

NIH Public Access

Author Manuscript

Curr Opin Urol. Author manuscript; available in PMC 2015 January 01.

Published in final edited form as:

Curr Opin Urol. 2014 January ; 24(1): 66–74. doi:10.1097/MOU.000000000000010.

Intraoperative Optical Imaging and Tissue Interrogation During Urologic Surgery

Mark Hsu^a, Mohit Gupta^b, Li-Ming Su^b, and Joseph C. Liao^{a,c,*}

^aDepartment of Urology, Stanford University School of Medicine, Stanford, CA 94305-5118 ^bDepartment of Urology, University of Florida College of Medicine, Gainsville, FL 32610-0247 ^cVeterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304

Abstract

Purpose of review—To review optical imaging technologies in urologic surgery aimed to facilitate intraoperative imaging and tissue interrogation.

Recent findings—Emerging new optical imaging technologies can be integrated in the operating room environment during minimally invasive and open surgery. These technologies include macroscopic fluorescence imaging that provides contrast enhancement between normal and diseased tissue and microscopic imaging that provides tissue characterization.

Summary—Optical imaging technologies that have reached the clinical arena in urologic surgery are reviewed, including photodynamic diagnosis, near infrared fluorescence imaging, optical coherence tomography, and confocal laser endomicroscopy.

Keywords

image-guided surgery; optical imaging; fluorescence imaging; in vivo microscopy; minimally invasive surgery

Introduction

Advances in modern imaging technologies have had significant impact on improving clinical diagnosis and surgical planning. Computed tomography (CT) and magnetic resonance imaging (MRI) are widely used to define the extent of the pathology and devise optimal surgical approaches. Given the static nature of the images and challenges of operating room integration, however, cross-sectional imaging modalities are largely utilized in preoperative settings. Intraoperatively, key factors that contribute towards successful surgical outcomes include dynamic recognition of anatomic landmarks (which are frequently distorted in diseased states), precise dissection of tissue planes, and avoidance of collateral damage to minimize surgical morbidities. While the surgeon's clinical acumen and

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.

Hsu et al.

technical ability remain paramount, there is significant interest to develop new imaging technologies that can augment the surgeon's ability to 'see' and improve surgical techniques and outcomes.

Optical imaging utilizes visible, ultraviolet, and infrared light to interrogate tissue of interest. Advances in device miniaturization and packaging in recent years have enabled selected imaging platforms to transition from research to clinical arenas. Advantages of optical imaging include superior spatial and temporal resolutions, as well as the relative ease of integration into the operating room environment and instrumentations of minimally invasive surgery (MIS), which includes endoscopic, laparoscopic, and robotic-assisted surgery. In many surgical disciplines including urologic surgery, MIS has increasingly replaced traditional open surgery as a standard approach even for complex cancer and reconstructive surgery. In contrast to traditional open surgery, MIS has decreased (or no) tactile feedback and relies on white light laparoendoscopes to illuminate the smaller operative fields. By integrating features such as fluorescence imaging to standard white light, physiologic differences between diseased and healthy tissues (i.e. vascular perfusion, neoplastic transformation) can be differentiated to facilitate intraoperative guidance. In addition, emerging 'optical biopsy' techniques can provide subsurface, micron-scale interrogation of the tissue microarchitecture with resolution down to the cellular level.

In this review, several promising optical imaging technologies that have entered the clinical arena will be highlighted, with spatial resolutions ranging from macroscopic (i.e. photodynamic diagnosis, near-infrared fluorescence) to microscopic (i.e. optical coherence tomography, confocal laser endomicroscopy) scale. Salient features including imaging principles, image acquisition techniques, contrast agents [1*], clinical applications, and integration into MIS and open surgery will be discussed. While urological cancer applications are the focus, these imaging technologies have been demonstrated in other surgical disciplines as well [2-6]. Finally, promising technologies in pre-clinical settings as well as future outlooks will be discussed.

Photodynamic Diagnosis

Photodynamic diagnosis (PDD) is a fluorescence-based optical imaging technology that uses photosensitive protoporphyrin analogues as contrast agents, a blue light source (375-440 nm) to induce fluorescence, and a fluorescent camera. Selective accumulation of the photosensitizer 5-aminolevulinic acid (5-ALA) or its more strongly fluorescent ester analogue, hexaminolevulinate (HAL), by cancer cells causes them to appear pink under blue light [7,8]. PDD has been applied principally in bladder cancer through intravesical contrast administration with integrated blue fluorescence cystoscope, with more limited experience in penile, prostate, and kidney cancer.

Bladder cancer

For endoscopic management of bladder cancer, PDD is approved as an adjunct to standard white light cystoscopy (WLC), which has several well known shortcomings including difficulty in visualizing some tumors, particularly flat lesions such as carcinoma in situ (CIS) (Figure 1), leading to incomplete resection and increased risk for tumor recurrence

and possible progression [9*]. Several prospective studies have demonstrated that PDD improved initial detection of papillary and CIS lesions compared to WLC [10-12], and a meta-analysis of three phase III studies showed that HAL had a higher CIS detection rate than WLC [13]. Impact of fluorescence-guided transurethral resection (TUR) on the recurrence rate of non muscle-invasive bladder cancer remains undetermined. One study showed a 10% benefit in 3-year recurrence risk for fluorescence TUR with patients randomized to white-light TUR, fluorescence TUR with 5-ALA, and fluorescence TUR with HAL [14], and others showed that fluorescence TUR improved recurrence-free risk at 1 year [15,16]. However, two studies found no significant difference in decreasing recurrence risk with fluorescence cystoscopy [10,12]. The cost-effectiveness of PDD in bladder cancer has been debated in recent literature [17,18]. Accounting for the cost of capital equipment and reagents versus the cost of a TURBT, it is estimated that the cost ratio is about 5:1, with 1 TURBT being avoided for every 5 fluorescent cystoscopy procedures to achieve cost equivalence [19].

