Confocal microendoscope for use in a clinical setting

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Abstract

A mobile confocal microendoscope for use in a clinical setting has been developed. This system employs an endoscope consisting of a custom designed objective lens with a fiber optic imaging bundle to collect in-vivo images of patients. Some highlights and features of this mobile system include frame rates of up to 30 frames per second, an automated focus mechanism, automated dye delivery, clinician control, and the ability to be used in an area where there is a single 110V outlet. All optics are self-contained and the entire enclosure and catheter can be moved between surgical suites, sterilized and brought online in under 15 minutes. At this time, all data have been collected with a 488 nm laser, but the system is able to have a second laser line added to provide additional imaging capability. Preliminary in vivo results of images from the ovaries using topical fluorescein as a contrast agent are shown. Future plans for the system include use of acridine orange (AO) or SYTO-16 as a nucleic acid stain.

Keywords: Confocal, endoscope, fiber, optics, microscopy, fluorescence, tissue, imaging

1. INTRODUCTION

Endoscopes have provided doctors with insight into cancer detection for the past 40 years. However, endoscopes are limited in that they cannot provide definitive histopathologic information, and therefore a biopsy of tissue is required. Because most cancers originate in epithelial tissues[1], confocal microendoscopy is well suited for minimal to moderately invasive imaging of external and internal locations. Its use in providing diagnostic information about potentially cancerous cells and in assisting in tissue selection for biopsy is of particular interest. Eventually it may be practical to use in-vivo confocal images for cancer detection in place of extraction biopsy.

In previous papers, we have reported on the development and use of a 3mm confocal microendoscope system with a catheter to obtain real time images of excised and in-vivo animal tissues[2, 3]. We have also reported on, and have recently expanded upon, a method of obtaining multi-spectral images of tissue with the confocal microendoscope[4, 5].

There is currently no suitable method to image and screen ovarian cancer. The five year survival rate for patients diagnosed with ovarian cancer is under 50 percent[1]. However, with early detection, the five year survival rate is increased to over 90 percent. The large difference between the two survival rates can be attributed to the identification of ovarian cancer before metastasis has occurred. Early diagnosis and removal of the ovaries often eliminates the need to add chemotherapy to the treatment regime. Additionally, many women with a family history of ovarian cancer or a personal history of breast cancer opt to have a prophylactic oophorectomy to avoid the possibility of developing ovarian cancer in the future. This high-risk group could benefit the most from having an effective screening tool for ovarian cancer. Confocal microendoscopy is perfectly suited for this purpose.

In this paper we present the design and construction of a newly completed confocal microendoscope system for in-vivo imaging of ovaries in a clinical setting. All optics and hardware are located on a mobile cart, enabling video rate cellular imaging of multiple locations in-vivo.

Endoscopic Microscopy II, edited by Guillermo J. Tearney, Thomas D. Wang
Proc. of SPIE Vol. 6432, 64320H, (2007) · 1605-7422/07/$18 · doi: 10.1117/12.701798

2. SYSTEM DESIGN

2.1 Requirements for clinical use

Prior to construction of the mobile confocal microendoscope system, it was necessary to meet with clinical and safety officials from the University of Arizona to make sure the final design satisfied both the specified imaging and safety requirements for clinical use. The system continues to use the existing confocal optics developed and reported previously[6], however, there were modifications that had to be made for the specific adaptation to hospital use.

The primary concern from a clinical standpoint was patient safety. This meant that it would be imperative to maintain a sterile environment and to have a minimal impact on the patient, both in the time required to image and in any foreign materials used in order to make a diagnosis. Three main requirements were identified: a sterile catheter, a safety study on the use of exogenous fluorophores in patients, and the need to integrate into existing clinical procedures. For ovarian imaging, the sterile catheter needed to be less than 5 mm in diameter into allow access to the peritoneal cavity via a trocar inserted through the patients abdominal wall.

University of Arizona departments involved with approval for use of the instruments in the hospital on living patients were the Clinical Engineering department and the Institutional Review Board (IRB). The Clinical Engineering department is concerned with electrical and laser safety of all instruments used in the hospital. All metal portions of the cart needed to be electrically grounded, and a hospital-grade isolation transformer was needed to isolate the electrical components of the cart from the hospital's power supply in the event of an electrical malfunction. Proper identification of the laser and passive beam-blocking mechanisms also needed to be included on the system. Finally, clinical engineering required that goggles be supplied to each person in the clinic.

