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# Probe-based confocal laser endomicroscopy intra-operative evaluation in ovarian cancer: definition of *in vivo* architectural patterns to determine resection strategies

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## ABSTRACT

***Objective:*** Probe-based confocal laser endomicroscopy (pCLE) is a novel real-time imaging technique that is potentially useful for accurately distinguishing between normal and cancerous tissues. The aim of this study was to describe the pCLE patterns of areas suggestive of tumors and evaluate the ability of the method to differentiate between normal and cancerous tissue during cytoreductive surgery for epithelial ovarian cancer.

***Methods:*** *In vivo* pCLE images and subsequent biopsies were acquired from various anatomical sites including the parietal and visceral peritoneum, ovaries, and omentum. Each endomicroscopic sequence was analyzed by highly experienced investigators using pCLE imaging for cancer diagnosis. Each pCLE sequence was compared with the histology of the corresponding specimens.

***Results:*** We enrolled 18 women with International Federation of Gynecology and Obstetrics stage III/IV high-grade serous epithelial ovarian cancer referred for primary or interval debulking surgery. A total of 112 biopsies were obtained for histologic analysis. The pCLE images of normal tissue showed a regular distribution of stromal fibers and consistent cellular architecture, regardless of the anatomical region, with vascularized areas characterized by regular vessels. Conversely, the extravasation of fluorescein, used as a contrast agent, was a distinguishing feature of malignant nodules, which were easily recognized by leakage and are typical of tumor-associated vessels. The leakage often surrounded the dark clusters of neoplastic cells. A substantial agreement between pCLE and histology emerged ($k = 0.66$), whereas only a fair concordance between the surgeon's intra-operative assessment and histology was found ($k = 0.30$).

***Conclusions:*** Our results suggest that pCLE is a promising intra-operative technique to assist surgeons in accurately detecting peritoneal metastases in patients with advanced epithelial ovarian cancer, enhancing surgical radicality while avoiding unnecessary resection.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

*Very few studies have explored fluorescence-guided surgery in patients with epithelial ovarian cancer to reach a balance between surgical radicality and tissue preservation.*

### WHAT THIS STUDY ADDS

*Probe-based confocal laser endomicroscopy (pCLE) technology enables in vivo assessment of the architectural appearance of both normal and cancerous tissue in patients with epithelial ovarian cancer.*

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

*pCLE may assist surgeons in accurately detecting peritoneal metastases during surgery for advanced epithelial ovarian cancer, thereby enhancing surgical radicality while avoiding unnecessary resections.*

*Keywords:*
Probe-based Confocal Endomicroscopy (pCLE);
Epithelial Ovarian Cancer;
Minimal Residual Disease;
Surgical Procedures;
Fluorescent Dyes

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## INTRODUCTION

Approximately 80% of patients with epithelial ovarian cancer are diagnosed at an advanced stage (International Federation of Gynecology and Obstetrics [FIGO] stages III-IV). In such cases, widespread intra-abdominal disease with peritoneal carcinomatosis is often present.[1] Patients with advanced epithelial ovarian cancer are usually treated with extensive cytoreductive surgery. The intra-operative identification of malignant tissue samples can be challenging for surgeons. Microscopic tumors do not indicate clear boundaries to distinguish them from the surrounding healthy tissue. In addition, peritoneal adhesions from previous surgeries or from the cancer itself can obscure visibility and complicate the detection of smaller tumors.[2] This is crucial to prevent unnecessary re-sections in patients who undergo extensive surgery, which carries a high risk of intra-operative and post-operative morbidity. Therefore, there is an urgent need for a real-time diagnostic modality that can accurately identify peritoneal metastases while minimizing the re-section of normal tissue.