Penile, kidney, and prostate cancer

Fluorescence imaging based on PDD has also been explored in other areas of urologic oncology. In a study of patients with penile carcinoma and premalignant lesions, 5-ALA was topically applied over the glans penis and shaft preoperatively, with PDD employed intraoperatively to guide Nd:YAG laser coagulation, with the potential of decreasing recurrence rate and optimizing organ preservation in penile surgery [20]. In kidney cancer, PDD with oral 5-ALA has been investigated a way to assess the margin status in the laparoscopic partial nephrectomy [21]. In prostate cancer, two small multi-institutional phase II nonrandomized studies showing that oral ALA administration preoperatively can be useful to detect positive surgical margins during prostatectomy [22,23]. Fukuhara, et al. showed that PDD of positive surgical margins could be detected using fluorescence laparoscopy during open retropubic prostatectomy, though this was a preliminary study where there was a high false-positive rate for hyperplasia and inflammation [24]. More studies will be needed to move these promising applications beyond early phase investigations.

Near-Infrared Fluorescence Image-Guided Surgery

The use of near infrared fluorescence (NIRF) image-guided surgery has been established in the fields of ophthalmology, cardiology and breast cancer surgery. Indocyanine green (ICG) is the most commonly used contrast agent for intraoperative imaging that absorbs and emits light in the near infrared portion of the electromagnetic spectrum (about 800 to 2500 nm). When injected intravenously, ICG binds to albumin and lipoproteins and remains predominantly intravascular until excreted by the hepatobiliary system. ICG has also been used to highlight sentinel lymph nodes when injected subcutaneously into the breast [25]. Recent studies have investigated the use of intraoperative NIRF imaging to enhance intraoperative decision making as applied to prostatectomy and partial nephrectomy. This has been in the realm of evaluating vasculature, tumor margin identification and lymph node dissemination of cancer. NIRF imaging systems have been developed that can be used in conjunction with open, laparoscopic, and robotic-assisted surgery.

Kidney cancer

ICG has been used during robotic-assisted partial nephrectomy to facilitate identification of hilar vessels, particularly tumor-specific vessels for selective arterial clamping (Figure 2). This may be particularly helpful in more challenging hilar-based tumors where tumor-specific vessels are more likely to be identified as compared to cortical tumors located in the periphery [26*,27]. Once identified, these tumor-specific vessels can be preferentially and selectively clamped resulting in only regional ischemia around the tumor in lieu of clamping the main renal artery with resultant global kidney ischemia. As a result this may translate into minimizing ischemia related renal injury and optimizing renal preservation of the affected renal unit.

To enhance identification of tumor margins during partial nephrectomy, Tobis and colleagues demonstrated use of intravenous ICG with simultaneous NIRF imaging at the time of partial nephrectomy using a NIRF system designed for open surgery [26*]. The renal tumors (both benign and malignant) were not only well demarcated by ICG but surprisingly hypofluorescent as compared to surrounding normal parenchyma. This differential uptake of ICG has been attributed to the presence of the membrane protein bitranslocase in normal renal proximal and distal tubule cells, but is lacking in renal cortical tumors [28]. The degree of hypofluorescence however could not be reliably used to differentiate malignant versus benign tumors according to Manny et al., who found in 100 consecutive patients undergoing robotic partial nephrectomy with NIRF guidance a positive predictive value of 87%, negative predictive value of 53%, sensitivity of 84% and specificity of 57% [27]. Others have suggested that non-standardized dosing of ICG may explain the relatively poor differential ICG fluorescence between tumor and normal tissue [29]. These studies were performed using the FireFly (Novadaq Technologies Inc., Bonita Springs, FL) NIRF camera system integrated with the daVinci Si robot (Intuitive Surgical, Sunnyvale, CA). Although the use of intraoperative fluorescence during partial nephrectomy appears appealing, at this time the true benefit remains unclear as pertains to tumor identification and reducing positive margins especially in experienced hands where positive surgical margin rates are 1-2%. This must be weighed against the additional \$100,000 cost of the NIRF integrated robotic camera system and \$100 per vial cost of ICG.

