Published in final edited form as:

Int J Gynecol Cancer. 2016 February ; 26(2): 248–254. doi:10.1097/IGC.0000000000000595.

Pilot Clinical Evaluation of a Confocal Microlaparoscope for Ovarian Cancer Detection

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Abstract

Objective—The aim of this study is to evaluate the performance of a confocal fluorescence microlaparoscope for *in vivo* detection of ovarian cancer.

Methods/materials—Seventy-one patients scheduled for open or laparoscopic oophorectomy were consented for the imaging study. High-resolution confocal microlaparoscopic images of the epithelial surface of the ovary were acquired *in vivo* or *ex vivo* following tissue staining using acridine orange. Standard histologic evaluation of extracted tissue samples was performed and used as the gold standard of disease diagnosis. Trained human observers from different specialties viewed the microlaparoscopic images, rating each image on a 6-point scale ranging from “definitely not cancer” to “definitely cancer.” Receiver operating characteristic curves were generated using these scores and the gold standard histopathologic diagnosis. Area under the receiver operating characteristic curve (AUC) was calculated as a performance metric.

Results—Forty-five of the consented patients were used in the final evaluation study. From these 45 patients, 63 tissue locations or samples were identified and imaged with the confocal microlaparoscope. Twenty of the samples were high-grade cancers and the remaining 43 samples were normal or non-cancerous. Twenty-three of the samples were imaged *in vivo* and the remaining 40 samples were imaged *ex vivo*. The average AUC score and standard error (SE) for detection of cancer in all images were 0.88 and 0.02, respectively. An independent-samples t-test

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was conducted to compare AUC scores for *in vivo* and *ex vivo* conditions. No statistically significant difference in the AUC score for *in vivo* (AUC=0.850, SE=0.049) and *ex vivo* (AUC=0.888, SE=0.027) conditions was observed; $t(6)=1.318$, $p=0.2355$.

Conclusions—AUC scores indicate that high-resolution *in vivo* images obtained by the confocal laparoscope can distinguish between normal and malignant ovarian surface epithelium. In addition, *in vivo* performance is similar to that which can be obtained from *ex vivo* tissue.

Keywords

confocal; ovarian cancer; microendoscope; endomicroscope; microscopy

Introduction

Ovarian cancer accounts for 5% of all cancer-related deaths among women, and has the highest mortality rate of all cancers of the female reproductive system¹. Existing screening strategies for the detection of ovarian cancer are largely ineffective and that identification of subclinical disease at the time of surgery is a significant problem^{2–7}. Real-time *in vivo* optical imaging has the potential to improve this situation⁸. Such techniques can be based on wide-field fluorescence imaging^{9, 10} or on microscopic approaches, such as optical coherence tomography^{11, 12}, two-photon microscopy^{13, 14}, and confocal fluorescence microlaparoscopy^{15–21}. As non-destructive techniques, these so-called “optical biopsy” methods are not limited in the number of sites that can be imaged from a given patient, thus allowing more thorough evaluation of organs and tissues within the peritoneal cavity.

Confocal microendoscopy is a high-resolution imaging technique that allows *in situ* visualization of cells within a thin layer of bulk tissue. The signal is based on reflected light or the excited emission from endogenous fluorophores or exogenously administered fluorescent dyes. Confocal microendoscopes are capable of imaging cellular-level structures in biological samples at depths up to $\approx 150 \mu\text{m}$, and have been applied in corneal, dermal, esophageal, and gastrointestinal disease diagnosis^{22–25}.

As described in previous publications, a confocal microlaparoscope has been developed for the detection of ovarian cancer^{15, 16, 26–29}. Previous work with *ex vivo* ovarian tissue samples demonstrated the accurate diagnosis of ovarian cancer with this device³⁰. A clinical study was carried out to further evaluate the system¹⁵. This manuscript presents the findings from the clinical study and the performance achieved using the confocal microlaparoscope for *in vivo* imaging of ovarian cancer.