In order to get IRB approval, a patient consent form was drafted, a detailed experimental protocol was written, and a justification for non-significant risk assessment was provided. Most ex-vivo ovarian tissue data previously obtained with the confocal microendoscope used acridine orange (AO) or SYTO-16 as a contrast agent, both of which preferentially stain the DNA of cells. Since there was insufficient data available on the safety of these dyes, it was impractical to begin using either of these dyes in-vivo. Fluorescein, which is already used in-vivo for ocular imaging, was approved by the IRB for topical use on the ovaries for an initial feasibility study on 10 women. Pending positive results of the dye safety examination (explained below) the MCME will be used to image either AO or SYTO-16 in 48 additional patients over a period of 2 years. The system was ultimately approved by the IRB for use in human.

A dye safety study on the effects of AO and SYTO-16 was initiated on 75 mice, and was conducted by the staff of the animal care facility at the university. The mice were broken into five groups of 15 animals: Two groups assessing the safety of AO, two groups assessing the safety of SYTO-16, and a control group. For the study, 250 μL of solution with various concentrations of dye (330 μM AO, 3.3 mM AO, 20 μM SYTO-16, 200 μM SYTO-16, and a control group with saline) were injected into the peritoneal cavities of the mice. The lower two concentrations of dye represent what we typically use on ex-vivo tissues to obtain high contrast images. The two higher concentrations represent a 10X increase of what we would use in-vivo.

Prior to in-vivo use in a clinical setting, we determined it would be best to test the system at the Arizona Simulation Technology and Education Center[7] (ASTEC) facilities at the University Medical Center. The ASTEC facility is open to medical students and clinicians for training and education on simulated patients. There are full models of patients as well as smaller models that can be used to simulate laparoscopy in the peritoneal cavity. This training allowed clinicians and the microendoscopy system operators to make sure they agreed on the procedures to be employed prior to the first clinical use.

2.2 Confocal imaging components

Figure 1 shows the layout of the optical components of the mobile confocal micro-endoscope[6] system. The optical components can be broken into three parts: the illumination optics, the catheter, and the detection optics. In the illumination optics, laser light is collimated from a fiber coupled diode pumped

solid-state (DPSS) Coherent Sapphire 488 nm laser[8]. This collimated beam is passed through a cylindrical lens which is focused by a 10X Olympus[9] microscope objective onto the proximal end of the catheter. The line of light is scanned across the objective entrance pupil by a scan mirror[10].

The catheter consists of an imaging fiber bundle and a miniature objective lens. The imaging bundle we use is an IGN 08/30-30,000 element fiber supplied by Sumitomo Electric Industries[11]. This fiber has a 1 mm external diameter with a 720 μm active area, and consists of 2 μm core fibers with a 3 μm center-to-center spacing. A 2.5 mm diameter tubing made of PEEK protects the imaging bundle and allows the imaging bundle to move freely inside, which is how focusing takes place. A 3 mm diameter, 13 mm long objective lens[3] images a selected plane in the tissue out of the distal face of the tube. The final element of the objective lens is a coverslip that is placed in contact with the object being imaged. The catheter allows imaging up to 200 μm deep in tissue by moving the distal end of the fiber with respect to the lens. This is done by moving the fiber at the proximal end relative to the outer PEEK tubing that is attached rigidly to the distal objective lens, similar to how a brake-cable mechanism operates. The laser light excites fluorescence in the tissue, and the emitted light is collected by the objective lens and transferred back through the imaging fiber bundle.

The detection optics relays the emitted fluorescent light from the proximal end of the imaging fiber bundle to a camera. The light first passes back through the Olympus objective lens and is de-scanned and passed by a dichroic beam splitter that allows the emitted fluorescence to pass. This light is then focused onto the confocal slit. Past this point a second scan mirror, which is synchronized with the first mirror, scans the image across the CCD detector (Photometrics Cascade II)[12].

