Optical and Cross-Sectional Imaging Technologies for Bladder Cancer

Bernhard Kiss, Gautier Marcq and Joseph C. Liao

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Abstract
Optical and cross-sectional imaging plays critical roles in bladder cancer diagnostics. White light cystoscopy remains the cornerstone for the management of non-muscle-invasive bladder cancer. In the last decade, significant technological improvements have been introduced for optical imaging to address the known shortcomings of white light cystoscopy. Enhanced cystoscopy modalities such as blue light cystoscopy and narrowband imaging survey a large area of the urothelium and provide contrast enhancement to detect additional lesions and decrease cancer recurrence. However, higher false-positive rates accompany the gain of sensitivity. Optical biopsy technologies, including confocal laser endomicroscopy and optical coherence tomography, provide cellular resolutions combined with subsurface imaging, thereby enabling optical-based cancer characterization, and may lead to real-time cancer grading and staging. Coupling of fluorescently labeled binding agents with optical imaging devices may translate into high molecular specificity, thus enabling visualization and characterization of biological processes at the molecular level. For cross-sectional imaging, upper urinary tract evaluation and assessment potential extravesical tumor extension and metastases are currently the primary roles, particularly for management of muscle-invasive bladder cancer. Multi-parametric MRI, including dynamic gadolinium-enhanced and diffusion-weighted sequences, has been investigated for primary bladder tumor detection. Ultrasmall superparamagnetic particles of iron oxide (USPIO) are a new class of contrast agents that increased the accuracy of lymph node imaging. Combination of multi-parametric MRI with positron emission tomography is on the horizon to improve accuracy rates for primary tumor diagnostics as well as lymph node evaluation. As these high-resolution optical and cross-sectional technologies emerge and develop, judicious assessment and validation await for their clinical integration toward improving the overall management of bladder cancer.

Keywords
Bladder cancer · Optical imaging · Cross-sectional imaging
Molecular imaging · Enhanced cystoscopy · Optical biopsy

1 Introduction

Imaging plays an integral role in all aspects of bladder cancer management. As the standard optical imaging modality, white light cystoscopy (WLC) is utilized for office-based identification of bladder tumors and enables transurethral resection (TUR) for local staging. Cross-sectional imaging, primarily computed tomography
(CT), complements WLC to assess the upper urinary tract and potential extravesical tumor extension and metastases.

Over the past two decades, new optical and cross-sectional imaging technologies have emerged to complement and augment current standards, particularly in improving cancer diagnostic accuracy. New technologies provide enhanced spatial and temporal resolutions and hold the potential to highlight the dynamic cellular and molecular differences between cancerous and non-cancerous tissues. This chapter aims to review the current state of the art of new and developing optical and cross-sectional imaging technologies for bladder cancer.

## 2 Optical Imaging

In 2017, white light cystoscopy (WLC) remains the standard for evaluation of bladder urothelium and management of non-muscle-invasive bladder cancer (NMIBC), both in the office setting with flexible cystoscopy and TUR in the operating room. Despite its central role, WLC has well-recognized limitations [54, 59]. For papillary lesions, WLC is unreliable for the determination of low- and high-grade cancer and cannot assess level of invasion [22]. Differentiation of non-papillary and flat malignant lesions, particularly carcinoma in situ (CIS), from inflammations can be difficult, with detection rates of CIS as low as 58–68% by WLC [30, 43, 83]. Furthermore, smaller or satellite tumors can be missed, which contributes to the up to 40% rate of residual bladder cancer found at the time of second-look TUR [6, 25]. Finally, indistinct borders and inadequate submucosal margins during TUR can lead to incomplete tumor resection and understaging [21, 51]. These limitations of WLC contribute to the increased risk of cancer persistence, recurrence, and in the case of high-grade bladder disease, progression to metastatic disease [11, 47, 49]. Hence, there is significant interest to develop adjunctive imaging techniques to augment conventional WLC for more precise diagnostic and surveillance of bladder cancer.

### 2.1 Enhanced Cystoscopy Technologies

Adjunctive optical imaging technologies that go beyond WLC may be classified based on their field of view and spatial resolution. Enhanced cystoscopy technologies survey a large area of the urothelium and provide contrast enhancement beyond WLC to distinguish suspicious lesions from benign transformations. Blue light cystoscopy (BLC) and narrowband imaging (NBI) are approved examples of this modality.

