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Abstract. Recent evidence suggests that ovarian cancer can originate in the fallopian tube. Unlike many other cancers, poor access to the ovary and fallopian tubes has limited the ability to study the progression of this deadly disease and to diagnosis it during the early stage when it is most amenable to therapy. A rigid confocal microlaparoscope system designed to image the epithelial surface of the ovary *in vivo* was previously reported. A new confocal microlaparoscope with an articulating distal tip has been developed to enable *in vivo* access to human fallopian tubes. The new microlaparoscope is compatible with 5-mm trocars and includes a 2.2-mm-diameter articulating distal tip consisting of a bare fiber bundle and an automated dye delivery system for fluorescence confocal imaging. This small articulating device should enable the confocal microlaparoscope to image early stage ovarian cancer arising inside the fallopian tube. *Ex vivo* images of animal tissue and human fallopian tube using the new articulating device are presented along with *in vivo* imaging results using the rigid confocal microlaparoscope system. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.JBO.19.11.116010](https://doi.org/10.1117/1.JBO.19.11.116010)]

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1 Introduction

Ovarian cancer is a devastating disease with an overall high morbidity and low 5-year survival rate.¹ Unfortunately, there are no known early detection strategies that have resulted in either earlier stage diagnosis or improved survival.^{2,3} As a consequence, many high-risk women choose to undergo prophylactic surgical removal of their ovaries and fallopian tubes to prevent future ovarian cancer. Although this procedure prevents ovarian cancer in 95% of the cases, there are significant downsides in terms of early menopause and cardiovascular disease in addition to removing the patient's ability to have children.⁴⁻⁷ Clearly, providing women in the high-risk population the option to undergo a minimally invasive laparoscopic imaging procedure to evaluate their ovary and fallopian tube health before deciding on prophylactic surgery would be beneficial if the imaging procedure had sufficient diagnostic sensitivity and specificity.

Although ovarian cancer was long thought to originate in the epithelial surface of the ovary, no precancerous or intraepithelial lesions have been identified in the ovary. New evidence suggests that ovarian and fallopian tube cancers derive instead from cells of Müllerian origin and there is strong evidence that many cancers in the ovary in fact arise from cells originating in the fallopian tube.⁸⁻¹¹ We have previously demonstrated a rigid confocal microlaparoscope system for imaging the epithelial surface of the ovary¹²⁻¹⁶ and our results as well as the findings of several other groups¹⁷⁻²⁰ demonstrate that the high resolution *en face* images obtained with confocal microendoscopes can be used for diagnosing disease.

To demonstrate the potential for confocal microendoscopy applied to the fallopian tubes, we first used our previously developed rigid clinical confocal microlaparoscope to image the fimbriae of the fallopian tubes. The preliminary *in vivo* and *ex vivo* images show potential to identify pathologic changes in the fallopian tubes, but the rigidity and the diameter of the confocal microlaparoscope make it difficult to image beyond the exposed surfaces of the fimbria. In order to obtain better access to the fallopian tube itself, we modified the rigid confocal microlaparoscope to include a small diameter articulating distal tip. The new articulating confocal microlaparoscope employs a bare fiber optic imaging bundle that is capable of visualizing cellular morphology and subcellular detail such as nuclear size and shape. In the following section, we briefly review the instrumentation of the rigid confocal microlaparoscope and present preliminary *ex vivo* and *in vivo* images of human fallopian tube images obtained with that system. The new articulating tip confocal microlaparoscope is described in Sec. 3 along with *ex vivo* images of fallopian tubes obtained using the new system. A discussion and conclusions of the work are presented in Sec. 4.