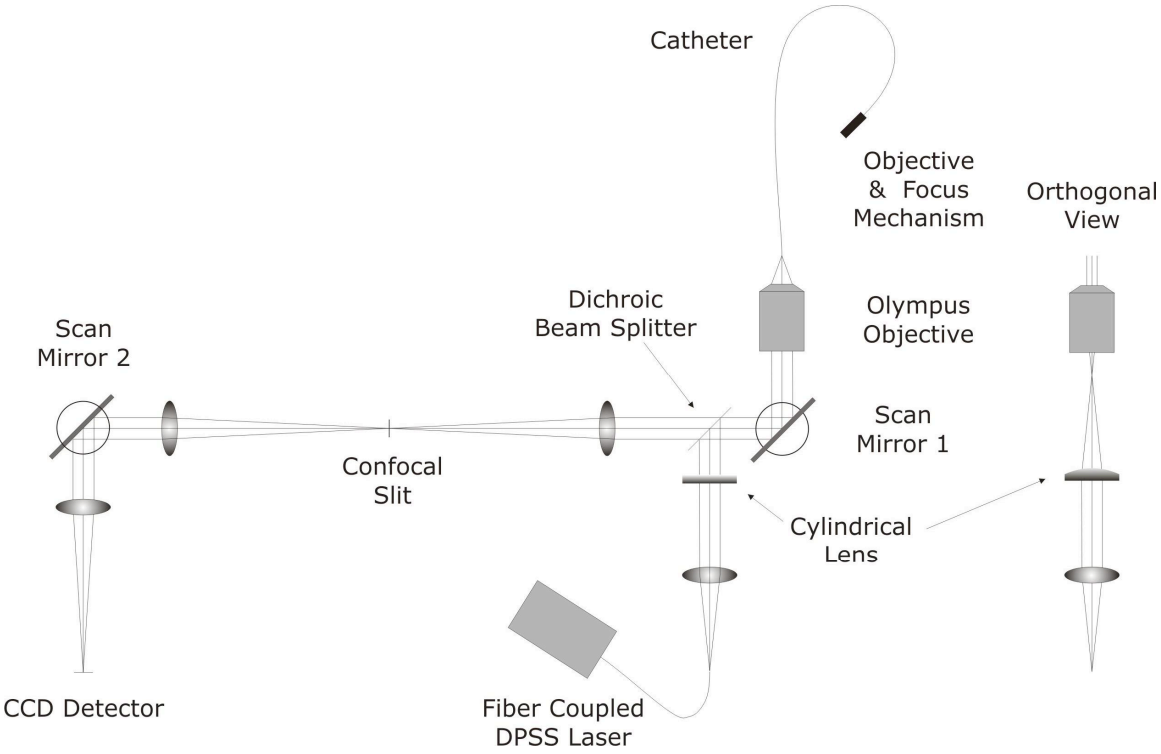

Fig. 1. Layout of the optical components used in the mobile confocal micro-endoscope (MCME) system. The orthogonal view shows the illumination optical path for the direction in which the cylindrical lens has power.

2.3 Sterilizable catheter for in-vivo imaging and dye delivery

Originally the catheter developed for the confocal micro-endoscope was not manufactured for in-vivo use. This presented some issues concerned with sterilizing the catheter and its miniature objective lens, if this same catheter was to be used for repeated clinical procedures. Application of dye to the in-vivo

imaging sites was also a concern, and a way to deliver dye to the tissue through the same imaging catheter would be optimal. A 4.8 mm diameter polycarbonate tube was designed to allow the 3 mm imaging catheter to slide down a channel inside it[13] (Fig. 2). A 100 μm diameter channel was included next to the imaging channel to deliver an exogenous fluorescent contrast agent to the tissue. A computer controlled syringe pump allows the operator to apply a quantitative amount (~1 μL) locally to the tissue site being imaged. The distal end of the polycarbonate tubing has a glass coverslip sealed to the face with a hole over the dye delivery channel. This ensures that the ETO sterilized polycarbonate tube is the only component that comes into contact with the patient.

The semi-rigid polycarbonate catheter is 19 inches in length. This is long enough to allow the surgeon to control the catheter's positioning within the patient from the outside. A sterile cover is pulled back over the PEEK tubing of the imaging catheter and dye delivery tubing to provide a completely sterile environment at the surgical table.