#### 2.1.1 Blue Light Cystoscopy (BLC)

Also known as photodynamic diagnosis or fluorescence cystoscopy, BLC provides wide field of view similar to WLC. It requires preoperative intravesical instillation
of a photosensitizer that is preferentially metabolized by neoplastic cells. Once taken up by the urothelium, the photosensitizer (i.e., protoporphyrin IX precursor) accumulates, whereby in neoplastic cells the absorption of blue fluorescent light (375–440 nm) induces emission of red light, thus allowing visualization of the neoplastic tissue [14, 64] (Fig. 1). In bladder imaging, two protoporphyrin analogues, 5-aminolevulinic acid (5-ALA), and its ester derivate hexaminolevulinate (HAL) have been extensively investigated clinically. HAL, which is more lipophilic with greater local bioavailability and superior fluorescence intensity, is approved for clinical use.

BLC has been demonstrated to improve detection of papillary lesions and CIS in numerous multi-institutional randomized studies [53, 76]. In a meta-analysis, the detection of CIS was significantly higher by the combination of BLC and WLC compared to WLC alone (87 vs. 75%) [57]. Furthermore, significantly reduced residual tumor rates were found in patients who underwent BLC-assisted TUR (relative risk of 2.77-fold higher for WLC compared to BLC) in meta-analyses [45, 86]. A prospective randomized multi-institutional study (n = 300) failed to demonstrate a significant benefit in tumor recurrence and progression for BLC compared to WLC after 12-month follow-up [84], while another prospective randomized study (n = 551) found an increased time to recurrence of 16.4 months with BLC using HAL compared to 9.4 months with WLC (p = 0.04) [38]. In a meta-analysis of the prospective trials, Burger et al. found significantly lower overall recurrence rates at 12 months with BLC compared to WLC in 1345 patients with NMIBC (34.5 vs. 45.4% pooled sensitivity, p = 0.006) [12]. Main limitation of BLC is the non-cancer-specific fluorescence from inflammatory lesions, previous biopsies, or pretreatment with bacillus Calmette–Guérin (BCG) [53, 59] leading to false-positive rate. Endoscopic images of high-grade pTa, carcinoma in situ (CIS) and inflammation under white light cystoscopy (WLC), blue light cystoscopy (BLC), and corresponding hematoxylin and eosin (H&E) histology.

Fig. 1  Blue light cystoscopy facilitates detection of papillary and flat bladder cancer but increases false-positive rate. Endoscopic images of high-grade pTa, carcinoma in situ (CIS) and inflammation under white light cystoscopy (WLC), blue light cystoscopy (BLC), and corresponding hematoxylin and eosin (H&E) histology.
false-positive diagnosis in 10–12% on a per-patient basis [88]. HAL-assisted BLC is currently not approved for patients within 90 days of intravesical chemotherapy or BCG instillations. In the 2016 AUA guidelines on NMIBC, HAL-assisted BLC received moderate recommendation [17].

2.1.2 Narrowband Imaging (NBI)

Narrowband imaging (NBI) was developed in 1999 [35] by Olympus (Tokyo, Japan) and first applied to gastrointestinal endoscopy [34]. The technology relies on a light filter that provides narrow (blue, green), instead of broad illumination (blue, green, and red) as in standard white light, thereby highlighting vascularized lesions (Fig. 2) [103]. In contrast to BLC, NBI does not need exogenous fluorescent dyes. A prospective randomized trial in 178 patients with NMIBC showed significantly lower recurrence rates at 3 and 12 months for patients who underwent NBI-assisted TUR than patient who underwent standard TUR [62]. At 3-month follow-up, the recurrence rates were 5.8% in the NBI group compared with 18.5% in the WLC-only group. At 1-year follow-up, the recurrence rates were 18.6% in the NBI group compared with 38.04% in the WLC. The recurrence rate of CIS was significantly lower in the NBI group (2.3 vs. 14.1%, \( p < 0.05 \)).

A meta-analysis [100] has shown that compared to WLC alone, NBI increased NMIBC detection by 9.9%, increased diagnostic sensitivity from 81.6 to 95.8%, reduced tumor persistence rate at 1 month at re-resection (RR = 0.43), and reduced recurrence rate at 12 months (RR = 0.81). A recent multi-institutional, prospective, randomized study of 965 patients with primary diagnosis of NMIBC found a significantly higher recurrence rate in patients with low-risk NMIBC treated by WLC-assisted compared to NBI-assisted TURBT (27.3 vs. 5.6%, \( p = 0.002 \)) at 12 months [69]. However, no overall difference in recurrence rates was found at 12 months (27.1% WLC vs. 25.4% NBI, \( p = 0.585 \)). Increasing sensitivity frequently leads to higher false-positive rates, and NBI was shown to have higher false-positive rates of 21.8–50% per patient compared to WLC [40, 100, 101] even if a recent study found an increased detection of CIS up to 28% without increased false-positive rates with NBI [58]. Table 1 summarizes performance of enhanced cystoscopy technologies compared to WLC alone when analyzing accuracy on the patient level as well as on the biopsy level.

