Imaging modalities aiding nerve-sparing during radical prostatectomy

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ABSTRACT

Nerve-sparing robot-assisted radical prostatectomy has decreased the post-surgical complications of prostate surgery, but has not eliminated it. The ability to view the microstructure will enable better surgical decisions and lead to better post-surgical outcomes. An ideal imaging modality should provide rapid image acquisition, be low cost, and be specific to the tissue being examined. This article aims to review the current literature to compare three main techniques: multiphoton microscopy (MPM), optical coherence tomography, and confocal microscopy, to see which of these techniques may be best applied in surgical procedures in the future. Embase and Medline were used as the primary databases. Combinations of various key words were used while researching the literature. These included: "Radical prostatectomy," "nerve-sparing," "nerve mapping," "multiphoton microscopy," "Confocal microscopy," and "Optical Coherence Tomography." Thereafter, the relevant results were selected and used in the review. Although optical coherence tomography is a low cost and compact modality, it lacks cellular resolution, while confocal microscopy offers great cellular resolution but lacks depth. MPM, on the other hand, provides sufficient depth and produces high-resolution images. The limitation of MPM is its lack of portability, however the advent of dual-modality MPM may be a way forward.

Keywords: Confocal microscopy; multiphoton microscopy; nerve-sparing imaging; optical coherence tomography; radical prostatectomy.

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Introduction

Since its introduction[1], the treatment outcomes of radical prostatectomy (RP) have continued to improve, particularly with the advent of robotic surgery at the start of the 21st century[2,3]. However, in an era of patient-centered care, the spotlight has fallen on the post-surgical complications, namely erectile dysfunction (ED), which has very serious physical and psychological effects on men.

Post-operative erectile function varies between 15%-87% of cases.[4] An important reason for this is the inability of the operating surgeon to identify and preserve the neurovascular bundle (NVB), worsened by the high level of variation of the microscopic cavernous neural tissue involved in erectile function.[5] It is, therefore, of great importance to be able to view the NVB, a collection of small blood vessels and nerves involved in supplying the penis to facilitate an erection, which runs posterolateral to the prostate and is in close proximity to its capsule.[6] Due to their close proximity to the surface of the prostate, the nerves are prone to injury during dissection surgery.[7] Moreover, the nerves involved in human erectile function take variable routes in different patients.[5] There are various neuronal damage mechanisms (reversible and irreversible) that can occur during RP. Neuronal damage mechanisms are broadly classified into five different grades (76).[8] Grade 1 is neuropraxia; Grade 2, 3, and 4 refer to the severity of axonotmesis (axonal damage); and grade 5 refers to neurotmesis, where there is complete nerve and nerve sheath damage and where spontaneous recovery is unlikely.[9] To minimize the risk of nerve damage during nerve-sparing robot-

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assisted RP (RALP), a number of hemostatic agents have been tried, such as Floseal®. However, Martorana et al.[10] reported that the use of Floseal® is detrimental to long-term erectile function recovery after nerve-sparing RALP. Therefore, a reliable and tailored approach to sparing the NVB during RP should be developed to reduce the rates of ED following RP.

Currently, stereoscopic magnification does not provide satisfactory enlargement to view the delicate microanatomy of the nerves surrounding the prostate.[11] Thus, real-time in vivo techniques are required to provide high-quality images without obstructing the view of the surgeon. An ideal imaging modality should provide rapid image acquisition, be low cost, and be specific to the tissue being examined. This article reviews the current literature and compares three of the available techniques: multiphoton microscopy, optical coherence tomography, and confocal microscopy, to assess which of these techniques provide for better appreciation of the NVB, with the goal of decreasing post-surgical ED.