Prostate cancer: lymph node metastasis

The identification of regional lymph nodes suspicious for cancer metastasis is relevant to many urologic cancers including prostate, bladder, penile and testis and may affect not only diagnostic and staging accuracy but also in some cases provide therapeutic benefit. In a pilot study, van der Poel and colleagues injected a self-assembled multimodal radioactive nanoparticle (i.e. 99mTc-NanoColloid) and ICG into the peripheral zone of the prostates of 11 men undergoing planned robotic prostatectomy for prostate cancer [30]. Following injection, single-photon emission computed tomography/computed tomography (SPECT/CT) imaging of the pelvic nodes was performed to highlight the putative sentinel lymph nodes draining the prostate in the preoperative setting. These same lymph node regions were then confirmed intraoperatively by both laparoscopic NIRF and gamma probe assessment. While background radioactive signal from the injected prostate gland posed a problem for acoustic gamma probe identification of sentinel nodes, the use of fluorescence

co-labeling assisted in identification of these nodes and was more precise. However, fluorescence imaging of relevant nodes was limited by overlying fat and blood preventing real-time detection of 15% of sentinel lymph nodes. Overall, 5 of 27 (18.5%) removed sentinel lymph nodes were detected outside of the standard extended lymphadenectomy template and would otherwise have been missed. In 36% of study patients, out of boundary nodes were found of which two contained nodal metastasis.

Low-dose methylene-blue, which is excreted in the urinary tract, has been applied as an alternative NIR contrast agent for ureteral visualization during complex open pelvic surgery to facilitate ureteral identification and avoid iatrogenic injury [31]. Future development may enable application during laparoscopic surgery.

Optical Coherence Tomography

Optical Coherence Tomography (OCT) is an example of emerging optical biopsy techniques that provides high-resolution, real-time, cross-sectional imaging of tissues (Figure 3). Analogous to B mode ultrasound, OCT relies on information gathered by reflected energy. In comparison, however, OCT utilizes near-infrared light rather than acoustical waves and, unlike ultrasound, does not require direct contact with tissue or a transducing medium. Backscattered light is combined with a reference signal to produce a high-resolution, two-dimensional map of tissue microstructure [34]. OCT thus provides images with a depth of penetration of 2 mm and spatial resolutions of $1-15 \mu m$, over one order of magnitude higher than conventional ultrasound [35,36]. OCT imaging in the genitourinary system was first demonstrated in 1997, and since then, multiple studies have emerged examining its use as a tool to guide clinical decisions. Early *ex-vivo* studies noted significant levels of correlation between OCT images and histologic architecture of urologic tissues sampled.

Bladder cancer

An integrated OCT platform (Niris, Imalux Corp.) has been developed with the imaging probe that fits in standard working channels of cystoscopes. The superficial imaging characteristics of OCT make it well suited for assessment of bladder cancer: OCT imaging can differentiate the multiple layers of the bladder urothelium, underlying lamina, and muscularis propria. Thus, OCT can be used alongside conventional cystoscopy to characterize regions of the bladder suspicious for CIS, and to differentiate between noninvasive (Ta), superficial (T1), and invasive (T2 or greater) disease [13,35,37]. Sensitivities and specificities of cancer diagnosis have been reported between 83%-100% and 72% to 89%, respectively [38,39]. The ability to image and detect muscle invasion at the time of biopsy or excision theoretically would be useful in identifying tumors in which resection including muscularis propria would be critical. Limitations, however, despite promising initial reports and high sensitivities for detection of muscle-invasive malignant lesions, include a 2mm depth of invasion limit. Thus, OCT may not always provide sufficient imaging to judge the invasion depth of a tumor. In addition, false positives from conditions such as radiation, inflammation and other benign conditions remain a problem.

Kidney cancer

OCT has also been investigated for renal imaging in evaluating kidney morphology and renal masses incidentally discovered by CT or MRI. Preliminary studies have begun to investigate OCT's ability to distinguish benign from malignant renal tumors and appear promising. Barwari *et al.* concluded that *ex-vivo* OCT attenuation coefficients were different between normal renal parenchyma and RCC tissue: RCC tissue showed a significant higher attenuation coefficient than normal parenchyma [40]. If these results are confirmed *in-vivo*, OCT may be applied to evaluate surgical margins after partial nephrectomy or combined with biopsy needles to provide a functional optical biopsy where cross-sectional images can be correlated with histopathology [37]. OCT has also been evaluated in transplant surgery to image donor kidney structures and to evaluate organ viability following physiologic insults and acute kidney injury [41]. Limitations in these studies, however, include small sample sizes, study designs, and the need for numerous images to evaluate the relatively large surface area of a kidney.

Prostate cancer

OCT also has demonstrated potential for prostate cancer in detecting extraprostatic invasion and in identifying the neurovascular bundle (NVB) during nerve-sparing radical prostatectomy. Surgically-treated patients with focal extracapsular extension, seminal vesicle invasion, and positive pelvic lymph nodes have been noted to have decreased tenyear prostate-specific antigen (PSA) failure-free survival in comparison to patients with T2 disease [42]. Despite advances in diagnostic imaging, however, it is still not possible to visualize the extent of microscopic disease reliably before definitive local therapy. OCT has a demonstrated significant ability for evaluation of surgical margins and extra-capsular extension: Dangle et al. correlated OCT images of post-prostatectomy ex-vivo specimens to detect positive margins and found a sensitivity, specificity, and negative predictive value of 70%, 84%, and 96% respectively [43]. Multiple studies have also shown significant correlation with OCT findings in comparison to histology in differentiating cavernous nerves from underlying prostate glandular architecture in a small animal model [36,33,32]. In addition, Aron et al. utilized in-vivo OCT imaging to provide real-time identification of the NVB during nerve-sparing radical prostatectomy[34]. However, the limitations of OCTs field of view make intraoperative mapping of the NVB for nerve sparing radical prostatectomy challenging. As most OCT studies have been done ex vivo, larger clinical in vivo trials are required to determine its ability to guide clinical decisions during urologic surgery and the potential benefits.