2 Imaging Fallopian Tubes Using the Rigid Confocal Microlaparoscope

2.1 Optical Scan Unit General Description

Both the rigid clinical system and the new articulating bare fiber system connect to the same optical scan unit. A detailed description of this confocal slit-scanning system can be found in previous publications.^{15,21} Briefly, a laser source provides an excitation wavelength of 488 nm. A cylindrical lens transforms the collimated laser beam into a line illumination profile that is scanned across the proximal end of an imaging fiber bundle by a

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scan mirror. Induced fluorescence from the exogenously labeled tissue is collected back through the fiber, de-scanned by the scan mirror, and imaged onto a fixed confocal slit aperture, which rejects light emitted from out-of-focus planes within the tissue. A second scan mirror and a camera lens image the signal onto a two-dimensional (2-D) CCD camera. The two scan mirrors synchronously scan the excitation line of illumination across the proximal end of the fiber bundle and map the emission light onto the CCD to produce a 2-D *en face* confocal image of the sample. The confocal images are read out of the camera at 30 frames per second and displayed on a monitor for the surgeon to view in the operating room.

2.2 Rigid Confocal Microlaparoscope

The rigid confocal microlaparoscope probe has a 5-mm diameter and 30-cm-long rigid tip on a handle that connects to the optical scan unit through a cable containing the fiber bundle and electrical wires that control the operation of the instrument. The rigid confocal microlaparoscope incorporates a 30,000 element fiber-optic imaging bundle with 4- μm center-to-center spacing, which transfers the scanned line illumination profile to the distal end of the probe. The fiber bundle has an imaging diameter of 720 μm , a minimum bending radius of 40 mm, and an NA of 0.35. A miniature achromatic objective lens images the distal end of the fiber bundle into the tissue. A focus mechanism in the handle of the microlaparoscope allows the user to focus from 0 to 200 μm below the surface of the tissue. During a laparoscopic procedure, the rigid catheter is inserted through a trocar and

routed to the ovary under guidance by a conventional wide-field laparoscope. The microlaparoscope is placed in contact with the ovary and fluorescent contrast agent is topically delivered to the tissue at the imaging site.

2.3 Imaging Results Using the Rigid Confocal Microlaparoscope

To evaluate whether we can identify pathologic changes in fallopian tube using confocal microendoscopy, we used the current rigid microlaparoscope to examine excised human fallopian tube samples. In addition, we were able to test the concept of *in vivo* fallopian tube imaging during several surgeries where the surgeon was able to manipulate the fallopian tube and position the 5-mm rigid tip of this microlaparoscope on the fimbriated end of the fallopian tube.

Figure 1 shows two *ex vivo* images of human fallopian tube stained with acridine orange and imaged with the rigid confocal microlaparoscope. The figure includes corresponding histology images taken from the regions where the confocal images were obtained. The diagnosis in both cases was normal fallopian tube. Images taken from the rigid confocal microlaparoscope have a full field-of-view of 0.45 mm in tissue space. Figure 2 shows *in vivo* images taken with the rigid microlaparoscope. As stated, the rigidity and diameter of this system make it hard to image inside the fallopian tube but the surgeon was able to image the fimbriae. Figures 2(a) and 2(b) were taken from a patient whose histologic diagnosis revealed normal tissue. Figures 2(c) and 2(d) were obtained from a patient who had a visible mass attached to

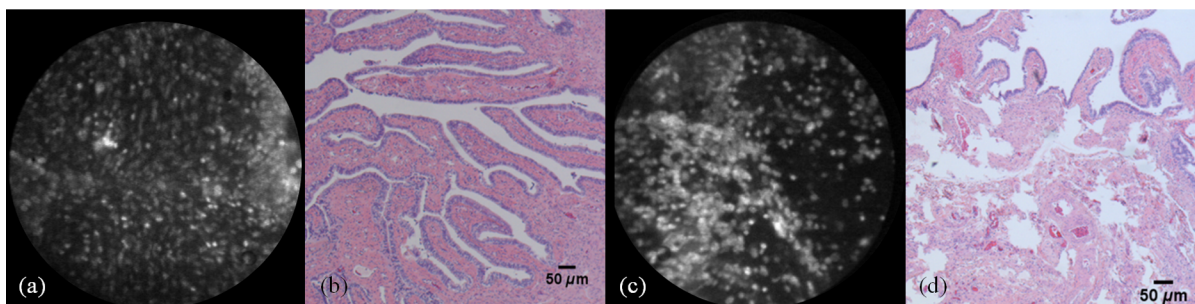


Fig. 1 *Ex vivo* confocal microlaparoscope images and corresponding histologic images of excised normal human fallopian tube stained with acridine orange. Images (a) and (b) are from a similar location on one patient. Images (c) and (d) are from a similar location on another patient. The confocal images (a) and (c) have a full field-of-view of 0.45 mm.

