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Emerging Endoscopic Imaging Technologies for Bladder Cancer Detection

Aristeo Lopez^a and Joseph C. Liao^{a,b,*}

^aDepartment of Urology, Stanford University School of Medicine, Stanford, CA 94305-5118

^bVeterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304

Abstract

Modern urologic endoscopy is the result of continuous innovations since early 19th century. White light cystoscopy is the primary strategy for identification, resection, and local staging of bladder cancer. While highly effective, white light cystoscopy has several well-recognized shortcomings. Recent advances in optical imaging technologies and device miniaturization hold the potential to improve bladder cancer diagnosis and resection. Photodynamic diagnosis and narrow band imaging are the first to enter the clinical arena. Confocal laser endomicroscopy, optical coherence tomography, Raman spectroscopy, UV autofluorescence, and others have shown promising clinical and pre-clinical feasibility. We review their mechanisms of action, highlight their respective advantages, and propose future directions.

Keywords

optical imaging; fluorescence imaging; in vivo microscopy; multimodal imaging; cystoscopy; bladder cancer

Introduction

Landmark innovations over the last two centuries have shaped modern urologic endoscopy and particularly endoscopic management of bladder tumors.[1•] Bozzini first conceptualized endoscopy with his *lichleiter* ('light conductor') to illuminate various accessible body cavities including the bladder.[2] Subsequent innovations include the first endoscopic surgery (i.e. extraction of a urethral papilloma) by Desormeaux in 1853, invention of the cystoscope by Nitze in 1894, and the development and refinement of the resectoscope by Stern and McCarthy in the early 1930's.[3] The introduction of flexible fiberoptic

Corresponding author: Joseph C. Liao, MD, 300 Pasteur Dr., Room S-387, Stanford, CA 94305-5118, jliao@stanford.edu, Phone: 650-858-3916, Fax: 650-849-0319.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

Dr. Aristeo Lopez declares no potential conflicts of interest relevant to this article.

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cystoscopes in the 1980's and more recently the digital videoscopes further decreased the morbidity associated with diagnostic cystoscopy.

Today, white light cystoscopy (WLC) is the cornerstone for office-based hematuria work-up, bladder cancer detection and surveillance, as well as detection of numerous other benign lower urinary tract disorders. Transurethral resection (TUR) under WLC is the standard for excisional biopsy and local staging of bladder cancer. While widely used, WLC has several well-recognized shortcomings. First, nonpapillary bladder cancer such as carcinoma *in situ* (CIS) may be difficult to visualize or differentiate from inflammation.[4] Second, smaller or satellite tumors may be missed. Third, bladder cancer may be incompletely resected therefore understaged.[5] Lastly, cancer grading and staging requires pathological analysis, which has a significant time delay. Suboptimal endoscopic management of early stage bladder cancer increases the risk of cancer persistence, recurrence, and progression.[6]

To address the shortcomings of WLC, new optical imaging technologies to improve detection and characterization of suspected bladder tumors are being developed. Table 1 summarizes the characteristics and properties of the technologies reviewed. PDD, NBI, CLE and OCT have been recently reviewed elsewhere [7] and their recent progress highlighted here. Several promising new technologies at the initial clinical feasibility (e.g. Raman spectroscopy, UV autofluorescence) or pre-clinical stage (e.g. scanning fiber endoscopy) will be introduced. Taken together, these technologies offer an exciting glimpse into future possibilities to improve optical diagnosis and endoscopic management of bladder cancer.

Photodynamic diagnosis (PDD)

Of the new imaging technologies, PDD has the most extensive clinical track record.[8] PDD is based on selective accumulation of heme-precursors, 5-aminolevulinic acid (5-ALA) or its ester derivative hexaminolevulinate (HAL), by cancer cells when introduced intravesically. Under blue fluorescence, bladder cancer appears red in contrast to the surrounding benign urothelium (Figure 1A).[9] PDD requires a specialized rigid cystoscope, light source and camera head, which can be used in conjunction with different size sheaths. Multi-institutional randomized clinical studies have demonstrated that PDD improves detection of both papillary tumors and CIS compared to white light [10, 11] and patients treated with PDD-guided transurethral resection of bladder tumor (TURBT) had longer recurrence-free intervals.[11, 12] Limitations of PDD include false positive fluorescence [10] from inflammatory lesions or previous biopsy sites. The contrast agent, HAL, is currently approved for single use and not for patients who received intravesical immunotherapy or chemotherapy within 90 days. More studies are needed to better define the optimal indications for PDD use and strategies to improve PDD's diagnostic specificity.