Confocal Laser Endomicroscopy

Confocal laser endomicroscopy (CLE) enables real time *in vivo* microscopy with the highest spatial resolutions $(1-5 \ \mu\text{m})$ among the optical imaging technologies available clinically. Based on the well-established principles of confocal microscopy, optical sectioning is achieved using a 488 nm laser as the light source and fluorescein, a FDA-approved drug, as the contrast agent. Tissue microarchitecture and cellular features can be resolved with images reminiscent of standard histopathology (Figure 4). The available clinical system (Cellvizio, Mauna Kea Technologies) utilizes miniaturized fiberoptic imaging probes

Hsu et al.

ranging from 0.85 mm to 2.6 mm diameter that be passed through working channels of standard endoscopes. The images are acquired as video sequences at 12 frames/sec, thus enabling dynamic imaging of physiologic parameters such as vascular flow.

In vivo applications of CLE in urological surgery has been reported in the lower urinary tract and bladder cancer in particular (Figure 4). Sonn et al conducted the initial *in vivo* pilot study in 27 patients undergoing cystoscopy and transurethral resection of bladder tumor. The feasibility of differentiating between normal and benign mucosa with cancer (low and high grade) with regard to cellular organization and vascularization was demonstrated [44]. In addition, protocols for both intravesical and intravenous administration of fluorescein were established [45]. To facilitate development of diagnostic criteria for CLE diagnosis of bladder cancer, an imaging atlas of the *in vivo* diagnostic features for normal, benign, and cancer has been established [46] comprised of both microarchitectural (flat vs. papillary, tissue organization, and vascularity) and cellular features (morphology, cohesiveness, and borders) [47]. Most recently, an interobserver agreement study of CLE was conducted among novice and experienced CLE users. CLE was found to be a highly adoptable technology for cancer diagnosis in novice CLE observers with moderate interobserver agreement, while the experienced CLE observers attained substantial levels of agreement for cancer diagnosis that was higher than white light cystoscopy along [47].

Using bladder cancer as a model with CLE, efforts are now being channeled toward defining diagnostic criteria for pathology in areas such as upper-tract urothelial carcinoma and prostate cancer (Liao et al, in preparation).

Molecular imaging

Molecular imaging represents a potentially exciting future arena in conjugating cancerspecific contrast agents to fluorophores to improve specificity of disease detection. The aforementioned optical imaging technologies can be coupled with molecular imaging agents for intraoperative tissue interrogation with molecular specificity [48]. Previous work in colon demonstrated that CLE may be coupled with molecular contrast agents such as fluorescently labeled peptides for endoscopic imaging of disease processes with molecular specificity [49]. Similarly, molecular imaging agents targeting high-grade bladder cancer targets may be used in conjunction with PDD or CLE to improve cystoscopic visualization and differentiation of malignant versus inflammatory/benign lesions.

Recently, Tsien and colleagues (2010) designed a unique nanoparticle vehicle termed activated cell penetrating peptides (ACPPs), that when attached to either a fluorescence or imaging (i.e. gadolinium) payload, can deliver the payload specifically to tumors [50]. This technology takes advantage of the fact that most tumors contain matrix metalloproteases (MMPs), which cleave specific surface bound proteins on ACPPs that allow for cell penetration and preferential uptake into tumor cells as compared to normal cells that do not contain MMPs. This work has significant implications in terms of delivery of targeted therapies to cancer sites as well as localization and imaging of cancer within the body. Their group has achieved high specificity in fluorescence labeling of implanted tumors in small animal models with improved resection and tumor-free survival as compared to animals

with tumors resected by traditional bright-field illumination. In addition, this same group has identified a NP41 peptide strongly associated with peripheral nerves, that when fluorescently labeled and injected systemically into mice provided 10-fold contrast between nerve and background tissues under fluorescence microscopy [51]. Taken together, tumor-and nerve-specific imaging has significant implications with regard to many urologic procedures such as nerve-sparing radical prostatectomy and cystectomy as well as retroperitoneal lymph node dissection for testis cancer.

Conclusion

New techniques in optical imaging and intraoperative tissue imaging in urologic surgery have emerged in recent years, and hold promise to revolutionize our clinical paradigm in guiding surgical resection, in particular with the advent of minimally invasive surgery. The implementation of innovative imaging modalities can provide the urologic surgeons with intraoperative capability to visualize diseased and healthy tissues with cellular and molecular specificity. The objectives of incorporating these technologies in the clinical setting will be dependent upon whether they are shown to improve patient outcome, reduce morbidity, are safe, and cost-effective. Prospective, studies will be needed to further define and validate the role of intraoperative imaging technology and its cost-effectiveness in the clinical setting.

Acknowledgments

The authors thank Kathleen E. Mach for critical review of the manuscript. M.S. acknowledges the support of the Urology Care Foundation and J.C.L. is supported in part by NIH R01 CA 160986.