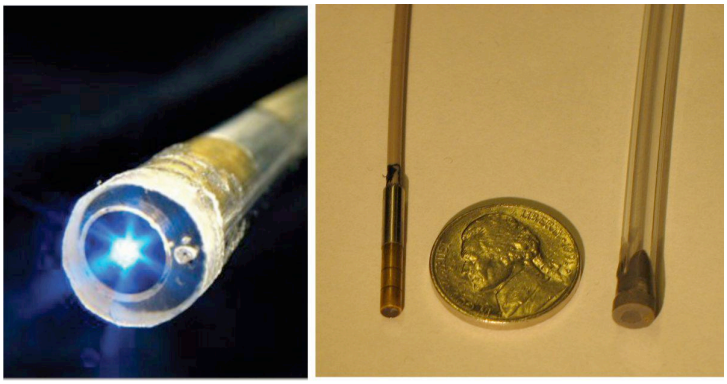

Fig. 2. Distal end of the polycarbonate tubing with the imaging catheter inserted and laser light coming through. The dye delivery hole can be seen on the right side of the tubing face (left). The imaging catheter and the polycarbonate tubing separated for reference (right).

An overview of the optical performance of the system with the laparoscopic polycarbonate catheter is shown in Table 1. Overall there has been no change in the imaging parameters with the addition of the polycarbonate catheter, though the rigidity of the tubing makes it easier to hold the catheter steady during imaging.

Magnification (tissue to fiber)	1.6
Full field-of-view in tissue	450 μm
Lateral resolution	3 μm
Axial resolution	30 μm
NA in tissue space	0.46
Range of focus in tissue	0 – 200 μm
Semi-rigid laparoscopic polycarbonate catheter length	19 inches
Polycarbonate tubing diameter	<5 mm

Table 1. An overview of the optical parameters of the clinical confocal microencoscope.

2.4 User interface screen

Operation and monitoring of the imaging system is performed through a custom user interface screen (Fig. 3). All the code for the display was written in Python and runs on an Apple Macintosh computer. When the camera and scan mirrors are running, a window shows real time images to both the computer operator screen and on a separate monitor in the surgical suite. The software operator can control how the data are normalized as well as control the laser power, function generator controlling the scan mirrors, the catheter focus position, and dye delivery.

The clinician also has access to a real-time automated diagnosis capability[14], which is based on texture analysis of the images. This has been developed as a preliminary tool, but will not be included in the trials. Eventually this capability may be included in future generations of the clinical system.

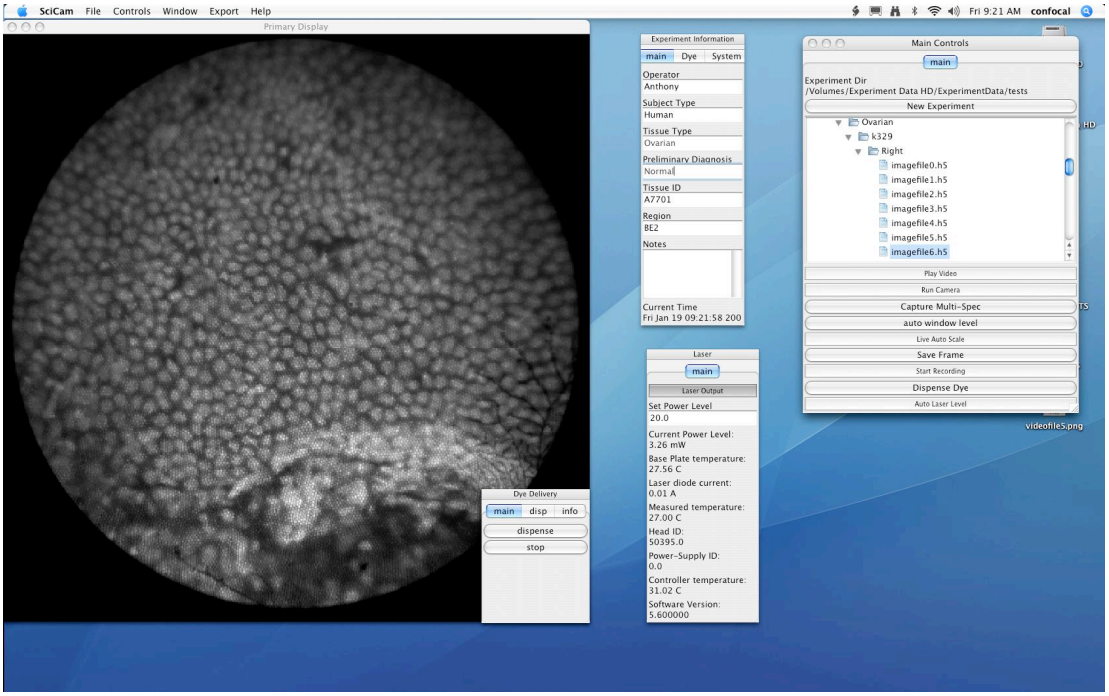

Fig. 3. User interface screen for system control. Clockwise from left are an image of ovarian tissue stained with AO; tissue and patient identification information; main controls to open files, operate the camera and focus the catheter; laser power control and information; dye delivery system control.