Materials and Methods

The imaging system employed in this study consists of a handheld microlaparoscope probe (see Figure 1) that connects to a custom confocal microscope on an endoscopy cart. The microlaparoscope probe consists of a 35-cm long, 5-mm diameter, rigid tube extending from a pistol-grip style handle. At the distal end of the rigid probe, a custom miniature objective lens images the tissue onto an imaging fiber bundle. A 750- μm diameter, 30,000-element imaging fiber bundle (Sumitomo Electric USA, Torrance, CA) runs through the rigid tube

and serves as an optical relay between the microlaparoscope probe and the line-scan confocal microscope on the endoscopy cart. The handle of the microlaparoscope has buttons that control motors in the handle to vary the focal depth in the tissue, deliver fluorescent dye to the tissue, and capture still images and video sequences of images. The endoscopy cart houses a laser source for illumination and an optical scan unit that contains the electronics, optics, and camera of the line-scan confocal microscope. Real-time confocal microlaparoscopic images are displayed at 30 frames-per-second on monitors in the operating room for the surgeons to view during the imaging procedure. The system provides 3- μm lateral and 25- μm axial resolution over a 450- μm field of view. The real-time imaging nature of the system allows data collection over a much broader field simply by moving the probe tip over the tissue surface.

Participants in the clinical study were recruited from a cohort of women who were at least 18 years of age, not pregnant, and who were undergoing laparoscopic or open oophorectomy. The study included patients with normal ovaries, benign neoplasms, and cancer. Standard surgical procedures were followed, with the addition of the confocal imaging protocol, which added no more than 10 minutes to the procedure. Prior to imaging with the confocal microlaparoscope, the ovary, with supplying vessels left intact, was placed in an endobag in order to isolate it from the peritoneal cavity. During imaging, dye was delivered directly to the tissue site under interrogation via hypodermic tubing that runs along the length of the microlaparoscope. The use of the endobag and localized dye delivery ensured that dye was not absorbed by tissue that remained inside the patient after the surgical procedure was completed. This specialized protocol was approved under the University of Arizona Institutional Review Board to allow the use of the fluorescent dye acridine orange (AO), which is a nucleic acid stain that is not yet approved for general human use. With these protective measures in place, the *in vivo* use of AO was granted under FDA Investigational New Drug approval 102603. For *in vivo* imaging, 1 μL of 330 $\mu\text{M/L}$ AO was administered topically to the surface of the ovary at each site just prior to imaging. After the ovary was removed, a tissue sample was taken from the imaged site for gold standard diagnosis by a pathologist with expertise in gynecologic malignancies.

Results

The image dataset

Seventy-one women were consented to participate in the clinical study. Technical issues prevented successful use of the confocal microlaparoscope in 4 cases. Five cases were not imaged due to unavoidable changes in patient status prior to surgery. Three cases resulted in image quality that was considered inadequate for use in the study. Nine cases had highly fibrotic or cystic tissue that was deemed inappropriate for the study. Five cases were excluded as a result of a missing histology slide (1 case), mishandled tissue leading to no histology slides being processed (1 case), a poor match between the imaged area and the tissue we were able to procure for pathological analysis (2 cases), or an inconclusive diagnosis from pathological analysis (1 case). This led to a total of 45 cases in the final study.

Out of the 45 patients included in this analysis, 14 were low-risk, 9 were high-risk, and 22 were diagnosed with cancer in one or both ovaries. All 9 of the high-risk patients had risk reducing prophylactic surgery; the low-risk patients had surgery for other indications. The 22 cancer cases included 1 transitional tumor (tumor not imaged), 1 mucinous carcinoma, 1 low-grade serous carcinoma, and 19 high-grade serous carcinomas. Four of the cancers were FIGO stage 1 or 2 and the remaining 18 were stage 3 or 4. Fifteen of the cancer patients had macroscopic dissemination and 7 of those had ascites. Eighteen of the 22 cancers had tubes or ovaries that appeared to the gynecologic oncologist performing the surgery to be cancerous under gross visual assessment.

For each patient, the confocal microlaparoscope was used to image one or more locations on one or both ovaries. By imaging multiple locations, 45 patients yielded 63 tissue samples, each with a corresponding gold standard histopathology diagnosis. Table 1 summarizes the final database. Note that, 16 of the 45 patients had cancer at the imaged location whereas 22 patients were diagnosed as having ovarian cancer. This 6 patient difference is a result of the specific locations that were imaged. Two patients presented with one cancerous and one non-cancerous ovary and in both cases, it was the non-cancerous ovary that was included in the confocal microlaparoscope analysis. In the remaining 4 patients, both ovaries were cancerous on final review of the tissue sections, but normal-appearing regions of the ovarian surface were imaged by the confocal microlaparoscope. The purpose of this pilot study was to demonstrate the accuracy of diagnosis using images collected by the confocal microlaparoscope compared to the corresponding gold standard histopathology diagnosis of tissue extracted from that same location. In a real clinical scenario, the confocal microlaparoscope would be used to image over a significant portion of the ovary rather than just one or two locations.