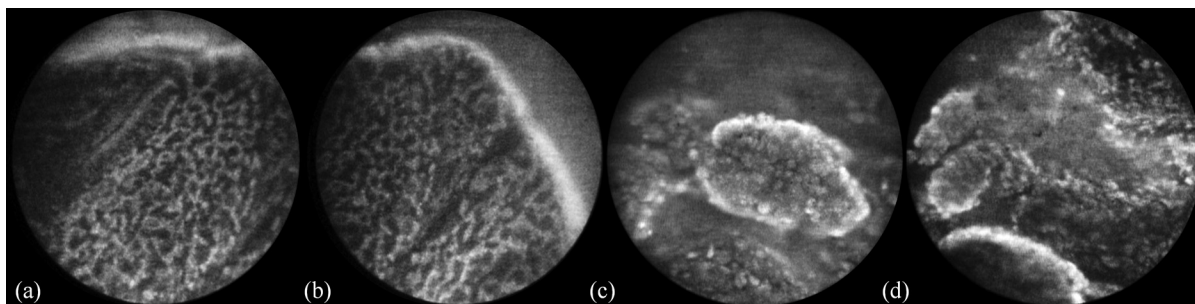


Fig. 2 *In vivo* images of human fallopian tube stained with acridine orange and imaged during open surgery with the rigid confocal microlaparoscope system. Images (a) and (b) are from a patient with healthy tubes and ovaries. Images (c) and (d) are from a patient diagnosed with high grade serous carcinoma in the fallopian tube and no ovarian involvement. The full field-of-view of images is 0.45 mm.

the fimbriated end of the fallopian tube. The mass was imaged *in vivo* with the rigid confocal microlaparoscope system and then diagnosed via frozen section as high grade serous carcinoma. Interestingly, the ovary associated with this fallopian tube had no evidence of cancer on pathologic examination. This particular fallopian tube tumor would have been visually detected and biopsied without the need for a high resolution imaging system. However, this case does call attention to the benefit of a high resolution imaging system to help diagnosis cases where fallopian tube pathology is less clinically evident.

These imaging results suggest that the confocal microlaparoscope has sufficient resolution to differentiate cellular structures in the fallopian tube. One can certainly visualize significant differences between the two *in vivo* cases in Fig. 2, presumably stemming from morphological changes in the cancerous tissue.

3 Imaging Fallopian Tubes Using the New Articulating Confocal Microlaparoscope Catheter

3.1 New Articulating Confocal Microlaparoscope Catheter

To enable better *in vivo* access to fallopian tubes, we designed and built a prototype laparoscopic imaging probe. This probe is a bare fiber bundle imaging system (i.e., no miniature objective lens), which has a thin 2.2-mm-diameter articulating distal tip. The fiber bundle has the same specifications as the one used in the rigid system. Since it is a bare fiber probe, the focal plane is static such that only the tissue in contact with the tip of the fiber bundle is imaged by the confocal instrument. The new articulating microlaparoscope catheter incorporates a dye channel to deliver exogenous contrast agent to the tissue. The smaller diameter distal tip and the ability to control the angle of the tip provide the size and flexibility needed to image inside the curved and delicate structure of the fallopian tube.