Probe-based confocal laser endomicroscopy (pCLE) is an endoscopic technique making it possible to integrate a confocal scanning microscope into a conventional flexible endoscope. pCLE provides high-quality imaging with resolution achieved by intravenous injection of fluorescein at approximately 1 μm of the mucosal layer.[3] The main clinical application for which pCLE was developed is the real-time histopathologic diagnosis of gastrointestinal lesions.[4,5] In our hands, this endoscopic technique provided additional structural and functional information to characterize the vascular network of tumor and thus analyze angiogenesis patterns in patients with gastric and rectal cancer.[6-8] More importantly, optical tissue navigation with pCLE could guide surgeons' resection strategies.[9−11]

To date, the potential role of pCLE for *in vivo* examination in patients with ovarian cancer with carcinomatosis remains unclear. To the best of our knowledge, this is the first study to examine the intra-operative identification and characterization of architectural and cytologic tissue patterns in patients with epithelial ovarian cancer using pCLE to assess whether the method can distinguish cancerous tissue from normal tissue and thus guide resection strategies.

## METHODS

### Patient Selection

All patients with FIGO stage III/IV serous high-grade epithelial ovarian cancer who were referred to our institution for primary or interval debulking surgery between January 2023 and December 2023 were enrolled in the study. The study protocol was approved by the Ethics Committee of the CRO-IRCCS of Aviano (institutional review board number: CRO-2019-62), and all participants provided written informed consent.

### Intra-Operative pCLE Evaluation

All surgeries were performed by gynecologic oncologists. *In vivo* endomicroscopic images and subsequent biopsies were obtained at the surgeon's discretion from various anatomical sites, including the parietal and visceral peritoneum, ovary, and omentum. Samples were obtained from areas without gross disease; in some cases, biopsies were specifically obtained from tissue without signs of carcinomatosis for comparison.

The real-time microscopic images were acquired at the beginning of the surgery, before debulking surgery, using the GastroFlex UHD Confocal Miniprobe connected to the Cellvizio system (Mauna Kea Technologies), which provided illumination at a wavelength of 488 nm. This Miniprobe was inserted through a port, and the distal tip of the probe was positioned perpendicular to the various anatomical sites. Images were acquired within the first 10 minutes after the intra-venous injection of fluorescein (3 mL of a 10% saline solution), as previously described.[6,12] pCLE acquisitions were performed for $\geq$3 minutes, generating real-time imaging of $>$1000 images.

In the initial group of patients, an experienced endoscopist performed an "online" analysis to identify benign and malignant tissue based on expertise acquired in pCLE imaging for cancer diagnosis. To improve the performance of pCLE in this novel area of investigation, an "offline" assessment was subsequently introduced in which images were stored digitally and reviewed using a dedicated software package (Cellvizio Viewer, Mauna Kea Technologies) by a pCLE expert blinded to any clinical or histopathologic information. The diagnostic performance of pCLEs was evaluated using histology as the reference standard.

### Data Analysis

The observer variability of the diagnostic evaluation was calculated using Cohen's $\kappa$ coefficient with the following classification: poor $<$0.2, fair 0.21 to 0.4, moderate 0.41 to 0.6, substantial 0.61 to 0.8, and excellent 0.81 to 1. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy, with corresponding 95% Clopper-Pearson CIs, were calculated for both surgeon and pCLE performance using histology as the reference standard.

In accordance with the journal's guidelines, we will provide our data for independent analysis by a selected team by the Editorial Team for the purpose of additional data analysis or for the reproducibility of this study in other centers if such is requested.

## RESULTS

A total of 18 patients agreed to participate. The median age was 67.5 years (range; 43-77 years). Half the patients underwent primary and interval surgery. In all cases, laparotomy was performed. In the interval surgery group, this was performed after 3 cycles of neoadjuvant platinum-based chemotherapy. A total of 112 biopsy samples were obtained for histologic analysis, and an average of 7 samples (range; 4-13) per patient (including *in vivo* pCLE and subsequent histologic analysis) were obtained. The number of biopsies was similar in women who underwent primary or interval surgery (range; 6.56 $\pm$ 0.73 vs 7 $\pm$ 2.87; $p = .66$).