References

- 1*. Van den Berg NS, van Leeuwen FWB, van der Poel HG. Fluorescence guidance in urologic surgery. Curr Opin Urol. 2012; 22:109–120. This review provides an excellent summary of different types of exogenous contrast agents used in clinical fluorescence imaging. [PubMed: 22262249]
- Marano A, Priora F, Lenti LM, Ravazzoni F, Quarati R, Spinoglio G. Application of Fluorescence in Robotic General Surgery: Review of the Literature and State of the Art [Internet]. World J Surg. 201310.1007/s00268-013-2066-x.
- Keereweer S, Sterenborg HJCM, Kerrebijn JDF, Van Driel PBAA, de Jong RJB, Löwik CWGM. Image-guided surgery in head and neck cancer: Current practice and future directions of optical imaging. Head Neck. 2012; 34:120–126. [PubMed: 21284051]
- 4. Orringer DA, Golby A, Jolesz F. Neuronavigation in the surgical management of brain tumors: current and future trends. Expert Rev Med Devices. 2012; 9:491–500. [PubMed: 23116076]
- Schols RM, Bouvy ND, van Dam RM, Stassen LPS. Advanced intraoperative imaging methods for laparoscopic anatomy navigation: an overview. Surg Endosc. 2013; 27:1851–1859. [PubMed: 23242493]
- Orosco RK, Tsien RY, Nguyen QT. Fluorescence imaging in surgery. IEEE Rev Biomed Eng. 2013; 6:178–187. [PubMed: 23335674]
- Zaak D, Karl A, Knuchel R, Stepp H, Hartmann A, Reich O, Bachmann A, Siebels M, Popken G, Stief C. Diagnosis of urothelial carcinoma of the bladder using fluorescence endoscopy. BJU Int. 2005; 96:217–222. [PubMed: 16001963]
- Celli JP, Spring BQ, Rizvi I, Evans CL, Samkoe KS, Verma S, Pogue BW, Hasan T. Imaging and Photodynamic Therapy: Mechanisms, Monitoring, and Optimization. Chem Rev. 2010; 110:2795– 2838. [PubMed: 20353192]

- 9*. Liu J-J, Droller MJ, Liao JC. New Optical Imaging Technologies for Bladder Cancer: Considerations and Perspectives. J Urol. 2012; 188:361–368. This review summarizes the challenges of white light cystoscopy for bladder cancer detection and resection, as well as a detailed review of optical imaging technologies for the bladder. [PubMed: 22698620]
- Schumacher MC, Holmäng S, Davidsson T, Friedrich B, Pedersen J, Wiklund NP. Transurethral Resection of Non–Muscle-Invasive Bladder Transitional Cell Cancers With or Without 5-Aminolevulinic Acid Under Visible and Fluorescent Light: Results of a Prospective, Randomised, Multicentre Study. Eur Urol. 2010; 57:293–299. [PubMed: 19913351]
- 11. Grossman HB, Gomella L, Fradet Y, Morales A, Presti J, Ritenour C, Nseyo U, Droller MJ. A Phase III, Multicenter Comparison of Hexaminolevulinate Fluorescence Cystoscopy and White Light Cystoscopy for the Detection of Superficial Papillary Lesions in Patients With Bladder Cancer. J Urol. 2007; 178:62–67. [PubMed: 17499283]
- Stenzl A, Burger M, Fradet Y, Mynderse LA, Soloway MS, Witjes JA, Kriegmair M, Karl A, Shen Y, Grossman HB. Hexaminolevulinate Guided Fluorescence Cystoscopy Reduces Recurrence in Patients With Nonmuscle Invasive Bladder Cancer. J Urol. 2010; 184:1907–1914. [PubMed: 20850152]
- Lerner SP, Liu H, Wu M-F, Thomas YK, Witjes JA. Fluorescence and white light cystoscopy for detection of carcinoma in situ of the urinary bladder. Urol Oncol Semin Orig Investig. 2012; 30:285–289.
- 14. Burger M, Grossman HB, Droller M, Schmidbauer J, Hermann G, Dr goescu O, Ray E, Fradet Y, Karl A, Burgués JP, et al. Photodynamic Diagnosis of Non–muscle-invasive Bladder Cancer with Hexaminolevulinate Cystoscopy: A Meta-analysis of Detection and Recurrence Based on Raw Data [Internet]. Eur Urol. 201310.1016/j.eururo.2013.03.059
- 15. Daniltchenko DI, Riedl CR, Sachs MD, Koenig F, Daha KL, Pflueger H, Loening SA, Schnorr D. LONG-TERM BENEFIT OF 5-AMINOLEVULINIC ACID FLUORESCENCE ASSISTED TRANSURETHRAL RESECTION OF SUPERFICIAL BLADDER CANCER: 5-YEAR RESULTS OF A PROSPECTIVE RANDOMIZED STUDY. J Urol. 2005; 174:2129–2133. [PubMed: 16280742]
- 16. Hermann GG, Mogensen K, Carlsson S, Marcussen N, Duun S. Fluorescence-guided transurethral resection of bladder tumours reduces bladder tumour recurrence due to less residual tumour tissue in T a/T1 patients: a randomized two-centre study: FLUORESCENCE-GUIDED TURB REDUCES BLADDER TUMOUR RECURRENCE DUE TO LESS RESIDUAL TUMOUR TISSUE AFTER TURB IN TA/T1 PATIENTS. A RANDOMISED 2-CENTER STUD. BJU Int. 2011; 108:E297–E303. [PubMed: 21414125]
- Witjes JA. Is Fluorescent Cystoscopy of Cost/Benefit/Therapeutic Value for Carcinoma In Situ of the Bladder? Yes. J Urol. 2013; 190:19–20. [PubMed: 23603096]
- O'Brien T. Is Fluorescent Cystoscopy of Cost/Benefit/Therapeutic Value for Carcinoma In Situ of the Bladder? No (a difficult task). J Urol. 2013; 190:20–21. [PubMed: 23602484]
- Fradet Y, Lotan Y. Cost-Effectiveness of Fluorescent Cystoscopy for Noninvasive Papillary Tumors. J Urol. 2012; 187:1537–1539. [PubMed: 22425096]
- Schlenker B, Gratzke C, Seitz M, Bader MJ, Reich O, Schneede P, Hungerhuber E, Stief CG, Tilki D. Fluorescence-guided laser therapy for penile carcinoma and precancerous lesions: Long-term follow-up. Urol Oncol Semin Orig Investig. 2011; 29:788–793.
- Hoda MR, Popken G. Surgical Outcomes of Fluorescence-Guided Laparoscopic Partial Nephrectomy Using 5-Aminolevulinic Acid-Induced Protoporphyrin IX. J Surg Res. 2009; 154:220–225. [PubMed: 19375717]
- 22. Adam C, Salomon G, Walther S, Zaak D, Khoder W, Becker A, Reich O, Blana A, Ganzer R, Denzinger S, et al. Photodynamic Diagnosis Using 5-Aminolevulinic Acid for the Detection of Positive Surgical Margins during Radical Prostatectomy in Patients with Carcinoma of the Prostate: A Multicentre, Prospective, Phase 2 Trial of a Diagnostic Procedure. Eur Urol. 2009; 55:1281–1288. [PubMed: 19328622]
- 23. Ganzer R, Blana A, Denzinger S, Wieland WF, Adam C, Becker A, Khoder W, Walther S, Stief CG, Zaak D, et al. Intraoperative Photodynamic Evaluation of Surgical Margins During Endoscopic Extraperitoneal Radical Prostatectomy with the Use of 5-Aminolevulinic Acid. J Endourol. 2009; 23:1387–1394. [PubMed: 19673655]