Narrow Band Imaging (NBI)

Similar to PDD, NBI is an endoscope-based imaging modality that the user can switch dynamically between NBI and white light during cystoscopy. NBI improves visualization of bladder neoplasia through enhanced visualization of mucosal and submucosal vasculature without the need for exogenous contrast agent. White light is filtered into blue (415 nm) and green (540 nm), which are wavelengths that hemoglobin absorbs (Figure 1B). NBI is

approved for clinical use, either in an integrated videocystoscope or through a camera head that can be attached to standard white light cystoscopes. Both flat and papillary tumors are more easily visualized with NBI compared to WLC due to the increased and aberrant vascularity associated with bladder cancer. Single center studies have found a detection rate of 94.7% vs. 79.2% for WLC.[13] Studies have shown the feasibility of NBI-assisted TUR[14] with decreased 1 year recurrence in comparison to standard TUR.[15] The results from an ongoing multi-center international study comparing NBI- and WLC-assisted TUR are currently pending.[16]

Confocal Laser Endomicroscopy (CLE)

Based on the well-established principle of fluorescence confocal microscopy, CLE is an optical biopsy technology that enables *in vivo* high resolution, subsurface imaging.[17–19] CLE is currently approved for gastrointestinal and pulmonary endoscopic applications and has been under investigational use in the urinary tract since 2009. Fluorescein, an FDA-approved drug with an established clinical safety profile, is required as the exogenous contrast agent.[20] Reusable miniaturized imaging probes ranging from 0.85-mm to 2.6-mm diameter are compatible with working channels of standard cystoscopes.[21] Real time microscopy of normal urothelium, inflammation, CIS, low-grade and high-grade urothelial carcinoma have been demonstrated with images comparable to conventional histopathology (Figure 1C).[18•]. A recent study demonstrated moderate interobserver agreement in image interpretation between novice and experienced CLE urologists with respect to cancer diagnosis.[19] Multi-center studies examining the CLE diagnostic accuracy for real time cancer diagnosis and grading remained to be completed. An advantage of CLE is that it may be coupled with fluorescently labeled peptides or antibodies against cancer-specific surface antigens, as it has been demonstrated in the gastrointestinal tract.[22•, 23] Similar endoscopic molecular imaging strategies may be deployed in the bladder to improve cancer diagnosis with molecular specificity.

Optical coherence tomography (OCT)

OCT is another optical biopsy technology that enables high-resolution, subsurface tissue interrogation. OCT is analogous to ultrasound in that cross-sectional images are generated (Figure 1D) using near-infrared light instead of sound waves as the source. Image resolutions of 10–20 μm and depths of penetration of 2 mm can be achieved, thus enabling the possibility of real-time cancer diagnosis and staging (i.e. differentiation between non-muscle and muscle invasive cancer).[24–26] Similar to CLE, there is a learning curve associated with image interpretation, which is based on loss of delineation between tissue layers (e.g. lamina propria). Other recent studies have reported an automated image processing algorithm to detect bladder cancer from OCT images [27] and application in the upper tract.[28]

Multimodal imaging

Given the different imaging characteristics, a combination of macroscopic (e.g. PDD and NBI) and microscopic (e.g. CLE and OCT) imaging modalities used in concert may improve diagnostic accuracy.[7] For example, PDD and NBI could be used to identify suspicious

lesions while CLE and OCT provide high-resolution characterization to provide grading or staging information. Feasibility of combining endoscope-based PDD and NBI with probe-based CLE has been reported (Figure 2).[29, 30] A recent study showed that PDD combined with OCT had increased the specificity compared to PDD alone.[31] In another study, PDD in combination with standard OCT did not improve the diagnostic accuracy significantly in detecting non-invasive bladder cancer, but the use of cross-polarization OCT and PDD decreased overall false positive and negative cases.[32]

Raman spectroscopy (RS)