Figure 1 presents representative images of normal ovaries and ovarian tumors. In normal tissue, the stroma was identified by the presence of a regular network of white fibers (Fig. 1A). This analysis allowed us to distinguish the normal epithelium, which was arranged in a regular pattern of evenly distributed cells. The vessels were clearly detectable owing to their normal size and geometry (Fig. 1A), and regular blood flow (data not shown). In contrast, the ovarian cancer tissue was characterized by the

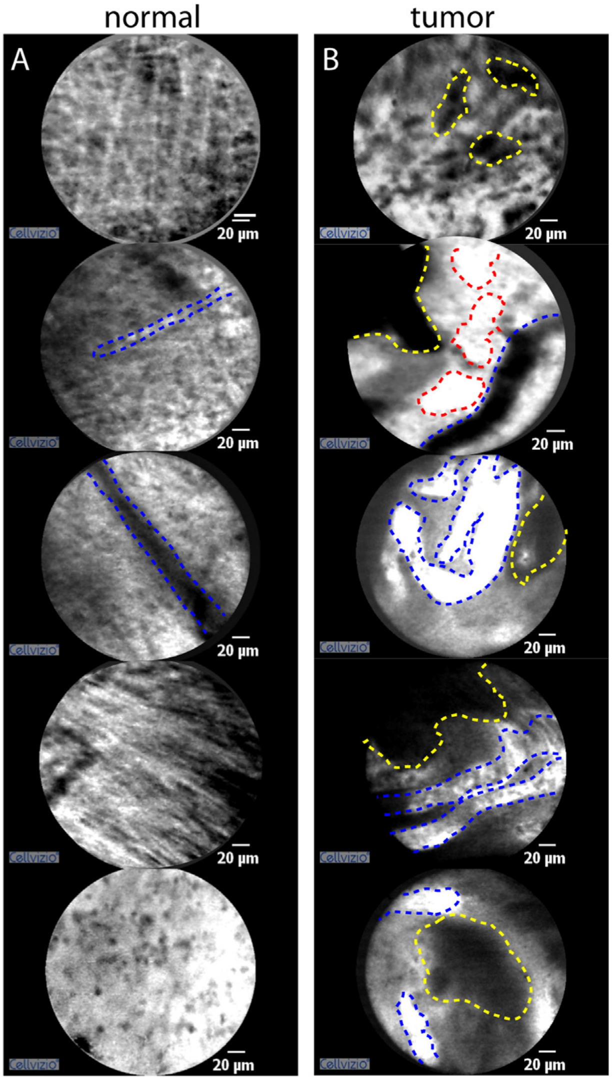


**Figure 1** Probe-based confocal laser endomicroscopy images of normal and neoplastic ovarian tissue. Images of the normal (**A**) and tumor (**B**) ovarian tissue as obtained by pCLE. The white network of connective and the homogeneous cellular distribution with normal vasculature (blue dashed lines) characterizes the non-pathologic ovary. In contrast, dark cell aggregates (yellow dashed lines), abnormal tortuous vessels (blue dashed lines), and leakage (red dashed lines) are typical features of ovarian tumor tissue. pCLE, probe-based confocal laser endomicroscopy.

presence of dark cellular agglomerates, often surrounded by tortuous and large vessels with occasional leakage (Fig. 1B).

The pCLE images of the normal parietal peritoneum revealed a uniform pattern, likely reflecting the stromal component of the inner peritoneal surface where the regular arrangement of the connective tissue was particularly prominent (Fig. 2A). In contrast, an inhomogeneous architectural organization was observed in the tumor counterpart, with irregular and dark cellular clusters often associated with regions of vascular leakage (Fig. 2B). In several imaged areas of the normal parietal peritoneum, pCLE revealed vascularized areas with regular flow characterized by normal vasculature (Fig. 2C). This was not the case for tumors localized in the peritoneum, in which dilated, distorted, and tortuous vessels, often associated with cellular clusters, were clearly detected. Several areas of leakage were observed (Fig. 2D).