Of the 63 samples, the gold standard histology diagnosis was cancer for 20 samples (from 16 patients). Seventeen of the cancer samples received a gold standard diagnosis of high-grade serous carcinoma, 2 samples were low-grade serous carcinomas, and 1 sample was a high-grade mucinous carcinoma. The remaining 43 samples (from 29 patients) were designated as non-cancerous with 42 samples displaying no abnormality and one benign serous neoplasm that was designated as non-cancer for this study.

Part of the purpose of this study was to compare the *in vivo* and *ex vivo* performance of the confocal microlaparoscope. As shown in Table 1, 23 samples (from 20 patients) were imaged *in vivo* and 40 samples (from 30 patients) were imaged *ex vivo*. This includes five patients that yielded both *in vivo* and *ex vivo* samples.

Multiple confocal images were collected at each tissue site, which led to a database of nearly 1000 images. Individual images were adjusted to achieve a consistent contrast and dynamic range across the entire database of images. A selection criterion was used to extract a reduced dataset for the study of 260 high-quality images, 67 from *in vivo* imaging and 193 from *ex vivo* imaging (see Table 1). Images were chosen based on the criteria that at least 30% of the imaged field of view contained tissue with distinguishable nuclei (e.g. the image contrast and resolution was sufficient). An additional selection criterion was to create a

manageable dataset that included a variety of image types (*in vivo*, *ex vivo*) and diagnoses (cancer, not cancer) but that did not include significant duplication.

Figure 2 shows examples of confocal microlaparoscope images that were obtained during this study. The top images were obtained from normal ovarian epithelial tissue and the bottom images are of ovarian cancer. This figure also compares *ex vivo* and *in vivo* images. The bright punctate structures in these images correspond to AO stained nuclei. These images show that normal tissue is distinguished by a regular and uniformly distributed cellular epithelium. Often, partially denuded epithelial and stromal layers are visible. Typically, a heterogeneous distribution of size and spacing of nuclei is indicative of ovarian cancer. The results demonstrated that the confocal microlaparoscope is capable of achieving comparable image quality *in vivo* and *ex vivo*. The results also showed that normal ovary and ovarian cancer have the same distinguishable features in both *in vivo* and *ex vivo* situations. The confocal microlaparoscope is also capable of displaying and recording real-time video during surgery (see **video link**, Supplemental Digital Content 1, which shows side-by-side videos of normal and cancerous ovaries during *in vivo* imaging).

Observer Study

For the observer performance study, twenty-nine images from the 260-image data set were selected to use as a training set. The training set contained the variety of morphological and image features representative of both normal and cancerous cases. Six images in the training set were *in vivo* and 23 were *ex vivo*. Twelve of the 29 images were taken from cancer cases, and the remaining 17 images corresponded either to a normal or in one case a benign diagnosis (which was classified as “non-cancerous” for the purposes of this study).

Five observers participated in the study: two researchers (AG, AR), two gynecologic oncologists (SC, KH), and one pathologist (WZ). The researchers had significant *a priori* familiarity with the types of images generated by the confocal microlaparoscope, while the gynecologic oncologists and pathologist were not as familiar with looking at these types of images. After studying the training set, a mock observer test was performed on rotated versions of the training images. If mistakes were made, these were discussed and clarified with the reviewer.

For the performance study, observers were asked to rate the remaining 231 test images on a scale from 1 to 6, with the corresponding definitions: 1 – “definitely not cancer”, 2 – “probably not cancer”, 3 – “possibly not cancer”, 4 – “possibly cancer”, 5 – “probably cancer”, and 6 – “definitely cancer.” These results were then tabulated for receiver operating characteristic (ROC) analysis, which was performed using the LABROC software package^{31, 32}. Observer scores were fit with a bi-normal distribution model for the two conditions, cancer and not cancer, and the AUC was computed by varying the decision threshold on the fitted data. The inverse of the information matrix was also calculated to determine the standard error (SE) of the AUC estimate. This calculation was performed for the full set of test images, as well as separately for the *in vivo* and *ex vivo* images.