2.5 System enclosure

All the hardware and optics for the confocal imaging system are inside of a mobile cart[15]. This cart has two internal shelves and an external top shelf. All the optical components are located on the middle shelf and the isolation transformer, battery backup supply, computer, laser, and scan mirror controllers are on the bottom shelf. Aluminum panels to cover the sides are attached, with one designed for quick release. The top of the cart has the computer monitor, keyboard and mouse, dye pump and a tool box for storing all sterilized polycarbonate catheters and laser safety goggles.

The system is designed so all control of interior components can done externally (Fig. 4). One side of the cart has the main power supply button. The front plate from the battery backup was removed and rewired so it could be mounted next to the main power control. A video port allows the computer to interface with a second monitor, and an Ethernet plug allows data to be transferred between the system and an external computer. The laser can also be turned on and off from a key switch with a removable key for safety purposes. Below the key switch is a laser operation indicator light that is on whenever the laser is powered on. To provide ventilation to the internal components, an air filter is in place that allows air to be drawn across the electrical compartment on the lower shelf.

On the opposite side of the cart are the catheter exit port and an exhaust fan. The catheter exit port is protected by a 90^0 angled PVC pipe to prevent the catheter from breaking if it is inadvertently bumped or hit. The angled PVC can be rotated to provide support to the catheter while it is being used to image a patient. Laser safety signs are located on both sides of the cart as per university clinical engineering requirements.