Similar to the findings in the parietal peritoneum, pCLE images of the normal visceral peritoneum revealed a regular distribution of stromal fibers and a well-organized cellular architecture in the presence of a number of regular vessels (Fig. 2E). Conversely, in cases of neoplastic involvement, abundant leakage and irregular and dark cell clusters were clearly detected using pCLE (Fig. 2F). Figure 3 presents representative pCLE images of normal (Fig. 3A) and tumor (Fig. 3B) mesenteric tissues. In this case, the regular architecture and vascular pattern of the normal tissue were easily recognizable. In addition, the normal adipose tissue was characterized by black, round, and regular structures surrounded by fluorescent fibrous extra-cellular matrix. In contrast, these structures appeared irregular in the tumor tissue; dark cell clusters were abundant, and fluorescein leakage occupied the interstitial space.

The presence of large vessels with regular flow (data not shown) and well-organized adipose tissue was a distinctive feature of the normal omentum (Fig. 3C). pCLE analyses of the metastases localized in the omentum showed irregular cell arrangements surrounded by dilated and tortuous vessels, from which fluorescein abundantly leaked into the intercellular spaces (Fig. 3D). In contrast, the blood vessels detected in the healthy tissue were functional, with no apparent signs of leakage.

A total of 123 pCLE sequences were collected from various tissue types: peritoneum, different anatomical sites (abdominal wall, pelvis, and visceral peritoneum) ($n = 81$), mesentery ($n = 16$), ovary ($n = 9$), and omentum ($n = 17$). The video sequences were associated with 112 bioptic samples to evaluate the concordance (Table 1). None of the patients included in the present analysis showed any intra- or post-operative complications after the intra-venous injection of fluorescein. Therefore, the use of fluorescein was both safe and effective, providing good contrast and confirming proper focus and dye delivery during the procedure. To the best of our knowledge, this was the first indication that pCLE using fluorescein as a contrast agent during laparotomic surgery is feasible and safe, as already reported in established pCLE applications in several other organs and different clinical settings.[6,13,14] The quality of images captured during *in vivo* laparotomic application was deemed adequate to accurately characterize tissue morphology according to a highly experienced investigator's opinion. The additional time required for the pCLE procedure was 9 minutes per patient (range; 5-12).

The diagnostic concordance between pCLE and histologic findings was 83.9%, which was higher than that between the surgeon's intra-operative assessment and histologic findings (66.1%). Table 1 lists the percentage of concordance based on the anatomical sites where the biopsies were performed. This analysis indicates that the evaluation of peritoneal carcinomatosis by the surgeon was not consistently reliable, with a concordance rate ranging from 53.8% to 88.9%. In contrast, pCLE imaging significantly increased the level of concordance with histology across all analyzed sites (concordance range; 75%-88.9%), particularly in the parietal peritoneum and mesentery. Table 2 presents the diagnostic accuracy of the surgeon's intra-operative assessment and pCLE compared with histology. Interestingly, a substantial agreement between pCLE and histology emerged (Cohen's $\kappa$ coefficient, 0.66), whereas only fair concordance between the surgeons' intra-operative assessment and histology was found (Cohen's $\kappa$ coefficient, 0.30).

