

## *New Technologies in Endourology*

# Fibered Confocal Microscopy of Bladder Tumors: An *ex Vivo* Study

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### Abstract

**Background and Purpose:** The inadequacy of white-light cystoscopy to detect flat bladder tumors is well recognized. Great interest exists in developing other imaging technologies to augment or supplant conventional cystoscopy. Fibered confocal microscopy offers the promise of providing *in vivo* histopathologic information to help distinguish malignant from benign bladder lesions. We report the initial use of this technology to visualize tumors in the human bladder.

**Materials and Methods:** We performed *ex vivo* fibered confocal imaging of fresh radical cystectomy specimens using the Mauna Kea Technologies Cellvizio® system. The findings were compared with results from standard histopathology.

**Results:** The bladders of four patients were imaged using the fibered confocal microscope. Normal and neoplastic urothelium manifested differences in cellular and vascular density.

**Conclusion:** This study demonstrates the feasibility of using fibered confocal microscopy to detect histologic differences between normal and neoplastic urothelium, and establishes a foundation for the use of fiber-based confocal microscopy in clinical studies.

### Introduction

**D**IAGNOSTIC CYSTOSCOPY is an indispensable part of the urologist's armamentarium to evaluate the lower urinary tract. Nevertheless, conventional white-light cystoscopy has numerous well-recognized shortcomings, including operator variability, uncertainty when differentiating inflammatory from malignant lesions, and difficulty identifying flat lesions, such as carcinoma *in situ* (CIS). Indeed, conventional cystoscopy may fail to visualize up to one third of tumors.<sup>1</sup>

For pathologic confirmation, suspected lesions must be biopsied with a delay of several days to obtain the result. To sample the remainder of the bladder, random biopsies may be performed, but this remains controversial in part because

of the relatively low yield.<sup>2–5</sup> Furthermore, biopsy entails an associated risk of bleeding and perforation, and provides a relatively small sample size.

Strong interest exists in the development of imaging technologies to augment conventional white-light cystoscopy. Examples include fluorescence cystoscopy with hexyl aminolevulinate (HAL), optical coherence tomography (OCT), and autofluorescent flexible cystoscopy. Multiple studies have shown that HAL fluorescence cystoscopy enables the visualization of more tumors in the bladder urothelium than can be seen by white-light cystoscopy alone.<sup>6,7</sup> Preliminary clinical studies of OCT demonstrate that noninvasive "optical sectioning" of bladder tumors may provide cancer staging information.<sup>8–10</sup> An initial report of autofluorescent cystoscopy indicates that this technique can help dis-

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tinguish CIS from normal urothelium without the need for an intravesical fluorescent agent.<sup>11</sup>

None of these evolving technologies, however, provides sufficient resolution to differentiate cellular details with molecular contrast. The ability to obtain noninvasive, real-time, *in vivo* urinary tract histopathologic information during cystoscopy would prove useful for early diagnosis, surveillance, and image-guided biopsy.

### Technology

First developed in 1957, confocal microscopy is a powerful imaging tool that provides high resolution, dynamic, sub-surface imaging of biological systems.<sup>12-14</sup> In contrast to conventional fluorescence microscopy, confocal microscopy images are not significantly contaminated by light scattered from other focal planes, thereby resulting in the ability to optically section tissues, improved localization of signals, and enhanced contrast.

In conventional confocal microscopy, a low-powered laser is focused onto a single point of the specimen, and the microscope then refocuses the emitted light from the specimen. Any out-of-focus light is removed from the image by passing through a pinhole, so only a thin optical section of the specimen is formed.<sup>15</sup> The illumination and detection systems are in the same focal plane and are termed confocal.<sup>16</sup>

Detection of only the light within the focal plane greatly improves image quality and allows for visualization of signals originating from greater tissue depths. Given the large size of conventional confocal microscopes, this approach has been largely restricted to research, rather than clinical, applications.

Recently, new instrument designs and advances in instrument miniaturization<sup>17,18</sup> have made possible the development of flexible, fiberoptic confocal microscopes that can be passed through the working channel of standard endoscopes. This enables *in vivo* microscopy and is referred to as "confocal endomicroscopy" or "fibered confocal microscopy."

Pilot clinical studies in gastrointestinal endoscopy<sup>19-29</sup> and bronchoscopy<sup>30</sup> have demonstrated excellent histologic resolution, producing images that resemble standard analyses via histopathology. In addition, a single *in vivo* study of fibered confocal microscopy in rat bladders has been reported.<sup>31</sup>

Contrast in confocal microscopy is generated through the use of fluorescent dyes and markers. In clinical studies, this has largely been provided by the dyes fluorescein<sup>19,32,33</sup> and indocyanin green.<sup>34</sup>

To our knowledge, application of *in vivo* confocal microscopy in the human urinary tract has not been previously demonstrated. We hypothesized that fibered confocal microscopy would be an adjunct to conventional white-light cystoscopy, providing images of normal and pathologic urothelium with cellular resolution that would improve the accuracy of cystoscopy.