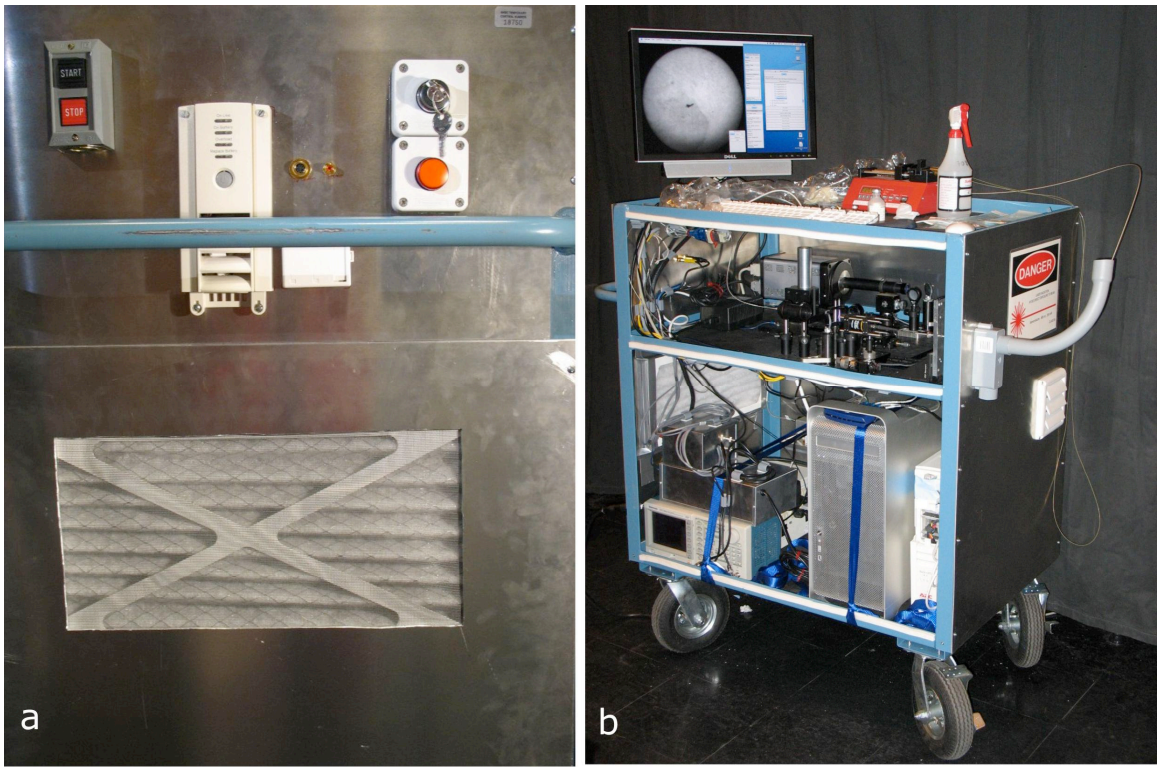

Fig. 4. The mobile confocal system is fully enclosed. One side allows control of the main power, battery backup, laser power, laser operation light, as well as video and Ethernet connections (a). All optics are located on the middle shelf of the cart and all hardware is on the ventilated bottom level (b).

3. Results and Discussion

To date the mobile confocal microendoscope has been used in two clinical oophorectomy procedures and in the ASTEC training facility. The ASTEC training sessions focused on the mechanics of using the catheter to ensure it remained sterile and to let the clinician practice keeping the catheter in contact with the ovaries during imaging (Fig. 5). One of the features of the ASTEC facility is the ability to make tissue phantoms out of rubber to accurately simulate the peritoneal cavity. Figure 5a is a picture of Dr. Kenneth Hatch practicing on the ASTEC system. The semi-rigid polycarbonate tube is held in his left hand, and he is guiding it into the 5 mm trocar. Figure 5b shows the polycarbonate catheter in contact with an ovary phantom (marked by arrow). This picture was taken from a laparoscope inserted into the peritoneal model.

There were several goals for the ASTEC training. It was important to minimize the amount of time required for setup and assembly of the system, the clinician needed to become familiar with the catheter, and communication terms needed to be established between the operator and the surgeon. The optical system remained stable during transport from our research lab to the clinic, so we were able to focus on the logistics of sterile catheter assembly. We also discussed positioning of the cart in the surgical suite and verified that the final design for the system would provide adequate space for the clinical staff and equipment.

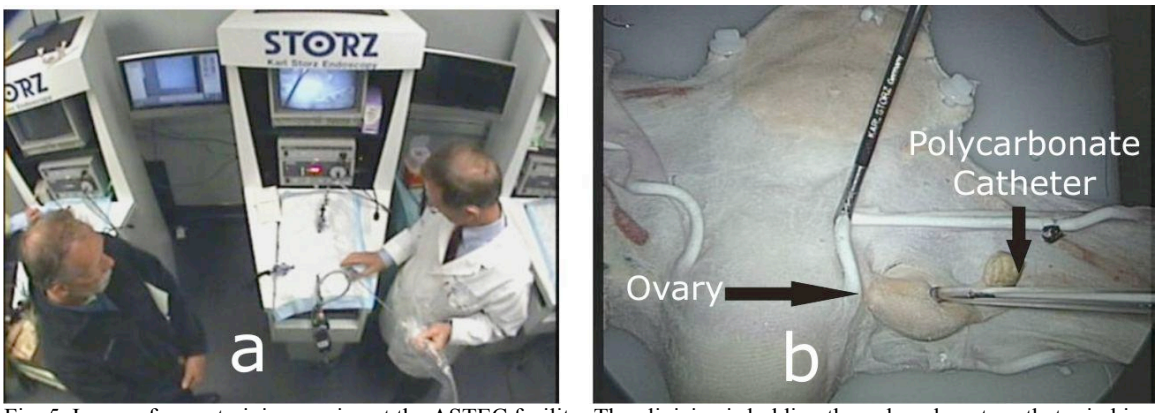

Fig. 5. Images from a training session at the ASTEC facility. The clinician is holding the polycarbonate catheter in his left hand and is standing in front of a model of the peritoneal cavity(a). The polycarbonate catheter in contact with a model ovary as seen by an laparoscope in the peritoneal model (b).

Confocal data have also been collected from two patients in-vivo using the confocal microendoscope. Three images from one of these trials are shown below (Fig. 6). Fluorescein was used as the fluorescent contrast agent in these images. There is some blurring of the images due to an issue with the focus control that is being resolved. This stage of the trials is not concerned with obtaining perfect images, but rather proving that the imaging system and catheter can be used in the clinic safely and reliably.