The articulating microlaparoscope, shown pictorially in Fig. 3, incorporates an ergonomic lightweight handle. The distal

tip articulates by flexing the handle relative to the direction of the rigid portion of the laparoscope probe. A ball and socket mechanism in the front portion of the handle serves as the pivot point. The flexible distal tip of the laparoscope is composed of 20 metal links that stack on top of each other. Four metal wires extend from a component aligned with the socket, through the ball, down a 30-cm-long rigid metal tube, through four small holes on the rims of the 20 metal links, and finally connect to the distal tip of the probe. As the surgeon changes the angle between the handle and the metal tube, the change in tension on the four internal drive wires causes articulation of the distal tip in a manner similar to that of a traditional flexible endoscope. Roughly 45 deg of angle between the handle and the rigid tube provides nearly 90 deg of distal tip articulation. The design also incorporates an articulation lock that allows the surgeon to set and maintain a specific angle at the distal tip. A single multipurpose button allows the surgeon to save still frames with a short press and deliver a predetermined amount of contrast agent with a long press. Video can be recorded via the computer-based controls on the optical scan unit. A small



Fig. 4 The handle of the articulating tip confocal microlaparoscope probe. The design incorporates an articulation lock that allows setting and maintaining a specific angle at the distal tip. A multipurpose button on top of the probe allows the operator to deliver contrast agent and grab static images.

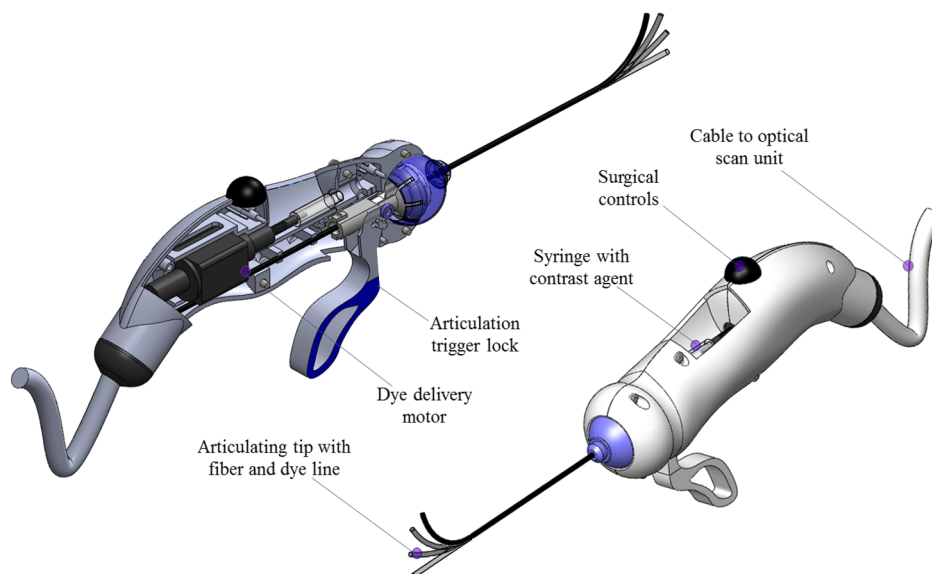


Fig. 3 The articulating confocal microlaparoscope. An internal motor controls the delivery of contrast agent through a hypodermic dye line to the imaging site. A single-multipurpose button is used to save still frames and delivery contrast agent. An articulation lock located on the underside of this design allows the clinician to fix the distal tip at a specific angle.

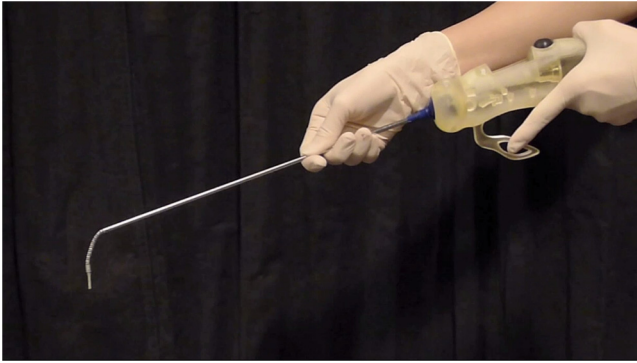


Fig. 5 Demonstration of the articulating distal tip of the new confocal microlaparoscope. (Video 1, MOV, 4.6 MB). [URL: <http://dx.doi.org/10.1117/1.JBO.19.11.116010.1>].

syringe loaded with 0.2 cc of contrast agent is inserted into a Luer Lock connector in a recessed cavity that guides the fluid down a 21 gauge hypodermic tube to the distal tip. The articulation mechanism was inspired by commercial instruments²² that are designed to overcome the limitations imposed by rigid laparoscopic instruments. The probe has a symmetrical design that allows for both left-handed and right-handed usage. The mechanical design for the new catheter was done in SolidWorks and then made using a rapid prototyping Objet350 Connex 3D printer. A photograph of the prototype device is shown in Fig. 4 and a demonstration of the articulating distal tip is provided in Fig. 5.