RS is based on the principle of inelastic scattering of photons following interaction with intramolecular bonds. Near infrared light (785–845 nm) is used to illuminate biological tissues and alter the vibrational state of molecules. The photons donate energy to molecular bonds and as a result exit the tissue at a different wavelength from the incident light. The change in energy level of the photons is known as a Raman shift.[33] Detection of multiple Raman peaks from the target tissue is plotted to create a spectrum of peaks, achieving a molecular “fingerprint” of the tissue examined without the need for exogenous contrast agent.[34] In *ex vivo* settings, RS has been shown to differentiate the normal bladder wall layers, identify low- and high-grade bladder cancer, and assess invasiveness.[35, 36] To evaluate the feasibility of *in vivo* bladder cancer diagnosis, a prototype fiberoptic Raman probe compatible with the working channels of endoscopes has been developed.[34•, 37, 38] In 38 patients suspected of bladder cancer, differentiation of cancer from normal urothelium was feasible in near real-time during cystoscopy.[34•] Recent research led to development of a non-contact Raman probe that detects signals even with imperfect centering of the probe and nonuniform topology of human tissue surface.[39] Recognized limitations of RS include time to obtain a spectrum (1–5 seconds), weak signals and limited field of view. Surface-enhanced Raman scattering (SERS) nanoparticles have been demonstrated to augment the relatively weak signals from Raman scattering. SERS substrates can be conjugated to cancer-specific antibodies to enable multiplexed targeted imaging during endoscopy.[40]

Ultraviolet (UV) autofluorescence

The basis of UV-induced autofluorescence is to differentiate normal, inflammatory and cancerous urothelium by differences in their intrinsic molecular contents. UV light is used to excite endogenous fluorophores (e.g. NAD and tryptophan) present in the tissue of interest. A pilot clinical study composed of 14 patients was recently published.[41•] A UV light-emitting probe (360 and 450 nm) was inserted via the working channel of a standard cystoscope and advanced in close proximity to the region of interest for tissue interrogation. For image processing, the signals were converted into an intensity ratio between the two wavelengths and color coded to facilitate real time interpretation.[41•] In the pilot study, real time differentiation of bladder cancer from benign/normal urothelium based on autofluorescence spectrum was demonstrated (Figure 3). Further studies are needed to investigate signal intensity across different tumor types (including flat lesions), reproducibility, and potential for UV-induced toxicity.

Multiphoton microscopy (MPM)

In MPM, fluorescence is achieved by nonlinear excitation of molecules after the simultaneous absorption of two or more photons of lesser energy.[42] MPM takes advantages of intrinsic tissue fluorophores (i.e. NADH, FAD and collagen) instead of exogenous contrast agents. In addition to the autofluorescence signals, MPM is also able to visualize non-centrosymmetric structures (e.g. collagen) via second harmonic generation (SHG). The exciting light is produced from a femtosecond pulsed Ti-Sapphire laser tuned to 780 nm while the detected autofluorescence and SHG signals are in the range of 420–530 nm and 355–420 nm respectively.[43] Interpretation of acquired images is simplified by color-coding the detected SHG and autofluorescence signals. Recent *in vitro* studies using MPM demonstrated high-resolution images from fresh TUR bladder tissues comparable to histopathology and differentiation of malignant and benign features. Similar to CLE, limitations of MPM include limited depth of penetration not suitable for cancer staging and lack of nuclear morphology on the images. Current research is focused on miniaturizing the MPM system into a compact probe suitable for *in vivo* use.[44–46]

Scanning Fiber Endoscopy (SFE)

SFE uses an ultrathin (1.2 mm tip) flexible endoscope that provides wide angle, full color, high-resolution images.[47] The tip contains a single-mode optical fiber that spirally scans red, green and blue laser light that is focused onto tissue. The backscattered light is collected by multiple optical fibers and an image is generated pixel by pixel.[48] The small diameter decreases the invasiveness and expands the versatility of how SFE may be deployed either as a standalone miniaturized endoscope or as a probe in conjunction with other imaging modalities.[49] Currently, SFE has been demonstrated in the gastrointestinal tract and in *ex vivo* bladder models.[50, 51•] A provocative application of SFE involves integrating an automated bladder-scanning device and an ‘image-stitching’ algorithm to generate a 2-D panoramic view of the mucosa. Figure 4 shows examples of such image *mosaicing* in a phantom model and *ex vivo* pig bladder.[50, 51•] With integration of automated scanning, SFE cystoscopy may be performed in the future by skilled technologists with the urologist reviewing the images in real time or offline. The panoramic view may also provide a systematic way for tumor mapping and survey the bladder longitudinally.

Telerobotic cystoscopy—cystoscope of the future?

As new imaging technologies enter into clinical use, new methods to integrate different imaging modalities with endoscopic surgical interventions may be needed. A telerobotic cystoscopy system has recently been developed and tested in *ex vivo* animal models (Figure 5).[52•] This system contains multiple instrument channels and offers the potential to integrate optical imaging, catheter-based ablative technologies, and new generation of micro-surgical tools with additional degrees of freedom. Such ‘smart’ endoscopic instruments may expand the spectrum of multi-modal endoscopic interventions that can be performed in the urinary tract.