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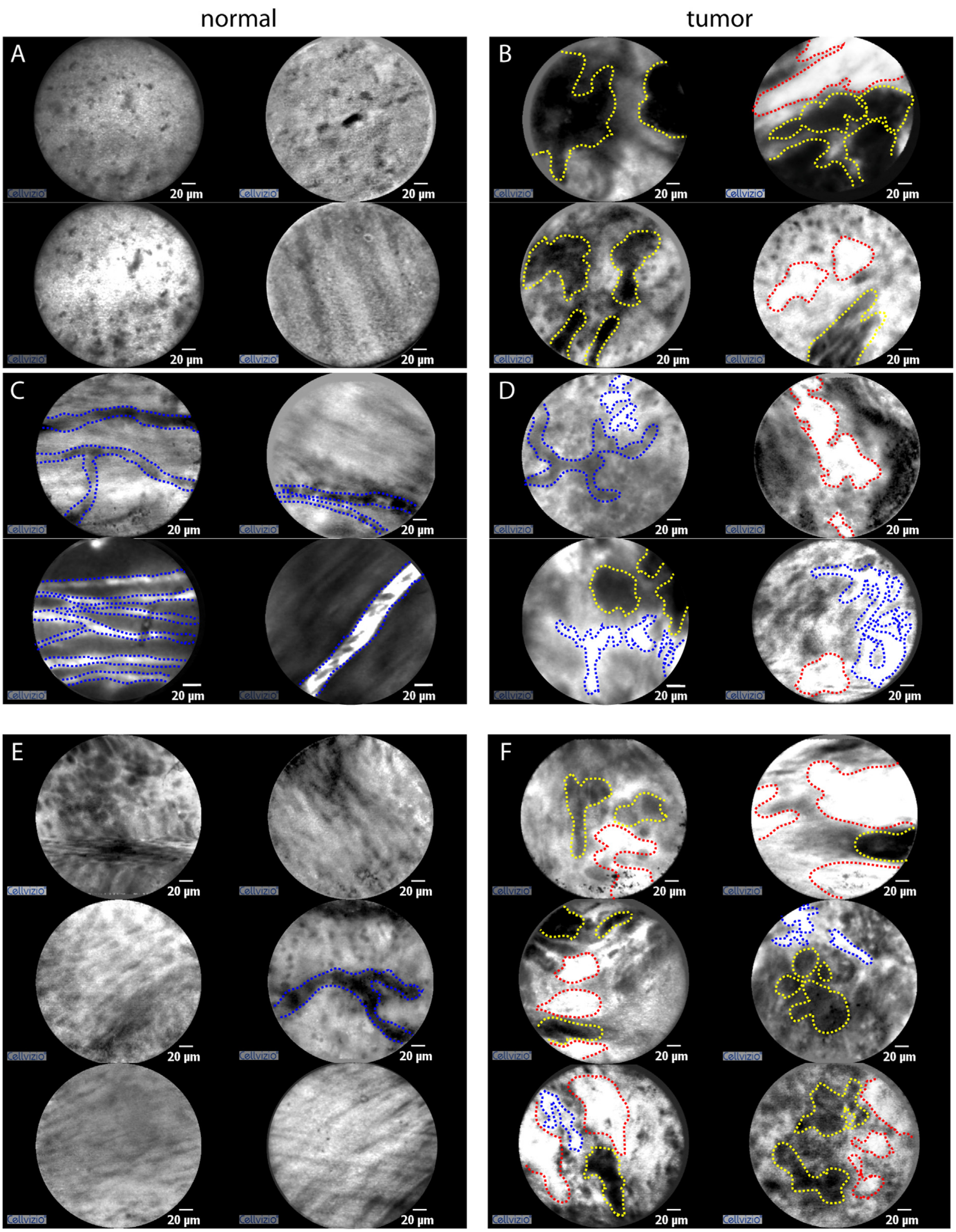


**Figure 2** Probe-based confocal laser endomicroscopy images of the parietal and visceral peritoneum. Areas of normal (**A, C,** and **E**) and tumoral (**B, D**, and **F**) tissues in the parietal (**A-D**) and visceral (**E-F**) peritoneal cavity imaged with pCLE. Homogeneous appearance and the presence of regular vessels (blue dashed lines) characterized healthy tissue. Dark cell aggregates (yellow dashed lines), distorted vessels (blue dashed lines), and leakage (red dashed lines) are distinctive for the presence of malignant nodules. pCLE, probe-based confocal laser endomicroscopy.