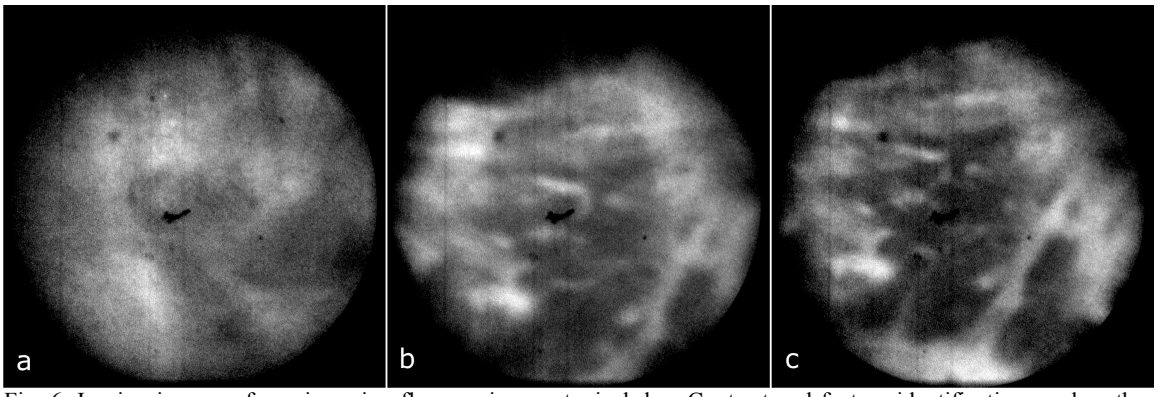

Fig. 6. In-vivo images of ovaries using fluorescein as a topical dye. Contrast and feature identification are less than optimal due to fluorescein's non-specific staining.

Experiments to date using the imaging system have been successful logistically, but the images obtained do not provide the same level of contrast we are accustomed to from our ex-vivo results wit AO (Fig. 7). Images obtained using AO as the fluorescent contrast agent give a clearer indication of the tissue health based on the size, shape, and distribution of the nuclei. These images show an excellent level of detail and contrast compared to the images taken using fluorescein. In images taken with AOwe are able to see the difference between epithelial layers (which have nuclei that are more round and evenly spaced), and the underlying stromal layers (which have nuclei that are elongated and more irregular). Images of malignancies have a heterogeneous distribution of nuclei.