3.2 Imaging Results Using the New Articulating Confocal Microlaparoscope

The spatial resolution of this bare fiber system is $\sim 7\text{-}\mu\text{m}$ lateral and $20\text{-}\mu\text{m}$ axial measured in water with a full field-of-view of 0.72 mm. The imaging performance of the prototype articulating tip probe was demonstrated using excised animal tissue as well as excised human fallopian tube tissue. Figure 6 shows preliminary *ex vivo* fluorescent confocal images obtained from a mouse [Figs. 6(a) and 6(b)] and a rat [Figs. 6(c) and 6(d)]. The articulating bare fiber probe was placed in contact with the tissue surface after acridine orange was topically applied through the contrast agent delivery channel. Figure 7 shows *ex vivo* images from human fallopian tube stained with acridine orange. Figures 7(a)–7(c) were obtained when the articulating distal tip was placed in contact with the fimbriae. Figure 7(d) was taken when the articulating distal tip was inserted inside the lumen of the fallopian tube. The corresponding histological diagnosis for the fallopian tube tissue in Figs. 7(a)–7(c) was postmenopausal normal fimbria. A histologic diagnosis for the tissue shown in Fig. 7(d) was not obtained since it was difficult to correlate the image location to the tissue site while the tip was inside the lumen of the fallopian tube. The images shown in Figs. 6 and 7 are high resolution and high contrast, and clearly demonstrate the powerful imaging capabilities of this new microlaparoscope probe. During *ex vivo* imaging, we found that the thin articulating tip can be inserted 2 cm beyond the fimbriated end of the fallopian tube relatively easily. Although the new articulating tip probe has not yet been tested *in vivo*, we expect that it will provide better access to the fallopian tubes and an image quality

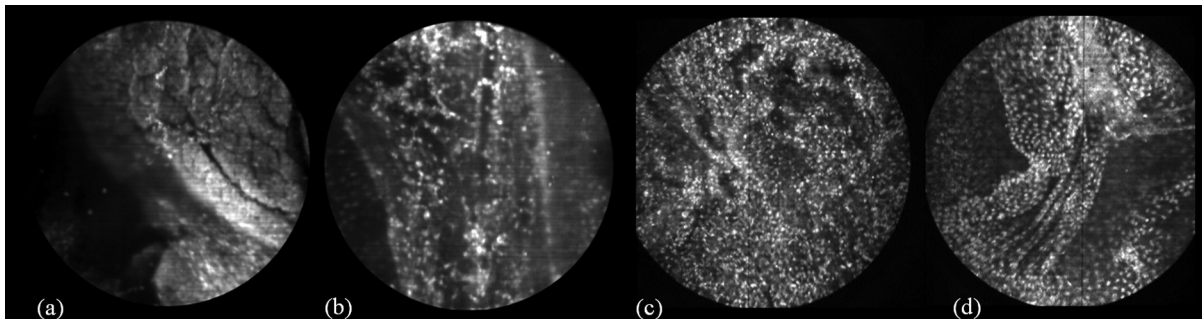


Fig. 6 *Ex vivo* animal tissue images obtained with the new articulating confocal microlaparoscope. All samples were stained with acridine orange: (a) mouse pancreas, (b) mouse abdominal wall, (c) rat spleen, and (d) rat spleen. The full field-of-view of images is 0.72 mm.