Conclusions

Modern endoscopy and endoscopic surgery of the urinary tract is the culmination of ingenuity and persistence by many innovators in urology, surgery, and engineering over the last two centuries. These innovations introduced the concept of minimally invasive surgery and revolutionized the management of bladder cancer. Recent advances in optical imaging and instrument miniaturization are poised to usher in an era of image-guided surgery, that holds the cancer recurrence and progression, and cost effective utilization of available health care resources.[53]

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Figure 1. New bladder imaging technologies with corresponding white light cystoscopy (WLC) (A) Bladder tumors appeared pink under blue fluorescence of photodynamic diagnosis (PDD); (B) Narrow band imaging (NBI) enhances visualization of aberrant tumor vasculature; (C) Confocal laser endomicroscopy (CLE) enables in vivo microscopy of a papillary urothelial carcinoma; (D) Subsurface imaging of a papillary urothelial cancer using optical coherence tomography (OCT) showing loss of normal urothelial layers. Image reproduced with permission from Elsevier.

Figure 2. Multimodal imaging using narrow band imaging and confocal laser endomicroscopy (A) Endoscopic view of the confocal imaging probe adjacent to a papillary tumor at the bladder dome. Yellow tinge corresponds with intravesical fluorescein as contrast agent for CLE. (B) Narrow band imaging highlights tissue and tumor vasculature. (C) Concomitant endomicroscopy image shows well-organized papillary border and monomorphic urothelial cells consistent with a low grade papillary urothelial carcinoma.

Figure 3. Tumor autofluorescence with an ultraviolet (UV) imaging probe

(A) Cystoscopic view of the imaging probe adjacent to normal urothelium and tumor; (B) Color-coding is integrated in real-time and facilitates interpretation of autofluorescence measurements and differentiation of normal (green) and malignant (red) tissue; (C) Histogram of the autofluorescent measurements showing the calculated mean diagnostic ratios of healthy tissue (0.91) and the papillary tumor (0.26). Image reproduced with permission from Elsevier.

Figure 4. Scanning fiber endoscopy (SFE) of a bladder phantom and excised pig bladder to generate surface mosaics of the urothelium

(A) A bladder phantom with painted on vessels was imaged using a conventional endoscope from which a (B) 3D mosaic was constructed. (C) A multi-modal mosaic was generated by SFE imaging of the same phantom with green fluorescent microspheres to mimic hotspots (arrows). Using the SFE, a mosaic (D/E) was constructed from roughly a thousand frames taken within an excised pig bladder. Image courtesy of Eric Seibel, University of Washington.

Figure 5. A prototype telerobotic system for the bladder

(A) Schematic of the dexterous bladder telerobotic system. The central rigid stem contains an optical scope and irrigation apparatus. (B) The dexterous end effector has multiple channels including its own optics and channels for laser and other tools such as tissue optical interrogation catheters. (C) The dexterous instrumentation manipulated to various areas of an *ex vivo* bovine bladder. Courtesy of Nabil Simaan and Duke Herrell, Vanderbilt University.

Table 1

Characteristics and Properties of Emerging Imaging Modalities

Name	Mechanism	Contrast Agent	Resolution	Depth	Status	Scope or Probe (diameter)	Image
PDD	Fluorescence	HAL	mm-cm	Surface	Clinical	Rigid scope within standard sheath
NBI	Absorption	None	mm-cm	Surface	Clinical	Flexible video- cystoscope (5.5 mm) or standard rigid cystoscope
CLE	Fluorescence	Fluorescein	1–3.5 μm	120 μm	Investigational (<i>in vivo</i>)	Probe (0.85–2.6 mm)
OCT	Scattering	None	10–20 μm	~2.5 mm	Clinical/ Investigational (<i>in vivo</i>)	Probe (2.7 mm)
Raman	Scattering	Optional (SERS)	-	2 mm	Investigational (<i>in vivo</i>)	Probe (2.1 mm)

Name	Mechanism	Contrast Agent	Resolution	Depth	Status	Scope or Probe (diameter)	Image
UV	Fluorescence	None	mm-cm	Surface	Investigational (<i>in vivo</i>)	Probe (3 mm)
SFE	Reflectance + Fluorescence	None	mm-cm	Surface	Investigational (<i>ex vivo</i>)	Scope/Probe (1.2 mm)
MPM	Fluorescence	None	*1–6 μm	*0.25–0.5 mm	Investigational (<i>ex vivo</i>)	Probe (3 mm)

* Spatial resolution and imaging depth were calculated from a custom built upright multiphoton microscope.

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