2.2 Optical Biopsy Technologies

Whereas enhanced cystoscopy technologies improve identification and enumeration of suspicious bladder lesions, their overall field of view and spatial resolutions are comparable to standard WLC and hence relatively minimal learning curve. In contrast, optical biopsy technologies provide cellular resolutions combined with subsurface imaging, thereby raising the possibility of real-time, optical-based cancer characterization including grading and staging. For integration into the clinical workflow, the high-resolution imaging data require real-time interpretation, thereby increasing the associated learning curve with the technology adaptation.
Confocal laser endomicroscopy (CLE) and optical coherence tomography (OCT) are examples of optical biopsy technologies with early stage clinical experience. Importantly, these technologies complement wide field imaging (i.e., WLC, BLC, and NBI) to provide a more comprehensive evaluation of tissue of interest.

2.2.1 Confocal Laser Endomicroscopy (CLE)
Confocal laser endomicroscopy (CLE) is based on the well-established confocal microscopy technique commonly used in laboratory settings [67]. Configuration of

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**Fig. 2** NBI-enhanced cystoscopy facilitates detection of papillary and flat bladder cancer but increases false-positive rate. a Small papillary tumor (pathology pTa) poorly visualized under WLC but improved under NBI (b); multi-focal papillary tumors (pathology pTa) under WLC (c) and NBI (d); e WLC image of CIS and NBI (f); g false-positive lesion near the right orifice identified by WLC; h same lesion identified by NBI (reactive tissue in pathology). Figures obtained from [15] with permission.

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Performance of each technique is given by sensitivity and specificity. BLC = blue light cystoscopy, WLC = white light cystoscopy, CIS = carcinoma in situ, NBI = narrowband imaging. Patient-level analysis shows the accuracy of the technology to diagnose all lesions in the bladder. Biopsy-level analysis shows the accuracy of the technology to diagnose one lesion in the bladder.
the technology into probe-based devices compatible with standard endoscopic instruments has enabled clinical translation and in vivo imaging of cellular and subcellular structures (2001). The CLE system (Cellvizio®, Mauna Kea Technologies, France) in clinical use has been coupled with endoscopy of the gastrointestinal, respiratory, and urinary tracts. The technology is based on a fiber-optic imaging probe coupled to a 488-nm laser-scanning unit. The reusable probe has a 1–5μm spatial resolution. Fluorescein is used as the contrast agent and can be applied either topically via intravesical instillation or systemically via intravenous injection [95]. Bladder application of CLE has been described in detail [19]. Pilot studies have shown that CLE can discriminate normal mucosa from benign lesions (scar, inflammatory lesion) and malignant lesion (CIS, low and high grade) after training (Fig. 3) [18, 99]. The inter-observer agreement to diagnose cancer was 90% in urologists experienced in using CLE compared with 80% in urologists not-experienced in CLE after one-hour training session [18]. To assess the learning curve of CLE image interpretation more broadly, crowdsourcing has been applied to assess the diagnostic accuracy to distinguish normal urothelium from cancer. After a shorting training module, of 92% diagnostic accuracy was obtained 1173 ratings from 602 participants [18, 20]. Current limitations of CLE include the lack of multi-institutional studies in order to validate diagnosis criteria and accuracy. Additional future directions include combining CLE with molecular imaging.

Fig. 3 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 micro-architecture. Inflammatory mucosa shows lymphocytic infiltrates. Figure from [42] with permission
modalities such as monoclonal antibodies bound to fluorescein-labeled monoclonal antibodies for targeted binding of cancer-specific antigens.