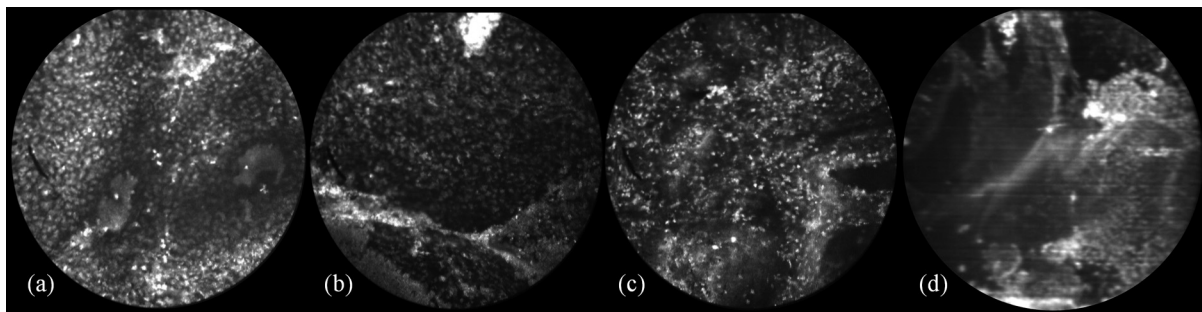


Fig. 7 *Ex vivo* images of excised human fallopian tube stained with acridine orange and imaged with the articulating tip confocal microlaparoscope. Images (a) to (c) were obtained from the fimbria of a patient with postmenopausal normal fallopian tubes. Image (d) was obtained by imaging inside the lumen of the fallopian tube extracted from another patient (diagnosis pending). The full field-of-view of images is 0.72 mm.

capable of visualizing cellular detail and the microstructural features of the tissue.

4 Discussion and Conclusions

A confocal microlaparoscope with a small diameter articulating distal tip was successfully developed to image inside the fallopian tube. The instrument is intended to allow the detection of early stage ovarian cancer arising in the fallopian tube. In this pilot study, tissues were topically stained with the fluorescent contrast agent acridine orange, which readily crosses the cell membrane and intercalates with DNA in the cell nucleus and RNA in the cytosol. Our work has shown significant promise in the identification of ovarian cancer using acridine orange¹⁶ and we have an investigational new drug approval from the US Food and Drug Administration (FDA) to test acridine orange in patients whose tissue is being surgically removed subsequent to imaging. However, there are concerns about the potential mutagenicity of acridine orange. For *in vivo* clinical use, we are investigating several FDA approved contrast agents including fluorescein sodium, methylene blue, and indocyanine green. The new articulating catheter can be used with any of these alternative contrast agents. The development of a safe and effective contrast agent for *in vivo* detection of ovarian cancer is a fundamental goal of our on-going research.

The results of *ex vivo* and *in vivo* imaging of human fallopian tube using our previously developed rigid clinical confocal microlaparoscope suggest the potential of the proposed confocal imaging technique, but access to the fallopian tube is limited by the geometry of the 5-mm-diameter rigid device. The new lightweight ergonomic confocal microlaparoscope with its articulating distal tip, will enable access to the distal end of the fallopian tube during laparoscopic surgery. The ability to image inside of the fallopian tubes up to a few centimeters past the fimbria is a significant advantage of this new instrument. Although the 7- μm lateral resolution of the articulating tip probe is less than the 4.5- μm lateral resolution of the rigid clinical microlaparoscope probe, the performance is sufficient to resolve subcellular structures, such as cell nuclei, and the important microstructural features relevant to ovarian cancer detection. In addition, the overall sensitivity of the bare-fiber articulating design is quite similar to that of the miniature objective-based rigid system. The bare-fiber system collects less light because it has a smaller NA but the bare-fiber system has a higher throughput because it eliminates the miniature objective, which has 15 optical surfaces.

Overall, the images obtained with this bare fiber-bundle imaging system are of comparable quality to images of ovarian tissue collected with the rigid clinical system. Ultimately, this articulating tip device may allow surgeons to image ovarian cancer arising inside the fallopian tube and to provide a means to detect the disease at an earlier stage when it is more amenable to effective treatment. *In vivo* clinical evaluation of the new articulating microlaparoscope system is the next step in demonstrating the potential of this instrumentation.

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