Figure 3 shows the ROC curves generated in the observer study. AUC and SE values are shown in Table 2. The average AUC between all observers was 0.876 with an average standard error of 0.024.

An independent-samples t-test (Welch's t-test) analysis was conducted to compare AUC scores between *in vivo* and *ex vivo* conditions. There was not a statistically significant difference in the AUC score for *in vivo* (AUC = 0.850, SE = 0.049) and *ex vivo* (AUC = 0.888, SE = 0.027) conditions for the number of samples used in this study ($t(6) = 1.318$, $p = 0.2355$).

Discussion

The results of the study indicate that images from the confocal microlaparoscope can be used *in vivo* to distinguish high-grade ovarian cancer from normal ovaries during laparoscopic or open surgery with reasonably high sensitivity and specificity. In addition, the results do not reveal a significant difference between making this diagnosis based on images acquired *in vivo* vs. *ex vivo*. The experimental data from the observer study were well represented by the bi-normal fitting model used in the ROC analysis and the standard error of the resulting AUC values are sufficiently low to provide a high confidence in these results. The consistency of AUC scores with respect to observer background indicates that the performance is not limited to classically trained pathologists. A relatively short training period appears to be sufficient to achieve accurate diagnosis. Moreover, it is expected that observer performance will improve as experience in viewing this type of imagery is gained and more subtle differences associated with pathology are appreciated.

While the observer study was performed on individual images shown to observers after the surgery was completed, experience in the operating room indicates that the system is capable of generating diagnostically useful information *in situ* at the time of surgery. By enabling disease diagnosis at the point of surgery without requiring the time-consuming process of frozen-section histology, optical biopsy via confocal microlaparoscopy has the potential to reduce operative times and facilitate a faster diagnosis. Moreover, the ability to scan over a larger area of tissue may increase the diagnostic accuracy by reducing sampling error.

As described in the Methods section, a variety of factors led to a reduction in the number of cases included in the final study. While protocols to identify and consent patients to this study were methodical and efficient, 15 consented cases were excluded from the final study due to factors that were beyond our control. As this was the first *in vivo* validation study for the confocal microlaparoscope, it was expected that some cases would be rejected due to hardware and methodology failures. Eleven cases were excluded for reasons of this nature. Experience with these complications enabled hardware and protocol improvements that increased the stability and reliability of the system.

One notable challenge in the study was ensuring that the sample removed for histopathological analysis was taken from the same region that was imaged *in vivo* by the confocal microlaparoscope. During open surgeries, the surgeon was able to indicate image

locations on the organ. However, during laparoscopic surgeries, when the ovary was removed in an endobag, it was more difficult to maintain the orientation of the tissue. The combination of this re-orientation during removal and the lack of distinguishing features on the ovary made it difficult to precisely identify the imaged locations for subsequent histopathological sampling. Matching histopathological analysis to the confocal imaging location was straightforward for the *ex vivo* imaging studies. Ultimately, similar AUC performance for *in vivo* and *ex vivo* imaging suggests that this was not a major factor in validating the instrument in this study. However, in the future, when the confocal microlaparoscope is used to evaluate the detection of early stage localized disease, careful alignment of imaging and histology will become more critical.

In general, it was slightly more difficult to obtain consistently high quality images *in vivo* compared to *ex vivo*. On occasion, the surgeon would slide the probe slightly beyond the small tissue region that had been stained by the topically applied fluorescent dye. Motion of the probe also occasionally made it difficult to keep the system in focus during an imaging session. As a result, *in vivo* images sometimes had lower signal and poorer image contrast than those taken *ex vivo*, where it was easier to control the application of dye and ensure that the probe remained stationary on the tissue site. Nevertheless, during *in vivo* imaging it was possible to pull back the probe and reapply dye when necessary. With the exception of one outlier (observer 5), nearly equal AUC performance was obtained for *in vivo* and *ex vivo* images, which indicates that the slightly lower contrast observed in the *in vivo* image data was not an impediment to making a correct diagnosis. Ultimately, the problem of localized dye delivery will be obviated by the identification or development of a safe and effective dye that can be delivered systemically or applied widely over the surface of the ovary.