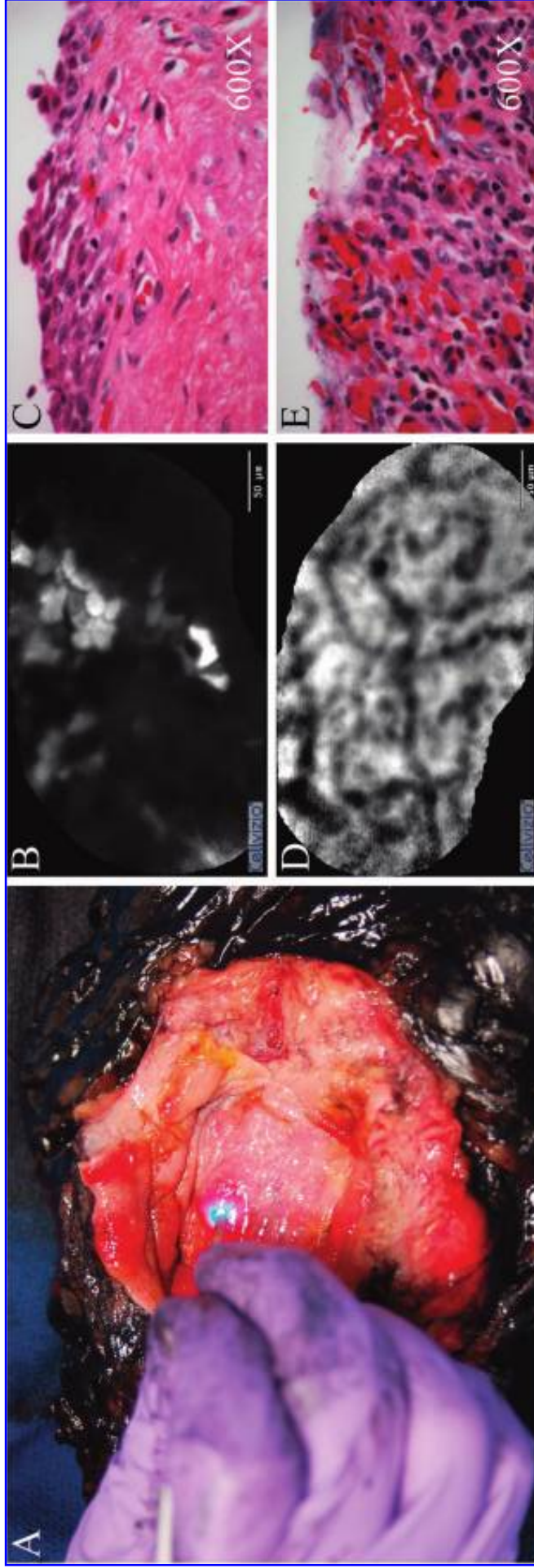
We report a study of fresh cystectomy specimens with biopsy proven bladder cancer using fibered confocal microscopy. The primary objectives of this study were to prove the feasibility of the technology and to document the presence of any morphologic differences between normal and cancerous bladder mucosa using a generalized stain and a confocal microscope.

### Materials and Methods

The research protocol was approved by the Stanford University Medical Center and Veterans Affairs Palo Alto Health Care System Institutional Review Board. Informed consent was obtained from patients with biopsy proven bladder cancer who were deemed suitable candidates for radical cystectomy. Immediately after cystectomy, the bladder specimens were brought to our laboratory for evaluation with the Cellvizio<sup>®</sup> fibered confocal microscope (Mauna Kea Technologies, Paris, France). To obtain optical contrast, 0.05% fluorescein sodium (Sigma-Aldrich, St. Louis, MO) was applied



**FIG. 1.** Fibered confocal microscope, composed of a Confocal Miniprobe<sup>™</sup> attached to the Laser Scanning Unit. (Image courtesy of Mauna Kea Technologies.)



**FIG. 2.** Representative images from a patient with multifocal carcinoma *in situ* (CIS). (A) Images being acquired through direct contact of the Confocal Miniprobe™ tip with the urothelium of the inked cystectomy specimen. (B) Cellular appearance of a normal area of bladder urothelium near the dome. (C) Corresponding normal bladder urothelium near the dome as seen on hematoxylin and eosin (H&E) stain. (D) Dense, arborized urothelial architecture suggestive of hemorrhagic small vessels later found by standard histology to represent CIS. (E) Corresponding appearance of the area seen on H&E stain showing denuded mucosa commonly seen with CIS. Scale bar represents 50  $\mu\text{m}$  in (B) and (D).



topically to the bladder mucosa, either by directly spraying it over the regions of interest after opening the bladder or by instilling 200-300 mL using a Foley catheter into the intact bladder. After instillation, fluorescein was left in contact with the tissue for 5 minutes, followed by rinsing or irrigation with phosphate buffered saline.