Multiphoton microscopy

Multiphoton microscopy (MPM) is the most studied modality out of the three in question. In MPM, samples are illuminated by near-infrared light. Each molecule absorbs two or more photons simultaneously, causing excitation of its electrons and consequently generating two types of emissions known as intrinsic tissue emissions: Auto-fluorescence (internal fluorophores, namely, NADH and FAD) and Second Harmonic Generation (SHG).[11] These emissions enable the detection of microscopic details that are otherwise difficult to see. This is an advantageous characteristic of MPM as it does not employ external dyes for labeling, which mitigates any toxic effects caused by the dyes.

Various studies have looked into the feasibility of MPM to effectively localize and track the course of a nerve. Yadav et al.[12] carried out an ex vivo study in which the prostates and cavernous nerves of 10 Sprague-Dawley rats were excised. Auto-fluorescence and SHG signals were collected, merged, and color-coded for better appreciation. The images were also correlated with histopathological slices using hematoxylin and eosin stain. They concluded that MPM was able to identify nerves and surrounding tissues and that it correlated well with the histopathology sections. Similarly, Tan et al.[13] also demonstrated that MPM images correlated strongly with histologic sections. Moreover, they were able to track the course of the nerve to a deep level (up to 500 micrometers) into the tissue as optical slices were taken.[13] Although the two above-mentioned studies are promising, they were conducted in rodents, in which the nerves lie on the surface and the anatomy is clear and distinct, unlike the human anatomy, where it is difficult to view the nerves on the surface of the prostate due to intervening fascia.[14]

In 2011, Tewari et al.[15] performed prostate imaging on 95 patients who underwent robot-assisted radical prostatectomy. Two types of samples were used: excised surgical margins and biopsies as well as sections from the excised prostate. The images were then cross-checked with histopathology slices, similar to the animal studies described above. The study concluded that structures such as blood vessels, connective tissue, and fat could be viewed with great clarity using MPM at low magnification. Nerves could also be traced by producing both lateral and vertical optical sections, resulting in the visualization of deeper tissues, thereby reducing the need for excision and biopsy. The images also matched their corresponding histopathology sections, illustrating the accuracy of the MPM system. Moreover, the authors also demonstrated the use of MPM in identifying benign and malignant alterations.[15] It was possible to distinguish between benign inflammation and more serious pathologies, enabling better-informed surgical decisions with regards to tissue removal. Another study conducted on human cadaveric prostates and peri-prostatic tissue set out to create an atlas of normal tissue appearance under MPM, suggesting that it was possible to appreciate prostatic architecture at both the glandular and cellular levels.[16]

Durand et al.[11] looked into the possibility of using MPM in real-time during surgery. For their study, they employed Sprague-Dawley rat models to identify prostatic and peri-prostatic tissue. Initially, images of the right lobe of the prostate and surrounding tissues were acquired. About 15 days later, another procedure was conducted and similar images were taken. Finally, the rats were euthanized and the prostate and its surrounding structures were excised and sent for histology. The left prostate lobe was used as the control. The study concluded that there was a clear identification of the peri-prostatic neural tissue and other surrounding structures and the images were reported to be stable between the day 1 and day 15. No other complications were reported as a result of the imaging process. This illustrates that MPM is an effective tool in providing in vivo real-time images. Moreover, the study also looked into the phototoxic effects of MPM, namely, the concerns of tissue damage by the laser used during the procedure. There were no abnormalities found between the two lobes, which reinforced the lack of photo-toxicity in MPM.[11] Nevertheless, a more in-depth post-surgical nerve function test should have been carried out to ascertain the phototoxic effects of MPM.

Despite these results, a few considerations have to be made. There was a possibility of a motion artifact occurring due to respiratory movements in some of the rats. Therefore, in some cases, the anesthetic had to be raised and changed to a stronger agent.[11] As trials have not yet been carried out on humans, it is not clear whether the artifacts will be present in humans or not, it can be argued that the rat anatomy is more compact as compared to humans, hence, there may be no motion artifact.