Ex vivo imaging was also logistically easier than *in vivo* imaging because dealing with excised tissue allowed us to spend an extended amount of time acquiring images without having to worry about delaying the normal surgery. This, along with the problem of localized dye delivery mentioned above, led to a slightly disproportionate number of *ex vivo* images in the final database. As shown in Table 1, 74% of the final database was comprised of *ex vivo* images whereas only 63% of the actual samples were imaged *ex vivo*. However, we feel that the group of images included in this study accurately represent the image performance of the confocal microlaparoscope under both *in vivo* and *ex vivo* conditions.

While we did not specifically select patients with high-grade serous carcinoma, it is the most common ovarian cancer and it was the most common subtype in the final image database. However, three of the cancer samples were not of the high-grade serous carcinoma subtype. Two samples, from a single patient, were low-grade serous carcinoma and one sample, from another patient, was a mucinous carcinoma. While the ROC analysis demonstrated an 88% AUC across all images, 73% and 65% of the low-grade serous and mucinous carcinoma images, respectively, were correctly diagnosed as cancer by the observers in this study. This reduced performance may be, in large part, due to the fact that the observers were not trained on low-grade serous and mucinous carcinomas since they made up such a small part of the final image database. Ultimately, it is important to note that confocal imaging in this study was intended to provide a determination of cancer vs. not-cancer rather than a diagnosis of disease subtype.

As noted in the Results section, 4 of the 22 cancer patients included in the final study had tubes or ovaries that were not recognized as cancer by the gynecologic oncologist on gross examination. However, three of these 4 cases were correctly identified as ovarian cancer by the confocal microlaparoscope. This highlights the potential utility of the confocal microlaparoscope to diagnose early stage cancers.

This pilot study was intended to demonstrate the ability of the confocal microlaparoscope to detect ovarian cancer. However, systems such as the confocal microlaparoscope have applications to many other cancers. For example, the diagnosis of early stage cancers not visible using standard methods, the identification of tumor margins, and the detection of subclinical metastases. Future studies will evaluate the confocal microlaparoscope approach using targeted fluorescent contrast agents to detect subclinical occult ovarian cancers and precancerous changes in high-risk women. The scope of this work is being expanded to include imaging of the fallopian tubes, which is believed by many investigators to be the site of origin for high-grade serous carcinoma³³.

In summary, this study demonstrates the feasibility of using the confocal microlaparoscope in the surgical setting. The results show that *in vivo* images are nearly identical to those obtained from *ex vivo* tissue. The diagnostic performance of the approach was demonstrated by the ability of observers to distinguish the cellular features of high-grade cancers from normal ovarian surface epithelium. Perhaps, most significant is the potential to use this approach for screening women at high risk for developing ovarian cancer. The confocal microlaparoscope, and its ability to assess ovarian health during laparoscopic imaging procedures, may become an attractive alternative to risk reducing surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

This research was supported by National Institutes of Health grant CA115780 and by American Cancer Society grant 4560700.

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Figure 1.

Figure 2.

Figure 3.

Table 1

Breakdown of image database.

	Total	Cancer	Non-Cancer	In Vivo	Ex Vivo
Patients	45	16 [‡]	29	20 [*]	30 [*]
Samples	63	20	43	23	40
Images	260	103	157	67 (19 cancer)	193 (84 cancer)

[‡] According to pathology reports, 22 patients had ovarian cancer. However, only 16 patients were diagnosed with cancer at the specific locations that were imaged. See text for more details.

^{*} Five patients were imaged both *in vivo* and *ex vivo*.

Table 2

AUC and SE values for all five observers.

	Total AUC	Total SE	In Vivo AUC	In Vivo SE	Ex Vivo AUC	Ex Vivo SE
Observer 1	0.851	0.026	0.838	0.050	0.848	0.031
Observer 2	0.909	0.019	0.880	0.039	0.924	0.020
Observer 3	0.859	0.025	0.887	0.038	0.867	0.028
Observer 4	0.875	0.028	0.888	0.052	0.885	0.034
Observer 5	0.886	0.022	0.759	0.067	0.919	0.021
Average	0.876	0.024	0.850	0.049	0.888	0.027