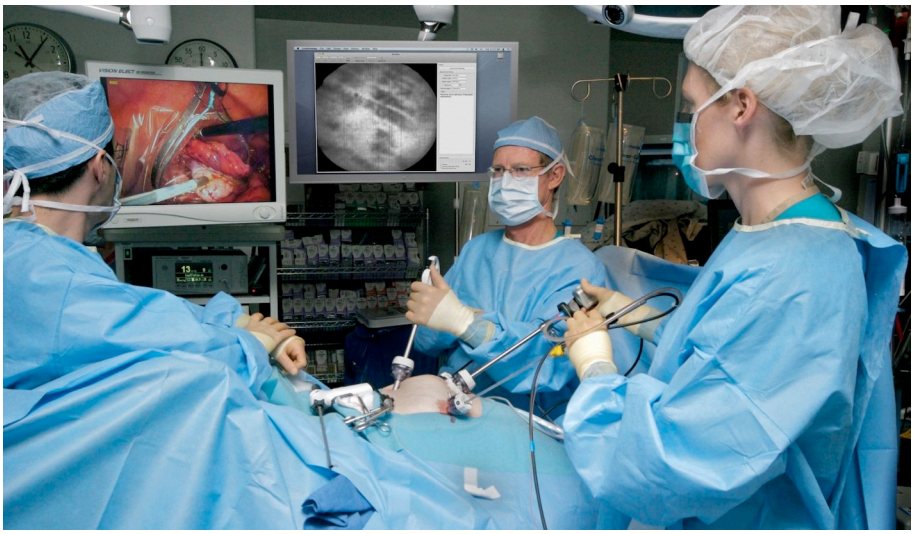


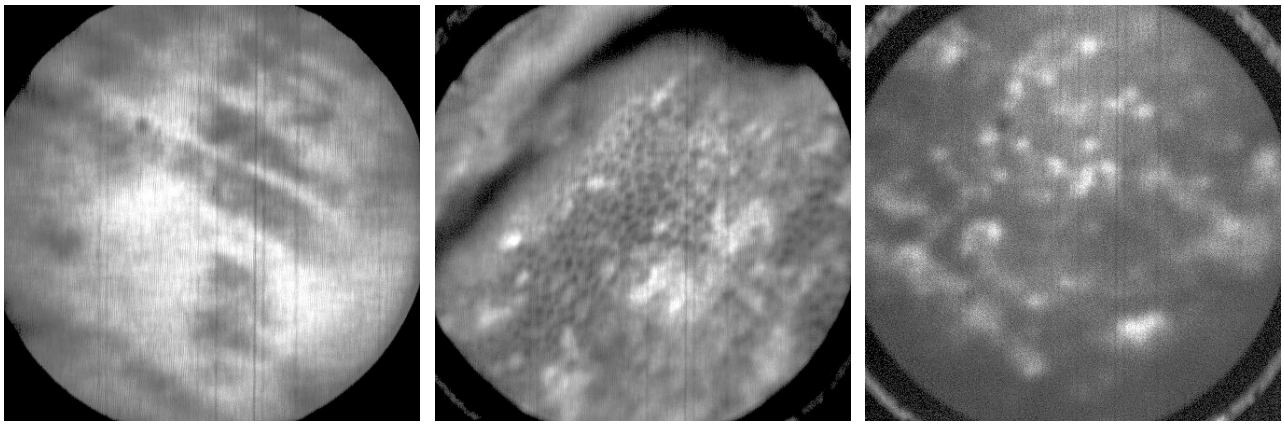
Figure 6. Clinical image of the confocal microendoscope system in use. The surgeon on the lower right is using the imaging catheter to image the patient's left ovary. The left most display shows the ovary in an endobag with the distal tip of the imaging catheter coming into contact with the ovary's epithelial surface. The display to the right shows the cellular images from the microendoscope using fluorescein as the contrast agent..

through the dye channel to the field of view. Figure 5 shows the dye delivery process in $1/30^{\text{th}}$ second intervals; full delivery occurs in approximately $1/6^{\text{th}}$ of a second.