Advantages of MPM

The distinct advantage of MPM is its ability to generate high-resolution images without the need for exogenous labeling.[12] These findings are supported by another study on human prostate and peri-prostatic tissue, where the authors confidently concluded that MPM provides high-resolution images without the need for external labeling, and that it is not phototoxic.[15] In addition to this, images need not be processed post-acquisition, therefore, it is possible to image both fresh and unstained specimens. There were no reports of tissue damage from previous studies.[17] From the study above, it is evident that there have been no complications post-surgery when MPM has been used, implying that its integration into surgical procedures will provide surgeons with added accuracy during operations.

Limitations of MPM

Although MPM has shown great promise in ex vivo models, there are still some possible challenges to its use in vivo. It has to be miniaturized so that it can be integrated with modern robotics. This may have further implications on increased costs when looking to include it into a system for robot-assisted radical prostatectomies. Moreover, there will be an additional cost of training the staff to use the imaging modality properly.[15] Signal interference by blood loss and fatty deposits may also be a challenge in real-time application in humans, although there were no reports of this phenomenon in rat models.[11] Image acquisition is also slow, with an image rate of 1 frame per second. Further, imaging uneven surfaces may prove to be a limitation.[11] However, surface contact is not needed at lower magnifications. As the equipment gets developed better, it would be possible to have probes that will acquire images at a faster pace than the ones used in the studies above.

Optical coherence tomography

Similar to MPM, optical coherence tomography (OCT) also provides high-resolution and real-time images. OCT works on the same principle as B-mode ultrasonography, but it employs near-infrared light as opposed to the sound waves used in ultrasonography.[17] A 2-D map of the tissue microstructure is made by illuminating the tissue with near-infrared light. The backscatter produced is collected and its intensity is analyzed.[18] OCT has been studied in the past for its potential use in various procedures such as cancer detection, optical biopsy, and organ-preventing resection.[6] In the present study by Aron et al.[6], the possibility for OCT to identify NVB during laparoscopic and robotic-assisted RP (LRP) was assessed. Between November 2005 to November 2006, 24 patients underwent LRP. The study used the Niris OCT probe to acquire in vivo images of the prostatic and peri-prostatic tissues. Following the excision of the prostate, images were taken again of the remaining structures in vivo. Ex vivo images of the prostate were also taken, which were used to determine the presence or absence of the NVB. The study demonstrated that it was possible to identify the NVB during LRP and also to differentiate between neural tissue and other tissue types using OCT. The authors advised the need for a follow-upappointment at 4 months post-surgery to determine an intact erectile function.[6]

The study conducted by Aron et al.[6] pointed out a few limitations of the OCT system. These included its limited capacity to image deeper structures, difficulty in differentiating between the types of tissues (adipose, lymphatics, and small blood vessels) that mimic nerve cells, and imprecise placement of the probe on the tissue. OCT, therefore, requires a high level of expertise and training.

In another study conducted by Fried et al.[14], the Niris OCT system along with the 8fr probe was employed to acquire real-time images of 6 male rats. Histological slices were taken for comparison and electrical stimulation was used to localize the cavernous nerve (CN). The CN appeared as a high-intensity linear structure overlying a less intense glandular prostatic structure.[14] They demonstrated that it was possible to track the course of the nerve by placing the probe at various angles. However, this study also reported limitations to the use of OCT. One restriction is the lack useful information after a depth of 1 mm is attained, even though the system that was used can attain a depth of 1.6 mm.[14] However, they argued that the OCT system used in this study belonged to the first generation of the machine, and possible developments in generations may provide better depth and clarity in the future. A similar study was conducted by Rais-Bahrami et al.[19], where they imaged rat models in vivo and thereafter acquired images of ex vivo human prostates. They concluded that OCT lacked consistent contrast when imaging the human prostate, which was likely due to the overlying prostatic fascia. Improvements in the quality of OCT images are necessary for clinical use. However, a study by Chitchian et al.[20] demonstrated the use of image algorithms for better image outcomes. Such techniques involved edge detection, segmentation, and wavelet denoizing which led to imaging of deeper structures, differentiation between different tissue types, and noise reduction, respectively.[7,20,21]