2.2.2 Optical Coherence Tomography (OCT)

OCT provides real-time high-resolution subsurface imaging of tissue using near-infrared light with wavelengths between 890 and 1300 nm. Analogous to B-mode ultrasound, the technique measures the backscatter properties of tissue layers thus providing a cross-sectional imaging with an image resolution of 10–20 µm and a depth of penetration of 2 mm [63]. Originally described for imaging of the retina [56], OCT has been demonstrated in a variety of organ systems including gastrointestinal, respiratory, and urinary tracts. In cancerous lesions, the anatomic layers of the urothelium are lost and therefore enable real-time cancer diagnostic. The reported overall sensitivity for cancer diagnosis is 84–100% and overall specificity 65–89% [32, 39, 44, 63, 75, 85]. Goh et al. reported 32 patients a 100% negative predictive value for the detection of muscle invasion [32], whereas another trial involving 24 patients at high risk for bladder cancer found a positive predictive value for tumor invasion into the lamina propria of 90% [63]. Schmidbauer and colleagues investigated combining enhanced cystoscopy (i.e., BLC) with OCT and found increased specificity in cancer diagnosis compared to BLC alone [82]. Current limitations include availability of clinical systems for bladder applications and relatively slow image acquisition time. Similar to CLE, further larger scale prospective studies are needed for OCT to demonstrate clinical utilities.

2.3 Molecular Imaging

Molecular imaging modalities enable visualization and characterization of biological processes at the molecular level, which may precede micro- or macroscale anatomic changes [37, 103]. Coupling of fluorescently labeled binding agents such as antibodies, peptides, or small molecules with optical imaging devices may translate into high molecular specificity. Urinary bladder, as an easily accessible hollow organ, is amenable to intravesical applications of therapeutic or imaging agents. The ideal molecular imaging agent has good safety profile, high sensitivity and specificity for cancer detection, suitable pharmacokinetics, and excellent in vivo stability.

Surface antigens are ideal targets for fluorescently labeled antibodies. Epidermal growth factor (EGFR) and prostate stem cell antigen (PSCA) show differential distribution and expression patterns in benign urothelium and bladder cancer [2, 13, 55, 65, 73]. Their relatively low expression rate in bladder cancer, however, makes them suboptimal targets for cancer molecular imaging. A recently described promising target is CD47, a cell surface protein involved in immune functions including neutrophil migration and T-cell co-stimulation, and a negative regulator of phagocytosis. Binding of CD47 on target cells with the native ligand SIRP-α on macrophages inhibits macrophage activation and phagocytosis. Blocking the CD47-SIRP-α interaction with an anti-CD47 antibody promotes phagocytosis of
the CD47 expressing cells and prevents metastasis in mouse xenograft models [16, 98]. CD47 expression is upregulated in bladder cancer, and it is expressed in more than 80% of bladder cancer cells and absent on the luminal cell layer of normal urothelium [16, 72]. In an ex vivo study to validate CD47 as an imaging target, fluorescently labeled anti-CD47 was instilled in 25 fresh radical cystectomy specimens followed by endoscopic fluorescence imaging of the intact bladders (Fig. 4) [72]. CLE and BLC were used as the imaging modalities and fluorescently labeled mouse monoclonal anti-CD47 as the targeting agent. Using the combination with BLC and anti-CD47 conjugated to a quantum dot (Qdot626) with matching spectra, an overall diagnostic sensitivity of 82.9% and a specificity of 90.5% were found for CD47-targeted imaging of bladder cancer.

pH low insertion peptides (pHLIPs) are a class of membrane-binding peptides that preferentially target acidic cells by inserting across cellular membranes at low extracellular pH [3, 97]. Due to increased metabolic activities, a wide variety of cancer cells exhibit acidic pH, thereby providing a versatile strategy for tumor targeting [8]. A recent study used a pHLIP conjugated to indocyanine green (ICG) for ex vivo imaging of bladder tumors from radical cystectomy specimen of 22 patients using a clinical grade near-infrared fluorescence (NIRF) imaging system (Firefly™) (Fig. 4). Sensitivity of targeting cancerous tissue versus normal urothelium was 97%, and specificity was 100% irrespective of urothelial tumor subtype. However, considering necrotic and previously treated tissues as false positives, the specificity was decreased to 80%. In vivo, studies and results particularly low-grade tumors are pending. While molecular imaging may represent the future given the improved cancer specificity, biosafety and regulatory hurdles remain to be overcome for clinical translation.