- 24. Fukuhara H, Inoue K, Satake H, Tamura K, Karashima T, Yamasaki I, Tatsuo I, Kurabayashi A, Furihata M, Shuin T. Photodynamic diagnosis of positive margin during radical prostatectomy: Preliminary experience with 5-aminolevulinic acid: Photodynamic diagnosis during radical prostatectomy. Int J Urol. 2011; 18:585–591. [PubMed: 21658132]
- 25. Sevick-Muraca EM, Sharma R, Rasmussen JC, Marshall MV, Wendt JA, Pham HQ, Bonefas E, Houston JP, Sampath L, Adams KE, et al. Imaging of lymph flow in breast cancer patients after microdose administration of a near-infrared fluorophore: feasibility study. Radiology. 2008; 246:734–741. [PubMed: 18223125]
- 26*. Tobis S, Knopf JK, Silvers CR, Marshall J, Cardin A, Wood RW, Reeder JE, Erturk E, Madeb R, Yao J, et al. Near Infrared Fluorescence Imaging After Intravenous Indocyanine Green: Initial Clinical Experience With Open Partial Nephrectomy for Renal Cortical Tumors. Urology. 2012; 79:958–964. This is the first clinical report detailing the use of near infrared fluorescence imaging with indocyanine green to identify the renal vascular tree intraoperatively as well as to differentiate the tumor boarder from normal renal parenchyma during partial nephrectomy. [PubMed: 22336035]
- Manny TB, Krane LS, Hemal AK. Indocyanine green cannot predict malignancy in partial nephrectomy: histopathologic correlation with fluorescence pattern in 100 patients. J Endourol Endourol Soc. 2013; 27:918–921.
- Mitsui Y, Shiina H, Arichi N, Hiraoka T, Inoue S, Sumura M, Honda S, Yasumoto H, Igawa M. Indocyanine green (ICG)-based fluorescence navigation system for discrimination of kidney cancer from normal parenchyma: application during partial nephrectomy. Int Urol Nephrol. 2012; 44:753–759. [PubMed: 22215306]
- 29. Angell JE, Khemees TA, Abaza R. Optimization of Near Infrared Fluorescence Tumor Localization during Robotic Partial Nephrectomy. J Urol. 201310.1016/j.juro.2013.04.072
- 30. Van der Poel HG, Buckle T, Brouwer OR, Valdés Olmos RA, van Leeuwen FWB. Intraoperative Laparoscopic Fluorescence Guidance to the Sentinel Lymph Node in Prostate Cancer Patients: Clinical Proof of Concept of an Integrated Functional Imaging Approach Using a Multimodal Tracer. Eur Urol. 2011; 60:826–833. [PubMed: 21458154]
- 31. Verbeek FPR, van der Vorst JR, Schaafsma BE, Swijnenburg R-J, Gaarenstroom KN, Elzevier HW, van de Velde CJH, Frangioni JV, Vahrmeijer AL. Intraoperative Near Infrared Fluorescence Guided Identification of the Ureters Using Low Dose Methylene Blue: A First in Human Experience. J Urol. 2013; 190:574–579. [PubMed: 23466242]
- Rais-Bahrami S, Levinson AW, Fried NM, Lagoda GA, Hristov A, Chuang Y, Burnett AL, Su L-M. Optical coherence tomography of cavernous nerves: a step toward real-time intraoperative imaging during nerve-sparing radical prostatectomy. Urology. 2008; 72:198–204. [PubMed: 18280549]
- Fried NM, Rais-Bahrami S, Lagoda GA, Chuang Y, Burnett AL, Su L-M. Imaging the cavernous nerves in the rat prostate using optical coherence tomography. Lasers Surg Med. 2007; 39:36–41. [PubMed: 17163481]
- 34. Aron L-M, Kaouk JH, Hegarty NJ, Colombo JR Jr, Haber G-P, Chung BI, Zhou M, Gill IS. Second prize: preliminary experience with the Niris optical coherence tomography system during laparoscopic and robotic prostatectomy. J Endourol Endourol Soc. 2007; 21:814–818.
- 35. Manyak MJ, Gladkova ND, Makari JH, Schwartz AM, Zagaynova EV, Zolfaghari L, Zara JM, Iksanov R, Feldchtein FI. Evaluation of superficial bladder transitional-cell carcinoma by optical coherence tomography. J Endourol Endourol Soc. 2005; 19:570–574.
- 36. Fried NM, Rais-Bahrami S, Lagoda GA, Chuang A-Y, Su L-M, Burnett AL III. Identification and Imaging of the Nerves Responsible for Erectile Function in Rat Prostate, In Vivo, Using Optical Nerve Stimulation and Optical Coherence Tomography. IEEE J Sel Top Quantum Electron. 2007; 13:1641–1645.
- Lee H-C, Zhou C, Cohen DW, Mondelblatt AE, Wang Y, Aguirre AD, Shen D, Sheikine Y, Fujimoto JG, Connolly JL. Integrated optical coherence tomography and optical coherence microscopy imaging of ex vivo human renal tissues. J Urol. 2012; 187:691–699. [PubMed: 22177199]
- Zagaynova, EV.; Streltsova, OS.; Gladkova, ND.; Shakhova, NM.; Feldchtein, FI.; Kamensky, VA.; Gelikonov, GV.; Snopova, LB.; Donchenko, EV. Optical coherence tomography in