Advantages of OCT

The unique benefit of an OCT system is its low cost and its ability to produce high-resolution images.[19] In addition to this, it is also portable and compact, therefore, it can be easily integrated into laparoscopes or robotic systems without obstructing the surgeon's surgical view.[14,19] It is also possible to distinguish clearly between neural tissues and other structures.[6] However, it has been argued that rats have less complicated prostate and peri-prostatic anatomy, which is not the case in humans due to the presence of intervening fascia. Therefore, OCT may have problems when imaging deeper tissues in humans. However, quality image processing can potentially overcome this.[7]

Limitations of OCT

Despite the use of OCT in preclinical studies, it has some drawbacks that must be overcome for it to be used in the operating

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Table 1. A summary of the available imaging modalities for nerve-sparing during radical prostatectomy

Modality	Advantages	Limitations
Multiphoton Microscopy	1. High-resolution images without the need for exogenous labeling 2. Images of fresh and unstained specimens can be obtained	1. Must be miniaturized to be integrated with modern robotics 2. Cost 3. Slow image acquisition
Optical Coherence Tomography	1. Low cost 2. Able to produce high-resolution images 3. Able to distinguish between neural and other structures	Poor resolution compared to histology slices and low depth penetration
Confocal Microscopy	Provides readily interpreted images	1. Lack of depth 2. Limited to 2 micrometers

theater. There have been issues with respect to poor resolution of images as compared to histology slices and low depth penetration, which may have unfavorable implications in tracking the course of the nerve.[14] This is made worse by other structures mimicking neural tissue.[6] A more accurate method of probe placement on the tissue needs to be designed, so small adjustments can alter the imaging field. Finally, it is expensive to acquire the expertize and training to use OCT during RP.

Confocal microscopy

Confocal microscopy (CM) involves the use of exogenous fluorophores to identify tissues. Fluorophores can be administered either systemically or locally[22] and can be selective for cells of interest, such as nerve cells, which can take up the fluorophore and subsequently allow the user to track the nerve pathway. In addition to this, they may be conjugated with other biomarkers, thereby enabling the detection of altered tissue architecture.[23] Fluorescent labeling (antegrade or retrograde) can be used to label axons after administering intracavernosal injections, to enable intraoperative imaging. When applied locally at the base of the penis, the fluorescent dye travels through a retrograde transport mechanism along the nerve.[24]

In their study, Boyette et al.[25] used a Cellvizio fiber-optic confocal fluorescent microscope (FCFM) to obtain in vivo real-time images to visualize the CN of male Wister rats following intracavernosal injections. The dye used was a cholera toxin subunit B-AlexaFluor 488 (CtB-488), a dye made by conjugating AlexaFluor 488 with cholera toxin.[26] The probe was directed on the nerve and moved along its length, while simultaneous real-time images were acquired. To determine if the imaging method had any adverse effects on the CN function, electrical stimulation of the nerve was performed and intracavernosal pressure was checked.

The study found encouraging results in that they were able to visualize the CN for up to 9 days after the initial induction. There was no evidence for the presence of the dye in the nerve at 10 weeks after the initial induction, although there were some traces found in the major pelvic ganglion.[25] Assessment of apoptosis was also carried out and the results were negative, with no presence of lipid peroxidation. Encouragingly, no adverse effects on the rats' erectile function were noted.[25]