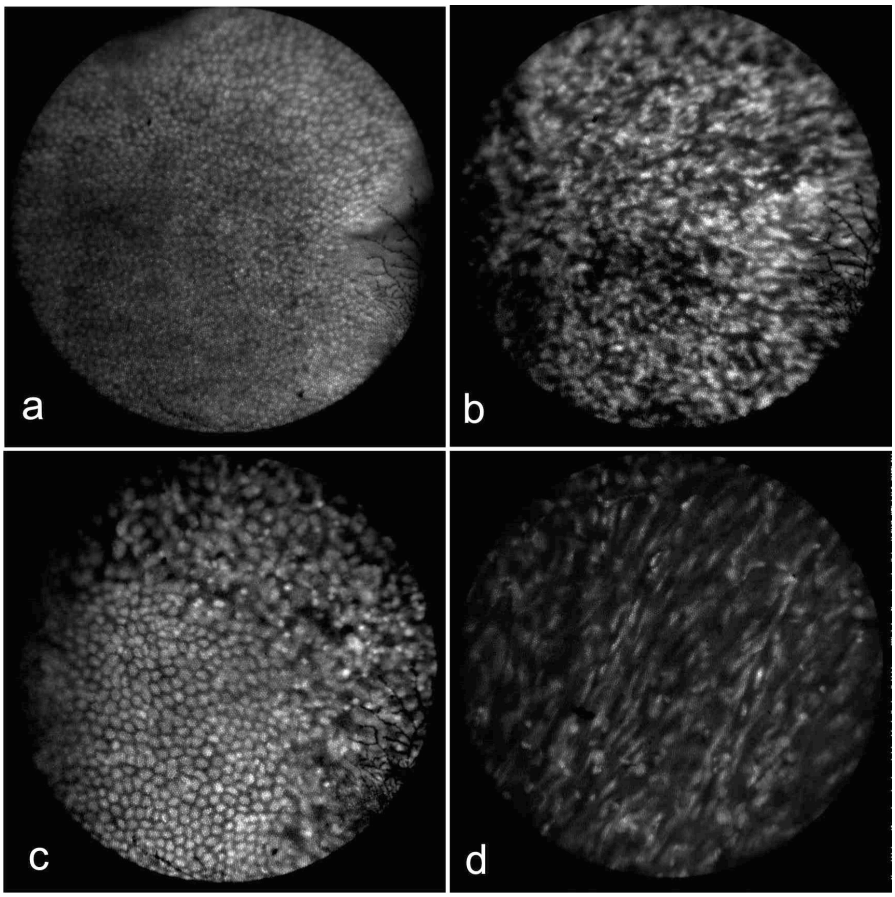

Fig. 7. Ex-vivo images of ovaries using AO applied topically. Images show epithelium (a), stroma (b), epithelium and stroma (c), and malignancy (d).

4. Conclusion

A mobile confocal micro-endoscope system has been built with the same optical parameters as previous bench-top systems. A rigid laparoscopic polycarbonate catheter that is able to be sterilized and reused has also been developed to protect the imaging catheter and deliver dye to tissue sites. The imaging system has been effectively used in a clinical setting to image ovaries in-vivo. All clinical staff involved with the procedures have expressed their satisfaction with the ease of use of the system, and the images collected have validated the system design. Future in-vivo studies will be done to further demonstrate the feasibility and ultimately prove the efficacy of the confocal microendoscope for ovarian imaging.

Acknowledgements

This work could not have been completed without the help of the UMC clinicians and their support staff, specifically Dr. Kenneth Hatch, Kathy Schmidt, and Christine Howison. This work was funded by NIH Grants CA 73095 and CA 115780 as well as ABRC grant 9711.

References

1. American Cancer Society, "Cancer Facts & Figures," http://www.cancer.org.
2. A. F. Gmitro and D. Aziz, "Confocal microscopy through a fiber-optic imaging bundle," *Optics Letters*, **18**(8), 565-567, (1993).
3. A. R. Rouse, A. Kano, J. A. Udovich, S. M. Kroto, and A. F. Gmitro, "Design and demonstration of a miniature catheter for a confocal microendoscope," *Applied Optics*, **43**(31), 5763-5771, (2004).
4. A. R. Rouse, A. F. Gmitro, "Multispectral imaging with a confocal microendoscope," *Optics Letters*, **25**(23), 1708-1710, (2000).
5. H. Makhlouf, Tanbakuchi, A. A., Rouse, A. R., Gmitro, A. F., "Design of a multi-spectral channel for in-vivo confocal microscopy," *in Proceedings of the SPIE The International Society for Optical Engineering: Endoscopic Microscopy II*, **6442**(Submitted for publication 2007), (2007).
6. A. R. Rouse, Tanbakuchi, A. A., Udovich, J. A., Gmitro, A. F., "Design of an in-vivo multi-spectral confocal microendoscope for clinical trials," *in Proceedings of the SPIE The International Society for Optical Engineering: Endoscopic Microscopy*, **6082**, (2006).
7. Arizona Simulation Technology and Education Center, http://www.astec.arizona.edu.
8. Coherent (5100 Patrick Henry Dr., Santa Clara, CA 95054).
9. Olympus America Inc. (3500 Corporate Parkway, PO Box 610 Center Valley, PA 18034).
10. Cambridge Technology Inc. (25 Hartwell Ave, Lexington, MA 02421).
11. Sumitomo Electric USA (21221 S. Western Ave, Suite 200, Torrence, CA 90501).
12. Photometrics (3440 E. Britannia Dr, Tucson, AZ 85706).
13. A. A. Tanbakuchi, Rouse, A. R., Udovich, J. A., Gmitro, A.F., "Surgical imaging catheter for confocal microendoscopy with advanced contrast delivery and focus systems," *in Proceedings of the SPIE The International Society for Optical Engineering: Endoscopic Microscopy*, **6082**, (2006).
14. S. Srivastava, Rodriguez, J. J., Rouse, A. R., Brewer, M. A., Gmitro, A. F., "Automated texture-based identification of ovarian cancer in confocal microendoscope images," *in Proceedings of the SPIE The International Society for Optical Engineering: Three Dimensional and Mulitdimensional Microscopy*, **5701**, 42-52, (2005).
15. DC Graves Co. Inc. (PO Box 583, Wakefield, MA 01880).