Fig. 4 Molecular imaging of human bladder tumors. Ex vivo molecular imaging of human bladder using anti-CD47-Qdot625 (anti-CD47) imaged with BLC and indocyanine green with pH low insertion peptide (pHLIP) agent imaged with da Vinci Si NIRF imaging system. The respective imaging systems for the two molecular imaging strategies are capable of detecting both a papillary tumors and b CIS with high sensitivity and specificity. Anti-CD47 images from [72] and pHLIP images obtained from [33] with permission.
2.4 Other Early Stage Optical Imaging Technologies

*Raman Spectroscopy.* Raman spectroscopy (RS) provides optical diagnostics through generation of tissue-specific spectra (i.e., molecular fingerprinting) without the need for exogenous contrast agents. RS is based on the Raman effect, a phenomenon of inelastic scattering of photons that occurs when the incident light is deflected by molecules [74]. These scattered photons are detected to generate spectra specific to the sample (i.e., cancer vs. non-cancer) [28]. For bladder cancer, ex vivo RS studies have demonstrated differentiation of normal urothelial layers, identification of low- and high-grade bladder cancer, and assessment of tumor invasiveness [23, 26]. In a pilot in vivo study of 62 suspicious lesions, an increase in the intensity of specific amino acid peaks and possibly in the DNA-specific peaks demonstrated sensitivity and specificity of 85 and 79%, respectively, for bladder cancer detection [26]. To further increase in sensitivity and specificity, surface-enhanced Raman spectroscopy (SERS), through coupling of molecular targeting nanoparticles, can significantly increase the overall signal-to-noise ratio and enable multiplexed detection of several molecular targets [94].

*Ultraviolet Autofluorescence.* Differences in tissue autofluorescence, derived from endogenous fluorophores and variations in tissue metabolism and cell types, have been investigated for optical diagnosis of bladder cancer using an ultraviolet (UV) laser [4, 81]. In a pilot in vivo feasibility study of 14 patients with bladder tumors, a UV imaging probe (360 and 450 nm excitation) was inserted in the working channel of a standard rigid cystoscope and placed in close proximity of suspicious bladder lesions. Compared to normal urothelium, decrease in overall fluorescence intensity was observed in bladder cancer, regardless of tumor stage and grade. The fluorescence signal was converted to an intensity ratio of the emitted light at abovementioned wavelengths and color coded, thus facilitating real-time interpretation [81].

*Three-Dimensional (3D) bladder reconstruction.* While cystoscopy video sequences contain a large volume of data, their documentations are generally suboptimal using non-standardized medical recordkeeping. A more precise strategy to document bladder tumors and suspicious mucosal changes may improve TUR surgical planning, tumor surveillance, trainee education and reduce inter-observer variance. Toward that goal, a variety of hardware and software-based approaches have been investigated. An “image stitching” algorithm has been described based on an ultrathin preclinical endoscope called scanning fiber endoscope [87]. Using ex vivo pig bladders, full-surface mosaics were generated with a projection error of 1.66 pixels on average and covered 99.6% of the bladder surface area. In another study using TUR videos derived from human subjects, the software algorithm successfully created panoramic images with a resolution of 4096 × 2048 pixel in 10 out of 12 cases [52]. Notable drawbacks include decreased illustration of the anterior bladder wall as well as low image quality in patients with significant gross hematuria. More recently, a Stanford group described a complete software-based strategy for high-resolution 3D reconstruction of the bladder using standard WLC videos using standard clinical hardware with only a minor modification to the
standard clinical scan pattern. The images were processed through a customized software algorithm called structure from motion (SfM) to generate a 3D point cloud, followed by mesh and texture generation (Fig. 5). The authors reported that successful reconstruction was achieved for 66.7% of the datasets, whereat the definition of successful was that at least 25% of the camera poses could be computed \[60, 61\] making this technique broadly applicable to endoscopy and thus may represent a significant advance in cancer surveillance opportunities for big data cancer research.

3 Cross-Sectional Imaging

Current roles for cross-sectional imaging in bladder cancer include evaluation of the upper urinary tract (UUT) and assessment potential extravesical tumor extension and metastases in patients with MIBC. For primary bladder tumor, cross-sectional imaging currently does not have the spatial resolution to replace cystoscopy, particularly for detection of small and flat urothelial lesions. For staging, cross-sectional imaging complements tissue diagnosis obtained via TUR under WLC and other optical imaging technologies. CT, and to a lesser extent magnetic resonance imaging (MRI), is the standard for staging.
CT staging of the primary tumor has been reported both overstage and under-stage in 23.4 and 24.7% of patients, respectively, and accuracy in predicting pathological tumor stage was 49% [92]. Furthermore, up to 25% of bladder cancer patients who were initially staged with clinical N0 disease preoperatively by cross-sectional imaging were found to have lymph node (LN) metastases in the final pathologic specimen [5]. Research on new cross-sectional imaging technologies, including new imaging agents and multimodal imaging, is progressing and poised for clinical translation in the near future.