However, there are still some concerns about the use of CM. The dye used in our procedure reached its optimum level after 9 days. The exact time it will take in humans is unknown, but it is speculated that it may take 38-45 days as the human nerve fibers are 5 times longer than that of rats.[26] In addition to this, there are no published data on the effects of CtB-488 on humans; its safety will need to be ascertained before it can be used clinically.[27] The authors also mention that there was a poor surgical view while using the probe, which may hinder the procedure.[25] These challenges need to be addressed before its use in surgery. Nevertheless, Lopez et al.[28] carried out a recent study involving 15 patients undergoing robotic-assisted RP. The images were produced in vivo and confocal video sequences were acquired. The findings were also correlated with histopathology specimens. Excitingly, intraoperative CLE of the NVB before and after nerve-sparing dissection revealed both intact dynamic vascular flow and axonal fibers. They concluded that confocal laser microscopy was feasible and safe to be used in vivo during robotic-assisted surgery, but that a study with a larger sample size would be required to thoroughly assess the benefits of CLE during RARP.[28]

Advantages of CM

Fiber-optic confocal fluorescent microscope has been used in humans during bronchoscopy to detect alterations in the basement membrane.[29] One aspect of FCFM is that it provides readily interpreted images, since it is able to identify the labeled nerve and no other tissue (such as connective and adipose tissue), thus eliminating doubts as to the identification of the imaged structure. Moreover, this type of imaging offers portability and adaptability to the current endoscopic equipment used in prostatectomies.[22]

Limitations of CM

One limitation is the lack of depth offered. According to

Ponnusamy et al.[26], OptiScan had a confocal microscope in clinical trials for prostatectomies, using a fluorescein dye they achieved a depth of 2 micrometers. This superficial examination using the confocal microscope may not prove useful in humans where the fascia can be obstructive in imaging techniques that require fine magnification. Another limitation is the use of a dye locally, allowing only one nerve fiber to be labeled at a time, which means that the microscopic CN fibers may not be labeled.[24]

Discussion

Radical prostatectomy is considered the gold standard in surgical treatment for organ-confined prostate cancer.[30] Post-surgical complications such as ED are still present despite the use of nerve-sparing RP. The main imaging techniques highlighted in this review may provide effective methods to overcome ED (Table 1), thus enabling better outcomes for the patient and reducing treatment costs. Despite the three modalities having the capabilities for imaging the nerves responsible for erectile function, there are currently some limitations that need to be addressed prior to their clinical application.

It is evident that OCT is a low cost system that is portable, compact, and provides better depth (1 mm) in comparison to the other modalities.[14,19] However, it lacks cellular resolution, which may allow different tissue types such as small blood vessels to mimic neural tissue.[11] This is not a problem in CM, which allows greater cellular detail as it uses dyes that target specific tissues, but it lacks depth.[11] Hence, making an image from the CN is more difficult because of the intervening fascia in humans. This is not the case with MPM, where sufficient depth (500 micrometers) and good cellular detail are available.[13]

In addition to this, it was difficult to identify a point-for-point location on the OCT in comparison to the histology, although this was not a problem in MPM. Similarly, CM used exogenous dyes, which have a questionable effect on humans.[6,27] Furthermore, some dyes that label the myelin sheath may not be able to label nerves that lack myelin, such as the smaller nerves involved in erectile function.[26] This, however, is not a problem for MPM, which uses intrinsic fluorescence. It also provides a high resolution such that cellular architecture such as prostatic acini can be identified, allowing its possible application in detecting malignant alterations with certainty.[11,15]

Multiphoton microscopy does stand out better than the other modalities discussed, however, this is not to say that MPM does not have any limitations. There is evidence of slow image acquisition in MPM as compared to CM (1 frame/second cf. 12 frames/second, respectively).[11,25] It is also apparent from the studies that MPM still needs to be miniaturized to be used in a clinical setting, unlike OCT and confocal microscopy[15]. The additional cost of training and equipment must also be taken into account. However, as technology continues to advance, better equipment will be offered at affordable prices. Excitingly, dual-modality MPM is also at the advent stages for possible clinical application in the near future.[31]

Future directions

This review highlights the advantages and disadvantages of MPM, OCT, and CM, the main imaging tools used in real-time surgery for nerve-sparing RARP. We concluded that MPM is capable of providing high-resolution images with appropriate depth for visualization of the neurovascular bundle. It is, therefore, plausible that this modality can be employed for clinical use in the near future. However, there is still a need to miniaturize the system to integrate it into a live surgical setting. A further recommendation that could have significant prospects in the future is dual-modality MPM, which provides high-resolution images within an easy to handle scope and displays a large field of view during surgery.