3. RESULTS

The confocal microendoscope system is currently being evaluated in clinical trials to image the epithelial surface of the ovary at the University Medical Center in Tucson, Arizona. The device was granted "non significant 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 sodium fluorescein as the contrast agent.[15] To date we have imaged 16 patients in-vivo.

Figure 6 illustrates how the confocal microendoscope system is used in our clinical trials. The current protocol entails imaging human ovaries in-vivo before oophorectomy or hysterectomy. The imaging protocol begins with



(a) (b) (c)

Figure 7. Images of ovary epithelium obtained in-vivo during clinical trials using fluorescein. The suboptimal cellular detail is a result of the minimal preferential binding exhibited by fluorescein.

the surgeon locating the ovary and isolating it in an endobag (visible on the left display in Figure 6) with the ovary still connected to the blood supply. Once the ovaries are isolated, the surgeon brings the imaging catheter into contact with the epithelial surface and four sites on each ovary are imaged. At each site the imaging catheter delivers a metered volume of fluorescein. The endobag serves to limit the extent to which the contrast agent can come into contact with any other organs of the patient. After the microendoscope imaging is done, the surgical procedure is completed as normal and the ovaries are removed. Finally, the removed ovaries are imaged again ex-vivo with additional contrast agents and biopsies are taken for correlated pathology.

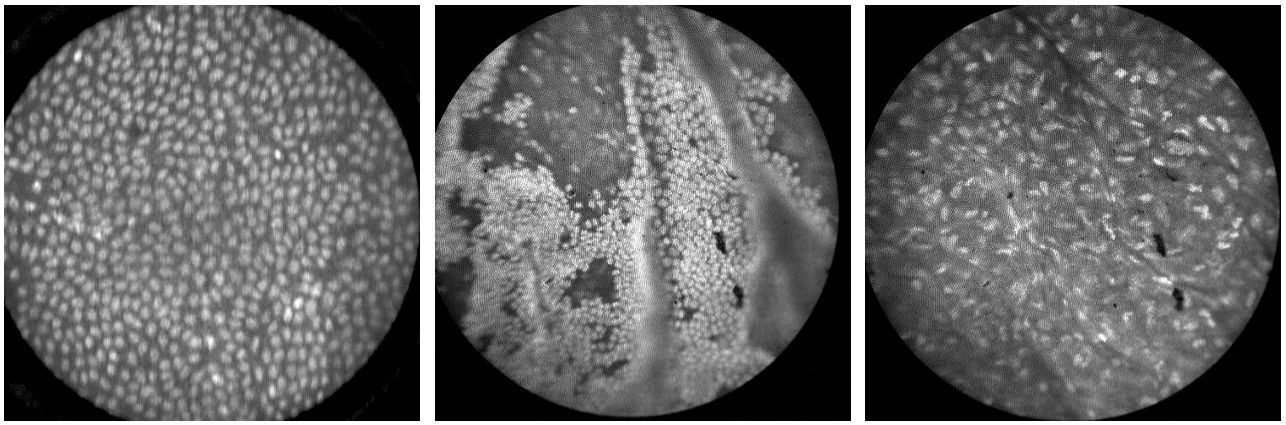
3.1. In-vivo images

For initial in-vivo clinical studies* fluorescein was selected because of its pre-existing approval for human use. The diagnostic utility of fluorescein when used as a topical contrast agent on the ovary is not very good as it provides little contrast and lacks preferential binding to cellular level structures of interest. However, it has served its purpose as a safe, pre-approved contrast agent allowing us to test the safety and feasibility of the confocal microendoscope system.