The Cellvizio fibered confocal microscope is composed of a 2.6-mm diameter Confocal Miniprobe™ attached to the Laser Scanning Unit (Fig. 1). Images were acquired at a rate of 12 frames per second through direct contact of the urothelium with the probe tip (Fig. 2A). The probes produce images 60  $\mu\text{m}$  in depth, with a lateral resolution down to 1  $\mu\text{m}$ , and a field of view of 240  $\mu\text{m}$  in diameter. Both normal and abnormal appearing areas of the bladder mucosa were imaged.

The specimens were then sent for standard pathologic assessment with hematoxylin and eosin (H&E) staining. Image processing was performed using the proprietary software from Mauna Kea Technologies. Confocal images of normal and abnormal areas of bladder mucosa were compared with the H&E sections of the same tissues.

## Results

Four patients were recruited for the study. On final pathologic evaluation, two specimens had high-grade invasive transitional cell carcinoma (TCC), one had high-grade invasive primary bladder adenocarcinoma, and one had multifocal CIS. Instillation of fluorescein through the Foley catheter yielded images of better quality than topically applied fluorescein alone. We noted microarchitectural differences between normal-appearing and tumor-bearing areas with respect to cellular density and vascular density.

Figures 2B–E show representative images from the patient with multifocal CIS, comparing a grossly normal area at the dome with a suspicious appearing erythematous patch near the trigone. In areas that appeared grossly normal, sheets of uniform cellular structures with uneven uptake of fluorescein were observed (Fig. 2B). These likely correspond to the intermediate cells seen in the H&E staining (Fig. 2C). In contrast, a dense arborized network in a background of irregular cellular structures was seen at the region of the erythematous patch (Fig. 2D). This region was confirmed to be CIS, characterized by denuded mucosa, local hemorrhage, and lymphocytic infiltrate (Fig. 2E). These arborized structures, which we speculate represent increased vascular density, were seen consistently in the suspicious regions of the bladders with TCC.

## Discussion

We report the first application of fibered confocal microscopy in the human urinary tract using a Confocal Miniprobe from Mauna Kea Technologies. Using fresh surgical specimens from patients with bladder cancer and topical fluorescein as contrast, we observed morphologic differences between normal bladder mucosa and cancerous areas that were confirmed using standard H&E sectioning. The data were obtained rapidly from relatively intact tissues, offering the prospect of using this microscope in the clinic.

The possibility of acquiring real-time histopathologic information of urinary tract pathology (ie, “optical biopsy”), if realized, would represent an important advance in endourology. While bladder biopsy only obtains a static, histologic snapshot of small pieces of tissue, fibered confocal

microscopy offers a dynamic survey of selected areas of the urothelium.

While promising, our pilot *ex vivo* study has several limitations, particularly the small sample size and the nonquantitative, descriptive nature of our image interpretation. Our preliminary experience with fibered confocal microscopy precludes us from making conclusive statements regarding the observed confocal images beyond empiric pattern recognition.

These observations argue that contrast agents with molecular specificity would be detectable using this microscope, and such agents may have a significant impact on detection and diagnosis. Such studies have been reported using peptide probes in the colon.<sup>35</sup> In addition, the recently devascularized bladder specimens, resulting in varying degrees of cell death, may explain the inconsistent staining of fluorescein.

Comparison with standard histopathologic analysis is limited because the fibered confocal microscope produces images of horizontal planes, parallel to the surface of the bladder, while vertical sections are displayed in routine histopathologic analysis.

Based on our *ex vivo* experience, we are initiating an *in vivo* study to assess bladder tumors using fibered confocal microscopy with fluorescein as a contrast agent. By imaging selected normal or abnormal-appearing areas of the bladder before surgical biopsy, this technology may improve the rate of positive findings in “random” bladder biopsy and reduce unnecessary biopsies.

At this time, the Cellvizio probe fits within the working channel of a standard resectoscope. Further advances in miniaturization, however, may enable future compatibility with flexible cystoscopes for office use.

## Conclusion

While the depth of penetration of fibered confocal microscopy will likely remain insufficient to provide information about muscle invasiveness, and the requirement for direct tissue contact with the probe makes imaging of the entire urothelium impractical, we anticipate that confocal endomicroscopy may complement other novel imaging modalities to improve conventional white-light cystoscopy, and that additional advances in the technology will increase its utility.

## Disclosure Statement

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#### Abbreviations Used

CIS = carcinoma *in situ*  
 HAL = hexyl aminolevulinate  
 H&E = hematoxylin and eosin  
 OCT = optical coherence tomography  
 TCC = transitional-cell carcinoma



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