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References

1. Young HH. The early diagnosis and radical cure of carcinoma of the prostate. Being a study of 40 cases and presentation of a radical operation which was carried out in four cases. 1905. J Urol 2002;167:939-47. [CrossRef]
2. Tosoian JJ, Loeb S. Radical retropubic prostatectomy: comparison of the open and robotic approaches for treatment of prostate cancer. Rev Urol 2012;14:20-7.
3. Lepor H, Walsh PC. Long-term results of radical prostatectomy in clinically localized prostate cancer: experience at the Johns Hopkins Hospital. NCI Monogr 1988;7:117-22.
4. Ficarra V, Novara G, Artibani W, Cestari A, Galfano A, Graefen M, et al. Retropubic, laparoscopic, and robot-assisted radical prostatectomy: a systematic review and cumulative analysis of comparative studies. Eur Urol 2009;55:1037-63. [CrossRef]
5. Takenaka A, Murakami G, Soga H, Han SH, Arai Y, Fujisawa M. Anatomical analysis of the neurovascular bundle supplying penile cavernous tissue to ensure a reliable nerve graft after radical prostatectomy. J Urol 2004;172:1032-5. [CrossRef]

Turk J Urol 2019; 45(5): 325-30
DOI: 10.5152/tud.2019.19007

6. Aron M, Kaouk JH, Hegarty NJ, Colombo JR, Haber G-P, Chung BI, et al. Second prize: preliminary experience with the Niris optical coherence tomography system during laparoscopic and robotic prostatectomy. J Endourol 2007;21:814-8. [CrossRef]
7. Chitchian S, Weldon TP, Fiddy MA, Fried NM. Combined image-processing algorithms for improved optical coherence tomography of prostate nerves. J Biomed Opt 2010;15:046014. [CrossRef]
8. Sunderland S. The anatomic foundation of peripheral nerve repair techniques. Orthop Clin North Am 1981;12:245-66.
9. Lee SK, Wolfe SW. Peripheral nerve injury and repair. J Am Acad Orthop Surg 2000;8:243-52. [CrossRef]
10. Martorana E, Rocco B, Kaleci S, Pirola GM, Bevilacqua L, Bonetti LR, et al. Does topical hemostatic agent (Floseal®) have a long-term adverse effect on erectile function recovery after nerve-sparing robot-assisted radical prostatectomy? Int Urol Nephrol 2017;49:1519-26. [CrossRef]
11. Durand M, Jain M, Aggarwal A, Robinson BD, Srivastava A, Smith R, et al. Real-time in vivo periprostatic nerve tracking using multiphoton microscopy in a rat survival surgery model: a promising pre-clinical study for enhanced nerve-sparing surgery. BJU Int 2015;116:478-86. [CrossRef]
12. Yadav R, Mukherjee S, Hermen M, Tan G, Maxfield FR, Webb WW, et al. Multiphoton microscopy of prostate and periprostatic neural tissue: a promising imaging technique for improving nerve-sparing prostatectomy. J Endourol 2009;23:861-7. [CrossRef]
13. Tan G, Herman M, Yadav R, Mukherjee S, Maxfield F, Webb W, et al. Imaging periprostatic tissue architecture with mulitphoton microscopy and second-harmonic generation to improve potency outcomes during nerve-sparing radical prostatectomy: a pilot feasibility study. Cancer Res 2009;3(3Suppl1):S33.
14. Fried NM, Rais-Bahrami S, Lagoda GA, Chuang Y, Burnett AL, Su LM. Imaging the cavernous nerves in the rat prostate using optical coherence tomography. Lasers Surg Med 2007;39:36-41. [CrossRef]