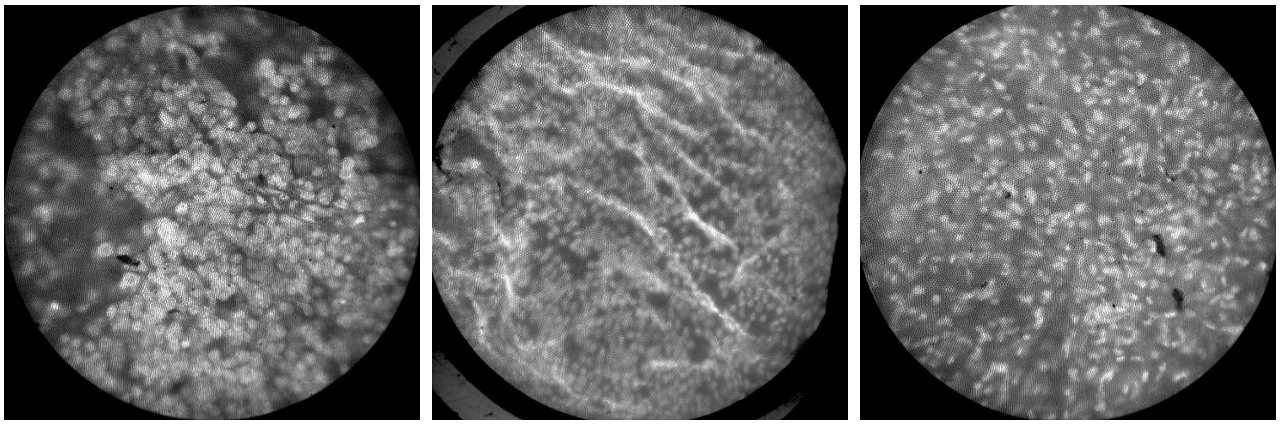
Figure 7 shows three examples of in-vivo images obtained with the confocal microendoscope system. The images demonstrate that the device functions as designed. The imaging catheter can deliver controlled volumes of dye to the image site and then display real-time image data to the surgeon. The focus mechanism works well; after an initial adjustment of the focus, the instrument can be moved to various sites on the ovary while maintaining good focus on the epithelial surface.

The full diagnostic utility of the confocal microendoscope system can only be realized with proper fluorescent contrast agents that provide useful preferential binding. Various contrast agents exist that provide superior diagnostic utility as compared to fluorescein. One such contrast agent is acridine orange. We are currently in the process of obtaining approval for in-vivo use of this contrast agent for the second stage of our clinical trials. In the following section ex-vivo images of human ovary obtained with the confocal microendoscope system using acridine orange are shown. We expect comparable results in-vivo.

*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.



(a) Normal epithelium (b) Partially denuded epithelium (c) Normal stroma



(d) Adenocarcinoma (e) Sclerotic (f) Sclerotic with endometriosis

Figure 8. Images of human ovary epithelium obtained ex-vivo using acridine orange. Sub-captions contain pathology diagnosis.

3.2. Ex-vivo images

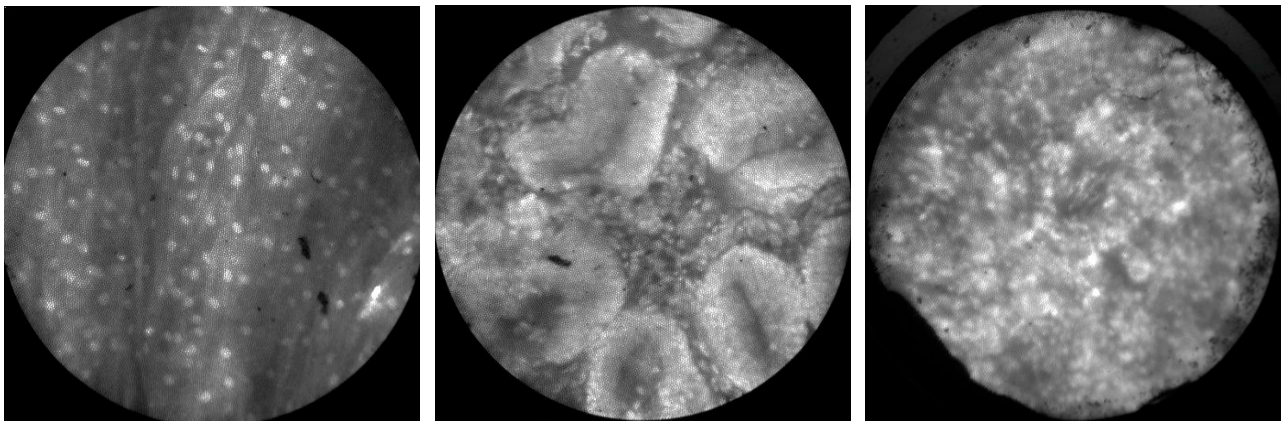
In addition to our in-vivo results, we have extensive studies[†] that include over 50 ex-vivo patients with ovaries imaged using acridine orange as a fluorescent contrast agent.