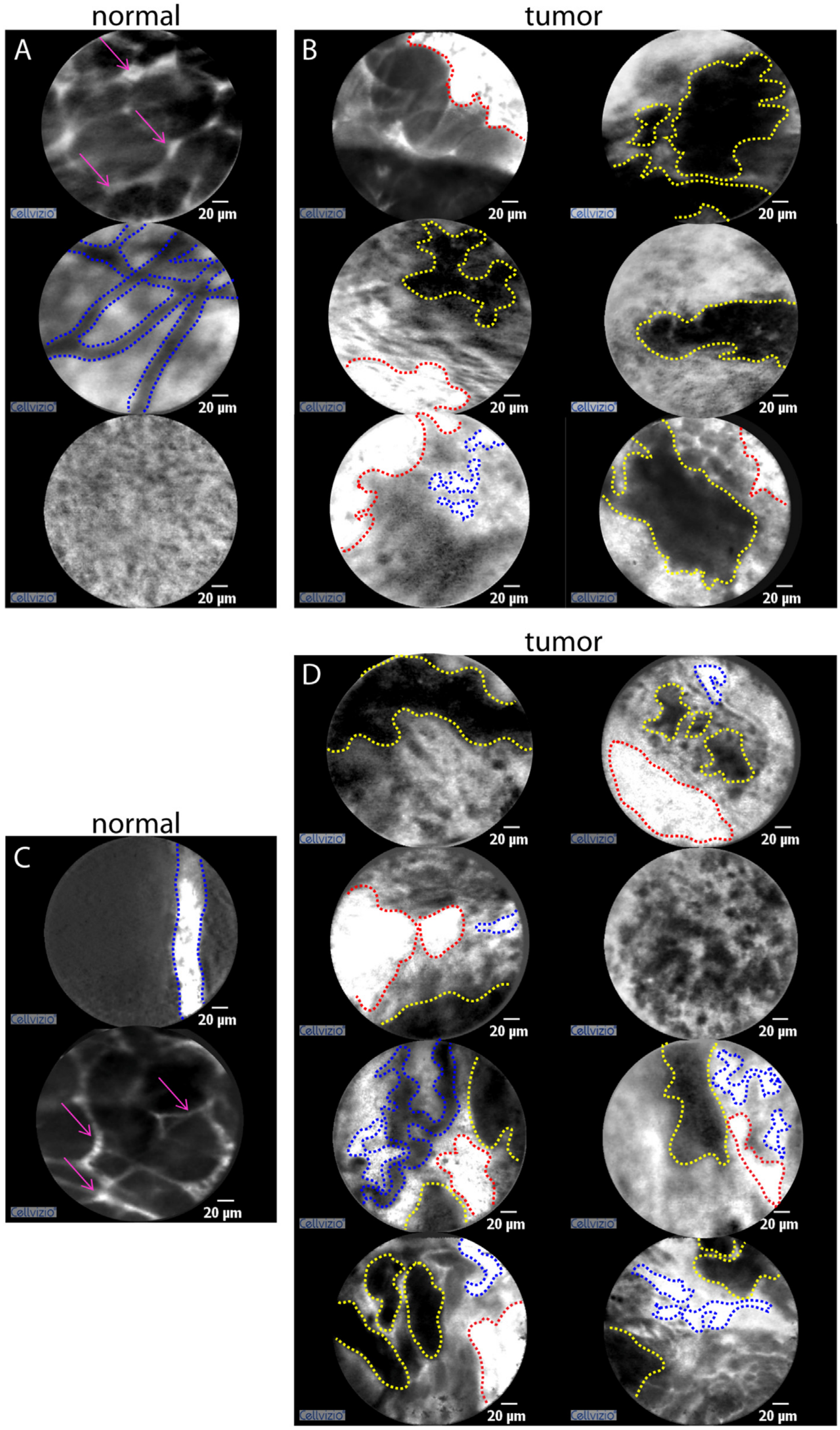


**Figure 3** Probe-based confocal laser endomicroscopy images of the mesentery and omentum. The normal adipose tissue (**A**, mesentery; **C**, omentum) is characterized by black, round, and regular structures surrounded by a fibrous extra-cellular matrix (pink arrows). The vessels are regular, as is the morphologic arrangement of the cells. In the tumor tissue (**B**, mesentery; **D**, omentum), large dark cell clusters (yellow dashed lines) and tortuous vessels (blue dashed lines) are abundant, and leakage (red dashed lines) is often present.