15. Tewari AK, Shevchuk MM, Sterling J, Grover S, Herman M, Yadav R, et al. Multiphoton microscopy for structure identification in human prostate and periprostatic tissue: implications in prostate cancer surgery. BJU Int 2011;108:1421-9. [CrossRef]
16. Herman M, Grover S, Srivastava A, Sooriakumaran P, Sterling J, Shevchuk M, et al. 941 fresh cadaveric tissue from human organ donors allows imaging of normal prostate and periprostatic tissue using multiphoton microscopy. J Urol 2011;185:e377-8. [CrossRef]
17. Rai S, Srivastava A, Sooriakumaran P, Tewari A. Advances in imaging the neurovascular bundle. Curr Opin Urol 2012;22:88-96. [CrossRef]
18. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence tomography. Science 1991;254:1178-81. [CrossRef]
19. Rais-Bahrami S, Levinson AW, Fried NM, Lagoda GA, Hristov A, Chuang Y, et al. Optical coherence tomography of cavernous nerves: a step toward real-time intraoperative imaging during nerve-sparing radical prostatectomy. Urology 2008;72:198-204. [CrossRef]
20. Chitchian S, Fiddy M, Fried NM. Wavelet denoising during optical coherence tomography of the prostate nerves using the complex wavelet transform. Conf Proc IEEE Eng Med Biol Soc 2008;2008:3016-9. [CrossRef]
21. Chitchian S, Weldon TP, Fried NM. Segmentation of optical coherence tomography images for differentiation of the cavernous nerves from the prostate gland. J Biomed Opt 2009;14:044033. [CrossRef]
22. Tuttle JB, Steers WD. Fiberoptic imaging for urologic surgery. Curr Urol Rep 2009;10:60-4. [CrossRef]
23. Peng Q, Berg K, Moan J, Kongshaug M, Nesland JM. 5-Aminolevulinic acid-based photodynamic therapy: principles and experimental research. Photochem Photobiol 1997;65:235-51. [CrossRef]
24. Pisipati S, Ali A, Mandalapu RS, Haines Iii GK, Singhal P, Reddy BN, et al. Newer concepts in neural anatomy and neurovascular preservation in robotic radical prostatectomy. Indian J Urol 2014;30:399-409. [CrossRef]
25. Boyette LB, Reardon MA, Mirelman AJ, Kirkley TD, Lysiak JJ, Tuttle JB, et al. Fiberoptic imaging of cavernous nerves in vivo. J Urol 2007;178:2694-700. [CrossRef]
26. Ponnusamy K, Sorger JM, Mohr C. Nerve mapping for prostatectomies: novel technologies under development. J Endourol 2012;26:769-77. [CrossRef]
27. Gupta AD, Han M. Imaging guidance in minimally invasive prostatectomy. Urol Oncol 2011;29:343-6. [CrossRef]
28. Lopez A, Zlatev DV, Mach KE, Bui D, Liu JJ, Rouse RV, et al. Intraoperative Optical Biopsy during Robotic Assisted Radical Prostatectomy Using Confocal Endomicroscopy. J Urol 2016;195:1110-7. [CrossRef]
29. Thiberville L, Moreno-Swirc S, Vercauteren T, Peltier E, Cavé C, Bourg Heckly G. In vivo imaging of the bronchial wall microstructure using fibered confocal fluorescence microscopy. Am J Respir Crit Care Med 2007;175:22-31. [CrossRef]
30. Heidenreich A, Bellmunt J, Bolla M, Joniau S, Mason M, Matveev V, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. Eur Urol 2011;59:61-71. [CrossRef]
31. Ouzounov DG, Rivera DR, Williams WO, Stupinski JA, Southard TL, Hume KH, et al. Dual modality endomicroscope with optical zoom capability. Biomed Opt Express 2013;4:1494-503. [CrossRef]