The images with acridine orange, shown in Figure 8, demonstrate the excellent diagnostic ability of the instrument. The epithelial surface of a healthy ovary is characterized by a homogeneous distribution of bright nuclei seen in Figure 8a. The epithelial surface of the ovary is delicate and partial denuding can occur, exposing the underlying stroma (Figure 8b). Below the epithelial surface, healthy stroma also exhibits a characteristically homogenous structure albeit with a different nuclear size distribution and shape (Figure 8c).

In the case of carcinoma, the tissue structure is visibly differentiable (Figure 8d) from healthy epithelial cells (Figure 8a). The epithelial surface is irregular and the high degree of heterogeneity indicative of ovarian cancer.

We have perviously shown[16] that the confocal microendoscope system can easily differentiate normal epithelium from ovarian cancer—providing a diagnostic advantage when the neoplasia is small and not visible at the gross anatomic level. It also appears that the confocal microendoscope system may also be able to visualize

[†]All tissue used in this research were collected in accordance with protocols approved by the Institutional Review Board of the University of Arizona.



(a) Normal Epithelium (b) Barrett's Esophagus (c) Tumor

Figure 9. Images of human esophagus epithelium obtained ex-vivo using acridine orange.

cellular changes that happen prior to the onset of cancer. Less distinct tissue changes such as tissue sclerosis and endometriosis may also be detectable (Figures 8e and 8f).

As previously discussed, any lumen or organ that is endoscopically or laparoscopically accessible is a suitable candidate for microendoscope imaging of the epithelium. Applications of the device to detect the transformation of normal esophagus to adenocarcinoma are also being investigated. To date, we have conducted ex-vivo imaging on more than 30 patients diagnosed with Barrett's Esophagus. Barrett's Esophagus is the premalignant lesion for adenocarcinoma of the esophagus. Due to a severe chronic gastroesophageal reflux disease, these patient experience a transformation of the normal squamous epithelium (Figure 9a) into tissue that closely resembles the intestine with columnar appearing mucosa and intestinal metaplasia (Figure 9b). Once this transformation has taken place, the individual is at higher risk for adenocarcinoma of the esophagus.[17] Moreover, the 5-year survival rate for this cancer is only between 10-15%.[3] The American Cancer Society estimates that approximately 529,000 new cases of adenocarcinoma of the esophagus were diagnosed in 2007 while more than 442,000 people died worldwide from the disease.[3] Yet again, one of the predominant factors causing low survival rates is the late detection of this disease.

Patients with Barrett's Esophagus identified with an increased risk for adenocarcinoma typically undergo endoscopic surveillance and biopsy every one or two years. Again, the confocal microendoscope system's ability to resolve cellular detail indicates that it would be a useful tool to improve the detection of dysplasia and adenocarcinoma in-vivo. Figure 9c illustrates the distinct difference when esophagus tissue has made the transformation to tumorous tissue.

4. CONCLUSIONS AND FUTURE WORK

We have built a mobile confocal microendoscope system capable of performing *optical biopsies* in-situ where unusual sites on epithelial tissue can be interrogated at the cellular level to characterize abnormalities. The system is currently being evaluated in clinical trials to determine its safety and efficacy for detecting ovarian cancer. To date, we have shown that the instrument is safe and that we can successfully image the ovaries in-vivo. Our in-vivo results indicate that the instrument functions as designed. Our ex-vivo results demonstrate that the instrument can resolve sufficient cellular detail to detect the transformation of normal epithelium and visualize cellular changes that happen with the onset of cancer.

Current efforts to acquire approval for use of more desirable in-vivo fluorescent contrast agents will likely allow us to demonstrate the same level of quality as seen in our current ex-vivo results. We are also continuing our investigations in multi-spectral imaging and computer aided analysis using the confocal microendoscope system.

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