diagnostics of precancer and cancer of human bladder [Internet]. Bartels, KE.; Bass, LS.; de Riese, WTW.; Gregory, KW.; Katzir, A.; Kollias, N.; Madsen, SJ.; Malek, RS.; McNally-Heintzelman, KM., et al., editors. 2004. p. 75-81.

- Hermes B, Spöler F, Naami A, Bornemann J, Först M, Grosse J, Jakse G, Knüchel R. Visualization of the basement membrane zone of the bladder by optical coherence tomography: feasibility of noninvasive evaluation of tumor invasion. Urology. 2008; 72:677–681. [PubMed: 18455778]
- 40. Barwari K, de Bruin DM, Faber DJ, van Leeuwen TG, de la Rosette JJ, Laguna MP. Differentiation between normal renal tissue and renal tumours using functional optical coherence tomography: a phase I *in vivo* human study: *IN-VIVO DIFFERENTIATION OF NORMAL AND TUMOURAL RENAL TISSUE BY OCT*. BJU Int. 2012; 110:E415–E420. [PubMed: 22574685]
- 41. Li Q, Onozato ML, Andrews PM, Chen C-W, Paek A, Naphas R, Yuan S, Jiang J, Cable A, Chen Y. Automated quantification of microstructural dimensions of the human kidney using optical coherence tomography (OCT). Opt Express. 2009; 17:16000–16016. [PubMed: 19724599]
- 42. Walsh PC, Partin AW, Epstein JI. Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. J Urol. 1994; 152:1831–1836. [PubMed: 7523730]
- Dangle PP, Shah KK, Kaffenberger B, Patel VR. The use of high resolution optical coherence tomography to evaluate robotic radical prostatectomy specimens. Int Braz J Urol Off J Braz Soc Urol. 2009; 35:344–353.
- Sonn GA, Jones S-NE, Tarin TV, Du CB, Mach KE, Jensen KC, Liao JC. Optical Biopsy of Human Bladder Neoplasia With In Vivo Confocal Laser Endomicroscopy. J Urol. 2009; 182:1299–1305. [PubMed: 19683270]
- 45. Chang TC, Liu J-J, Liao JC. Probe-based Confocal Laser Endomicroscopy of the Urinary Tract: The Technique [Internet]. J Vis Exp. 201310.3791/4409
- 46. Wu K, Liu J-J, Adams W, Sonn GA, Mach KE, Pan Y, Beck AH, Jensen KC, Liao JC. Dynamic Real-time Microscopy of the Urinary Tract Using Confocal Laser Endomicroscopy. Urology. 2011; 78:225–231. [PubMed: 21601243]
- Chang TC, Liu J-J, Hsiao ST, Pan Y, Mach KE, Leppert JT, McKenney JK, Rouse RV, Liao JC. Interobserver agreement of confocal laser endomicroscopy for bladder cancer. J Endourol Endourol Soc. 2013; 27:598–603.
- 48. Van Dam GM, Themelis G, Crane LMA, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJG, van der Zee AGJ, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results. Nat Med. 2011; 17:1315–1319. [PubMed: 21926976]
- Hsiung P-L, Hsiung P-L, Hardy J, Friedland S, Soetikno R, Du CB, Wu AP, Sahbaie P, Crawford JM, Lowe AW, et al. Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy. Nat Med. 2008; 14:454–458. [PubMed: 18345013]
- Nguyen QT, Olson ES, Aguilera TA, Jiang T, Scadeng M, Ellies LG, Tsien RY. Surgery with molecular fluorescence imaging using activatable cell-penetrating peptides decreases residual cancer and improves survival. Proc Natl Acad Sci U S A. 2010; 107:4317–4322. [PubMed: 20160097]
- Whitney MA, Crisp JL, Nguyen LT, Friedman B, Gross LA, Steinbach P, Tsien RY, Nguyen QT. Fluorescent peptides highlight peripheral nerves during surgery in mice. Nat Biotechnol. 2011; 29:352–356. [PubMed: 21297616]