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**Table 1** Percentage of Concordance of Biopsy Sites With Histologic Analysis

| Biopsy site | N | Concordant biopsies | | % of concordance | |
|---|---|---|---|---|---|
| | | Surgery | pCLE | Surgery | pCLE |
| Omentum | 16 | 10 | 12 | 62.5 | 75.0 |
| Parietal peritoneum | 31 | 18 | 26 | 58.1 | 83.9 |
| Visceral peritoneum | 43 | 31 | 37 | 72.1 | 86.0 |
| Mesentery | 13 | 7 | 11 | 53.8 | 84.6 |
| Ovary | 9 | 8 | 8 | 88.9 | 88.9 |
| Total | 112 | 74 | 94 | | |
| % of concordance | | 66.1 | 83.9 | | |

Abbreviation: pCLE, probe-based confocal laser endomicroscopy.

**Table 2** Observer Diagnostic Statistic Agreement

| Histology | Surgery | | pCLE | |
|---|---|---|---|---|
| | Positive ($n = 66$) | Negative ($n = 46$) | Positive ($n = 74$) | Negative ($n = 38$) |
| Positive[a] ($n = 66$) | 47 | 19 | 61 | 5 |
| Negative[b] ($n = 46$) | 19 | 27 | 13 | 33 |
| Sensitivity (95% CI) | 0.71 (0.59-0.81) | | 0.92 (0.82-0.97) | |
| Specificity (95% CI) | 0.59 (0.43-0.73) | | 0.72 (0.56-0.84) | |
| Accuracy (95% CI) | 0.66 (0.56-0.75) | | 0.84 (0.76-0.90) | |
| NPV (95% CI) | 0.59 (0.43-0.73) | | 0.87 (0.71-0.95) | |
| PPV (95% CI) | 0.71 (0.59-0.81) | | 0.82 (0.71-0.90) | |
| Cohen's k (95% CI) | 0.30 (0.12-0.48) | | 0.66 (0.52-0.80) | |

Abbreviations: NPV, negative predictive value; pCLE, probe-based confocal laser endomicroscopy; PPV, positive predictive value.
[a] Positive = neoplastic.
[b] Negative = healthy.

## DISCUSSION

### Summary of Primary Results

In this study, we demonstrated that pCLE analysis is more effective in detecting malignant nodules than a surgical approach solely based on macroscopic observation and palpation. Moreover, no difference in pCLE performance was observed between women who underwent primary and those receiving interval surgery, suggesting that pCLE may be equally reliable in both scenarios.

### Results in the Context of Published Literature

We have found characteristic and reproducible patterns in both cancerous and benign nodules on the peritoneum and on the ovarian surface and omentum. Notably, the presence of leakage was a characteristic pattern in tumor areas. Malignant nodules can thus be identified by the extravasation of the contrast agent, reflecting the abnormal functionality of tumor vessels.[6] As previously described in other cancers, analysis of the vasculature can be a valuable tool to identify malignant tissue.[6-8,15] In our analysis, the presence of tortuous, dilated vessels with irregular spatial geometry and blood flow seemed to be another distinctive feature of neoplastic abdominal nodules. Interestingly, good concordance was observed between the *in vivo* pCLE assessment and histology. In contrast, less concordance was found between the surgeon's intra-operative evaluation and histology.

Only a limited number of studies have evaluated the morphologic characteristics of the ovary using pCLE, typically after topical rather than intra-venous fluorescein administration, which affects the quality of the analysis.[16,17] The recent development of fluorescence-guided surgery is showing promising results, but its application in ovarian cancer has been largely limited to animal models.[18] Few studies in women diagnosed with epithelial ovarian cancer that were performed for efficacy and safety are encouraging, but evidence on the clinical relevance of these observations remains missing.[19,20] In this scenario, in which achieving a balance between surgical radicality and tissue preservation is challenging, non-invasive imaging technologies may enable the use of "optical biopsies" as an alternative to traditional tissue biopsies for diagnostic purposes. pCLE has facilitated dynamic imaging in fields such as gastroenterology, pulmonology, and urology.[6,7,14,21]