3.1 Primary Bladder Cancer Diagnosis Using CT or MRI

CT urography (CTU) represents the cornerstone of urologic imaging for hematuria work-up. Sensitivity and specificity of CTU to correctly diagnose the source of hematuria showed large variations and are reported between 78 and 95% and 83 and 99%, respectively [10, 80, 93]. However, CTU shows inadequate accuracy in diagnosing small and flat lesions (i.e., CIS) [96]. Furthermore, cross-sectional imaging performed right after a TUR further decreases diagnostic accuracy to 60% [41]. Thus, CTU for primary bladder cancer diagnostics is clearly inferior to optical imaging of the bladder.

Newer MRI sequences, including dynamic gadolinium-enhanced MRI (DGE-MRI) and diffusion-weighted MRI, have been investigated for primary bladder tumor. In a prospective study in 122 patients with bladder cancer who were scheduled for a radical cystectomy, Daneshmand et al. investigated the diagnostic accuracy of preoperative DGE-MRI to predict final pathological staging [24]. The authors report an overall accuracy of 74%, sensitivity of 87.5%, and specificity of 47.6% to correctly diagnosing organ-confined disease. The authors concluded that this technology still lacks significant predictive power.

Another group investigated diagnostic accuracy of diffusion-weighted MRI in bladder cancer to differentiate between NMIBC and MIBC (Fig. 6). DW-MRI is an imaging sequence that analyzes tissue diffusion properties, which provides information on the microstructure of the underlying tissue, without the need for exogenous contrast agent. The authors report a diagnostic accuracy between 78.8 and 81.7% within two different radiologists [50]. In a study comparing WLC to CTU or MRI, respectively, and CTU to MRI, the authors conclude that cross-sectional imaging (either by CTU or MRI) is not able to replace WLC and that MRI showed better accuracy rates compared to CTU (sensitivity 76.9 vs. 61.5%, specificity 93.4 vs. 94.9%, respectively) [31].

3.2 Lymph Node Imaging Using MRI or CT

CT and MRI are the standard for preoperative detection of LN metastases in patients with invasive bladder cancer. These conventional imaging techniques rely mainly on morphologic criteria including LN size, shape, and morphological
features including LN-calcification or necrosis. However, the size of non-metastatic LNs varies widely and may overlap with the size of LN containing metastases. A lack of consensus regarding the normal size limit diagnostic of pelvic LN metastases is another shortcoming [66]. Using the short-axis diameter of the LN, which is generally used as criterion for metastases, the sensitivity and specificity vary between 78 and 97%, respectively, with a 6 mm cutoff [71] to 86 and 78%, respectively, with a 5 mm cutoff [66].

Small metastases often remain undetected, and enlarged LNs due to reactive hyperplasia may be misinterpreted as metastatic LNs [89]. Accordingly, upstaging in pelvic urologic malignancies of clinical N0 to pathological N+ is frequently found despite the fact that negative preoperative imaging and diagnostic accuracy are low [7, 29]. Importantly, accuracy of LN imaging may only be drawn if a meticulous PLND has been performed and reported. Otherwise, the true rate of positive and negative LNs, and thus the accuracy of the imaging, remains unknown. A meticulous PLND depends on more than just the number of removed LNs. However, the reported number of removed LNs may represent a surrogate if a complete PLND was aimed and the benchmark imaging technique compared to.

**Fig. 6** Cross-sectional imaging of primary bladder tumor using multi-parametric MRI. (1) A large papillary NMIBC (pathology low-grade Ta) on T2 W-MRI (a) and DW-MRI sequences (b). DW-MRI shows a high-intensity area; (2) MIBC showing focal disruption of the muscle layer under T2 W-MRI (c) and a high-intensity area without submucosal components on transverse DW-MRI (d); (3) MIBC with perivesical fat invasion in T2 W-MRI (e) and DW-MRI (f), showing a high-intensity area with an irregular margin on transverse DW-MRI. Modified from [50] with permission. NMIBC = non-muscle-invasive bladder cancer; MIBC = muscle-invasive bladder cancer; T2 W = T2 weighted; DWI = diffusion weighted.
3.3 Diffusion-Weighted MRI (DW-MRI)

In a prospective study to evaluate the diagnostic performance of DW-MRI for LN staging, 120 patients with bladder or prostate cancer and normal-sized LNs on conventional imaging techniques (CT and/or MRI) were evaluated with DW-MRI [91]. The authors found a sensitivity and specificity ranging between the three different radiologists who independently evaluated the images and were blinded for the pathological results from 64 to 79% and 79 to 85%, respectively, on a per-patient basis. This study shows that detection of small LN metastases in normal-sized LNs that would have been missed with conventional imaging modalities is enabled with DW-MRI alone. Currently, DW-MRI cannot replace a meticulous PLND in terms of accuracy because of the possibility of obtaining false-negative results with DW-MRI.