Key points

- Optical imaging offers excellent spatial and temporal resolutions, as well as the relative ease of integration into the operating room environment and instrumentations of minimally invasive surgery.
- Photodynamic diagnosis and near infrared fluorescence provide real time intraoperative surgical guidance through differential contrast enhancement of regions of interest.
- Optical coherence tomography and confocal laser endomicroscopy are examples of optical biopsy technology which enable high resolution, subsurface imaging with spatial resolutions comparable to standard histology.
- Molecular imaging represents a exciting future arena in conjugating cancerspecific contrast agents to fluorophores to improve specificity of disease detection.

Hsu et al.



Figure 1.

Photodynamic diagnosis (PDD) in bladder. White light cystoscopy (a) of the right lateral wall / bladder dome regions showed a diffuse area of non-papillary tumor. Under PDD (b), pink fluorescence delineates the extent of neoplastic region which was pathologically confirmed to be carcinoma in situ (CIS).

[This figure is original work.]



Figure 2.

Near infrared fluorescence imaging (NIRF) during robotic partial nephrectomy using intravenously injected indocyanine green demonstrating (a) arterial phase enhancement of the primary, secondary and tertiary renal arterial branches of the kidney and (b) hypofluorescent renal mass with surrounding normal fluorescent renal parenchyma. This figure is reproduced with permission from:

Tobis S, Knopf JK, Silvers CR, Marshall J, Cardin A, Wood RW, Reeder JE, Erturk E, Madeb R, Yao J, et al.: Near Infrared Fluorescence Imaging After Intravenous Indocyanine Green: Initial Clinical Experience With Open Partial Nephrectomy for Renal Cortical Tumors. *Urology* 2012, **79**:958–964.



Figure 3.

Optical coherence tomography (OCT) of the bladder and prostate. (a) OCT of normal bladder mucosa showing distinct layers of bladder wall (OG = optical gap, U = urothelium, LP = lamina propria, MP = muscularis propria, White bar = 1 mm); (b) OCT images of a rat seminal vesicle [32] and (c) prostatic adipose tissue [33]; (d) OCT and histologic images of the rat cavernous nerve, oblique section with overlying vacuoles seen within the prostate gland [33].

This figure is reproduced with permission from:

a) Fried NM, Rais-Bahrami S, Lagoda GA, Chuang A-Y, Su L-M, Burnett III AL: Identification and Imaging of the Nerves Responsible for Erectile Function in Rat Prostate, In Vivo, Using Optical Nerve Stimulation and Optical Coherence Tomography. *IEEE J. Sel. Top. Quantum Electron.* 2007, **13**:1641–1645.

b) Rais-Bahrami S, Levinson AW, Fried NM, Lagoda GA, Hristov A, Chuang Y, Burnett AL, Su L-M: **Optical coherence tomography of cavernous nerves: a step toward real***time intraoperative imaging during nerve-sparing radical prostatectomy. Urology* 2008, **72**:198–204.

c) Fried NM, Rais-Bahrami S, Lagoda GA, Chuang A-Y, Su L-M, Burnett III AL:
Identification and Imaging of the Nerves Responsible for Erectile Function in Rat
Prostate, In Vivo, Using Optical Nerve Stimulation and Optical Coherence
Tomography. *IEEE J. Sel. Top. Quantum Electron.* 2007, 13:1641–1645.
d) [This figure is original work.]

Hsu et al.



Figure 4.

Optical biopsy of bladder mucosa using probe-based confocal laser endomicroscopy (CLE). CLE of normal, low/high grade papillary bladder cancer, CIS, and inflammation shown with corresponding white light cystoscopy (WLC) and hematoxylin and eosin (H&E) staining of the biopsy. Low grade cancer shows characteristic organized papillary structure whereas high grade cancer and CIS show pleomorphic cells and distorted microarchiture. Inflammatory mucosa shows lymphocytic infiltrates. [This figure is original work.]