Robotic developments combining microscopy and laparoscopy have also been attempted.[22] The insertion of the endomicroscopy probe trans-vaginally for the examination of the peritoneum in a porcine model was successfully proposed.[23] However, most laparoscopic attempts aimed at showing the diagnostic potential of the pCLE imaging technique for the peritoneal lesions were performed as *ex vivo* studies.[24] One of the first attempts to image ovarian tissue *in vivo* was performed by Tanbakuchi and colleagues,[16] who set up a confocal microlaparoscope with topically applied fluorescein to provide real-time diagnostic information and aid physicians in screening and/or surgical procedures. Tummers and colleagues[25] conducted a clinical study that indicated the possibility of detecting ovarian cancer metastases during the intra-operative procedure using near—infrared (NIR) fluorescence imaging and indocyanine green. The effectiveness of intra-operative NIR fluorescence imaging was limited for numerous false-positive lesions without distinguishing among malignant, reactive, or benign tissues.[25] However, in a recent study, Achimas-Cadariu and colleagues[26] showed that indocyanine green was efficacious in identifying additional residual malignant foci during interval surgery of apparently intact peritoneum not deemed clinically suspicious under white-light inspection. pCLE was also proved to be a minimally invasive procedure able to detect lesions of the fallopian tube, making it a useful tool for ovarian cancer prevention and early detection.[27]

### Strengths and Weaknesses

pCLE is a valid and safe endoscopic technique for real-time detection of malignant peritoneal nodules in patients with epithelial ovarian cancer. One potential limitation of pCLE is the restricted tissue depth accessible with the Miniprobe, which allows visualization of only the superficial layers. Nevertheless, given the superficial spread of peritoneal carcinomatosis in ovarian cancer, pCLE seems well suited for its intended application. A specific limitation of this study was the relatively small number of cases from a single institution and the recruitment of patients with high-grade serous ovarian cancer. Therefore, a larger cohort is required to substantiate the diagnostic accuracy of the technique. In addition, the interpretation of pCLE images is a critical factor for accurate assessment; however, it is subjective and reliant on the investigator's experience, which may affect the reproducibility of the method. Consequently, it is essential to establish a consensus and develop standardized protocols for pCLE-based diagnosis and image evaluation of cancerous and benign peritoneal and visceral tissues.

The effect of pCLE on surgical time requires further investigation. In our cohort, we found that using pCLE caused only a modest increase in operative time, which could potentially be minimized if the procedure were used routinely. Moreover, this modest increase in average surgical time could be more than offset by the time saved by avoiding unnecessary resection.

### Implications for Practice and Future Research

Several factors contribute to the use of pCLE in diagnosing peritoneal metastases. First, the development of more efficient and human-approved fluorescent agents could significantly enhance its applicability. Second, ongoing research on artificial intelligence and machine learning may contribute to the development of standardized image analysis algorithms, thereby minimizing subjectivity and improving the reproducibility of pCLE image interpretation. Another intriguing potential application is the combination of pCLE with minimally invasive surgery to compensate for the lack of tactile feedback and potentially to improve R0 re-section rates in patients with early stage ovarian cancer.

## CONCLUSIONS

pCLE may serve as a promising intra-operative technique to assist surgeons in accurately detecting peritoneal metastases in advanced ovarian cancer, thereby enhancing surgical radicality and avoiding unnecessary re-sections. However, further research including patients with other cancer subtypes is required to confirm the results of this study. Given the potential of neoadjuvant chemotherapy to alter tissue structure, future studies with larger patient cohorts are advisable to better investigate pCLE outcomes, particularly in this subset of patients.

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**Declaration of Competing Interests** None declared.

**Acknowledgments** PS and NC contributed equally to the study.

**Provenance and Peer Review** This work was internally peer reviewed.

**Ethical Approval** This study involved human participants and was approved by the Ethics Committee of the CRO Aviano Fondazione IRCCS Istituto Nazionale Tumori (institutional review board number: CRO-2019-62). Participants provided informed consent before participating in the study.

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