3.4 Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIO) with MRI

USPIOs (e.g., ferumoxtran-10) are iron oxide nanoparticles with a diameter <50 nm which can be used for MR contrast-enhanced imaging [70]. The iron oxide crystalline core of the USPIO produces strong susceptibility leading to a signal decrease in T2-weighted images. USPIOs are transported through the vascular endothelium into the interstitial space after intravenous injection. Subsequently, the particles are taken up by macrophages and transported into LNs. Therefore, lymphotropic USPIO can be used as MRI contrast agent for detection of metastases in normal-sized pelvic LNs. Non-malignant LNs, which contain significant amounts of USPIO within the macrophages, appear hypointense. Malignant LNs, in contrast, have fewer macrophages and show a total or partial lack of USPIO uptake, thus not showing a change in signal intensity after USPIO injection. The lack of USPIO uptake in metastatic LNs, therefore, is indicative of metastatic LNs, highlighting and potentially facilitating the identification of metastases in normal-sized LNs [9].

An initial study using ferumoxtran-10 as USPIO for preoperative staging in BC reports about sensitivity and specificity of 96 and 95% [27]. Although the numbers are very encouraging, no backup PLND was performed in this study and thus the true false-negative rate remains unknown even more because PLND was mostly limited to enlarged (which would have been detected by conventional MRI as well) or suspicious LNs in selected patients.

A Swiss group combined USPIO and diffusion-weighted DW-MRI for the detection of metastases in normal-sized pelvic LNs of patients with BC or PC and clinically staged N0 [9]. Combining those two techniques enables characterization of metastatic LNs according to a high signal intensity on DW-MRI and a lack of signal decrease after USPIO injection. After an extended PLND (median 39 LNs removed per patient), pathologically confirmed LN metastases were found in 20 patients and the long-axis diameter of the LN metastases was ≤ 5 mm in 83 and
≤3 mm in 50%. On a per-patient basis, sensitivity and specificity ranged from 65 to 75% and 93 to 96%, respectively, between the three different radiologists who independently evaluated the images and were blinded for the pathological results. Thus, the combination of USPIO and DW-MRI improved the detection rate of LN metastases in normal-sized LNs. Nevertheless, 25–35% of the LN-positive patients were incorrectly staged as LN negative, which remains too high. Furthermore, two MRI examinations are needed and USPIOs are not without side effects and have limited commercial availability [48].

3.5 Positron Emission Tomography (PET)

The diagnostic efficiency of PET does not depend on traditional parameters such as size or shape, but on the increased metabolic rate of specific tissues and their volume. As an intravenous imaging agent, 18F-fluorodeoxyglucose (FDG) highlights anatomic regions of increased metabolic activity. In bladder cancer, FDG-PET is predominantly used for detection of LN metastases. In a small 2010 prospective study, 51 bladder cancer patients received a FDG-PET/CT scan before radical cystectomy and pelvic LN dissection (a mean of 16 LN per patient removed) and the authors described a sensitivity and specificity in the detection of pelvic LN metastases of 46 and 97%, respectively [90]. Thus, the false-negative rate was over 50%, and even in some enlarged metastatic LNs up to 25 mm in size, no abnormal FDG uptake was found. Recently, another trial [1] compared preoperative FDG-PET/CT and conventional CT with pathological results in 54 patients who underwent radical cystectomy and PLND (a mean of 28 LNs removed per patient), and the same low sensitivity for both imaging techniques (41 vs. 41%) in the detection of regional LN metastases was reported. In line with these results is another study comparing preoperative CT scan with FDG-PET/CT in 207 patients with BC who underwent radical cystectomy and PLND (a mean of 17 LNs removed per patient) [36]. Although an increased sensitivity detecting LN metastases from 45% using CT alone to 69% with FDG-PET/CT was found, the additional diagnostic yield of 5% on a per-patient basis was small. Thus, FDG-PET/CT provides no or only minimal additional benefit in the loco-regional LN staging in BC (as the authors of this study claim). Other trials, however, have reported an increased detection rate of metastases using FDG-PET/CT for preoperative LN staging. The sensitivity rates in these trials varied from 50 to 70% [46, 79]. Only one of these trials [79], however, reports a median of 12 resected LNs per patient; the others did not perform (or did not report) a backup PLND. So, the true rate of false-negative LN metastases remains unknown. In summary, for patients with BC, currently available FDG-PET/CT techniques offer no substantial diagnostic benefit for the detection of pelvic LN metastases [48].

Recently, FDG-PET in combination with MRI has been investigated prospectively in a small pilot study of bladder cancer patients [78]. In a series of 22 patients
with known bladder cancer, Rosenkrantz et al. report increased accuracy of primary tumor detection from 77 to 86%, increased accuracy of detection of metastatic pelvic lymph nodes from 76 to 95%, and increased accuracy of detection of non-nodal pelvic malignancy from 91 to 100% when combining $^{18}$F FDG-PET with MRI compared to MRI alone (Fig. 7). The combination of multi-parametric MRI which offers high-contrast resolution with $^{18}$F FDG-PET which offers metabolic information seems to be a promising technology allowing to increase accuracy rates significantly. However, the sample size is small and a pathologic evaluation of the LNs has not been done in all cases. Therefore, final conclusions on the real advantages of this new combination of technologies are much too premature.

Fig. 7 Cross-sectional imaging the example of $^{18}$F-FDG-PET/MRI in pre-TURBT setting. (1) **Tumor detection**: A 68-year-old man with muscle-invasive high-grade bladder cancer on prior biopsy, undergoing simultaneous $^{18}$F-FDG-PET/MRI. a and b, Axial T2-weighted images show regions of mild nonspecific mural thickening (arrow, b) that was considered equivocal for the presence of tumor. (2) **Lymph node detection**: A 62-year-old man with prior biopsy showing high-grade non-muscle-invasive bladder cancer, undergoing simultaneous $^{18}$F-FDG-PET/MRI. c On postcontrast axial T1-weighted image of the pelvis, potential pelvic lymph nodes are difficult to differentiate from surrounding vessels and bowel loops. d Fused $^{18}$F-FDG-PET/MR image shows marked increased activity within numerous pelvic lymph nodes (arrows), which raised suspicion for nodal metastases. The nodes decreased in size following treatment with systemic chemotherapy. (3) **Non-nodal malignancy**: An 82-year-old man with prior biopsy showing high-grade non-muscle-invasive bladder cancer, undergoing simultaneous $^{18}$F-FDG-PET/MRI. e Axial T2-weighted image shows a left acetabular lesion (arrow) that was considered possibly degenerative, given its proximity to the hip joint, and equivocal for bone metastasis. f Fused PET/MR image shows corresponding marked increased metabolic activity (arrow), raising suspicion that the lesion represents a bone metastasis. Subsequent bone biopsy demonstrated metastatic urothelial carcinoma. From [78] with permission
4 Conclusion

Judicious applications of optical and cross-sectional imaging technologies play a paramount role in the management of patients with bladder cancer. Over the past 20 years, significant advances have taken place in both areas to improve their diagnostic yield for patients with NMIBC and MIBC. Level 1 evidence exists that enhanced cystoscopy such as BLC and NBI improves tumor detection and resection and reduces recurrence. However, in case of repeated intravesical chemotherapy instillations or post-BCG results are not that expedient and further validation in this setting has to be undertaken. To improve preoperative planning and standardize documentation at the same time, computer-assisted diagnostic algorithm (e.g., 3D reconstruction, machine learning) to enhance image processing will be increasingly studied. Numerous other emerging technologies (e.g., CLE, OCT) are promising and hold the potential to find their way into clinic to complement the currently available optical imaging technologies in the future, but at this time they still lack clinical efficacy data. Diagnostic accuracy, however, might be taken to another level in the future through molecular targeted imaging.

In cross-sectional imaging, while new contrast agents (e.g., USPIO) improve the detection of micro-metastatic disease and multimodal imaging (PET-CT and PET-MRI) provides superb anatomic and functional information, for local staging TUR with tissue diagnosis remains the standard for the foreseeable future. However, as resolution of MRI technology continues to improve and with integration of molecular tracers, noninvasive cross-sectional imaging may play an important adjunctive role in the future for the diagnosis of primary tumor. In terms of lymph node staging accuracy, none of the currently used cross-sectional imaging technologies have the potential to substitute pelvic lymph node dissection. Thus, similar to optical imaging, molecular targeting agents in combination with high-resolution cross-sectional imaging modalities might be anticipated to increase diagnostic accuracy.

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References


