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<td><strong>Author(s)</strong></td>
<td>Sivasubramanian, Kathyayini; Periyasamy, Vijitha; Austria, Dienzo Rhonnie; Pramanik, Manojit</td>
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Photoacoustic cystography using handheld dual modal clinical ultrasound photoacoustic imaging system

Kathyayini Sivasubramanian, Vijitha Periyasamy, Dienzo Rhonnie Austria, and Manojit Pramanik*

School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459

ABSTRACT

Vesicoureteral reflux is the abnormal flow of urine from your bladder back up the tubes (ureters) that connect your kidneys to your bladder. Normally, urine flows only down from your kidneys to your bladder. Vesicoureteral reflux is usually diagnosed in infants and children. The disorder increases the risk of urinary tract infections, which, if left untreated, can lead to kidney damage. X-Ray cystography is used currently to diagnose this condition which uses ionising radiation, making it harmful for patients. In this work we demonstrate the feasibility of imaging the urinary bladder using a handheld clinical ultrasound and photoacoustic dual modal imaging system in small animals (rats). Additionally, we demonstrate imaging vesicoureteral reflux using bladder mimicking phantoms. Urinary bladder imaging is done with the help of contrast agents like black ink and gold nanoparticles which have high optical absorption at 1064 nm. Imaging up to 2 cm was demonstrated with this system. Imaging was done at a framerate of 5 frames per second.

Key words: Photoacoustic imaging, clinical imaging system, photoacoustic cystography, handheld imaging

1. INTRODUCTION

There are many urinary bladder disorders such as urinary incontinence vesicoureteral reflux, cystitis, glomerulation, bladder cancer, which can be monitored with urinary bladder imaging [1-3]. These diseases vary in severity from causing minor discomfort to being fatal. Vesicoureteral reflux (VUR) is the reverse flow of urine from the bladder to the kidney through the ureters. Urine usually flows from the kidney to the bladder through the ureters. The valve present at the junction of the ureters and bladder prevents the reverse flow. When reflux occurs, urine from the bladder moves to the kidney causing further infection, scarring etc. The reflux occurs while voiding urine or at rest [4]. While majority of the urinary tract infections occur in women more frequently, bladder cancer occurs more commonly in men. The cancer begins on the wall of the bladder and eventually spreads, which is fatal to the patient. Therefore, imaging the urinary tract (ureter, bladder and urethra) is very important for early diagnosis [1, 5].

Some of the common clinical bladder imaging techniques include cystography, cystourethroscopy, ultrasound imaging and, optical imaging. Cystography is the gold standard for bladder imaging to evaluate bladder cancer, VUR, bladder polyps, hydronephrosis, etc. In cystography, a radio opaque contrast is injected into the bladder using urinary catheter and X-ray images of the bladder are obtained before, during, and after voiding to diagnose bladder related diseases [6, 7]. The sensitivity of the technique is not very high and injection of ionizing radiations increases the discomfort of the patient [8]. Cystourethroscopy (CUSC) is another commonly used imaging modality to diagnose and follow-up on the urinary bladder wall and the urethra. Ultrasound (US) imaging is used for bladder imaging very frequently. US imaging allow higher imaging depths compared to CUSC, and are used to image the lower urinary system in women to detect genuine stress urinary incontinence. Additionally, US imaging also gives information of bladder wall lesion and bladder cancer [9]. Optical imaging such as multiphoton microscopy, fluorescence was used to study urinary bladder [10-12].
Currently used bladder imaging techniques have several limitations including use of ionising radiation, invasive imaging, shallow imaging depth, poor resolution, and inadequate sensitivity. Therefore, a non-invasive, safe and better imaging alternative is required. Photoacoustic imaging (PAI) is a fast growing imaging technique. It combines the rich optical contrast with high ultrasound resolution [13-20]. It can be easily made into multimodal imaging platform by combining with other imaging techniques like ultrasound imaging, and fluorescence imaging. PAI is being looked into as an option for bladder imaging as its non-invasive and non-ionizing in nature [21]. PAI uses a pulsed laser as the excitation source; after absorption of light by the chromophores (tissues), a small temperature rise occurs. The rise in temperature causes thermoelastic expansion, which generates pressure waves. The pressure waves are detected with an ultrasound transducer and images are formed by reconstructing the data. PAI uses intrinsic contrast agents from the body (blood, melanin, water etc.) or extrinsic contrast agents like organic dyes and nanoparticles [22-28]. For bladder imaging the urine does not provide any intrinsic contrast, therefore extrinsic contrast agents are needed. Dyes like methylene blue and indocyanine green (ICG) have been used for bladder imaging [29]. Vasculature of urinary bladder is imaged using photoacoustic microscopy [30, 31].

These studies were done with laboratory photoacoustic imaging systems using single element transducers for image acquisition. The clinical translatability of this is very less. The first clinically compatible PA-US system reported more than a decade ago by Erpelding et al. This system did not use a clinical ultrasound platform available commercially [32]. Thus, making the system hard to replicate. Also, since the ultrasound machine is not FDA approved, the clinical translation is limited. There is a growing need to demonstrate PA-US imaging of bladder in the commercial clinically approved ultrasound imaging system with accessibility to data. Only in the last few years, commercial clinical ultrasound machines with access to raw channel data is available. Availability of raw channel data very essential for acquiring and reconstructing photoacoustic images. Therefore, only recently it has become feasible for researcher to use clinical ultrasound and photoacoustic imaging together [33]. With these systems PAI can be easily translated to clinics. Clinical ultrasound systems with combined light delivery and image acquisition are being explored for various biological applications recently [33-36].

In this study, real-time photoacoustic imaging of urinary bladder was combined with ultrasound imaging to obtain structural and functional information. A clinical dual mode ultrasound and photoacoustic imaging system was used for bladder imaging for the first time. For deep tissue imaging, 1064 nm light was used as the photoacoustic excitation source. Contrast agent (black ink) was injected into the urinary bladder to show the structural PA imaging. Sagittal and transverse sections of urinary bladder were shown in PA and ultrasound modes up to a depth of 2 cm.

2. METHODS

For photoacoustic imaging, the excitation is provided by the light from a frequency doubled nanosecond pulsed Nd:YAG pump laser (Continuum, Surelite Ex, San Jose, California, USA). The laser produces 5 ns pulses at a pulse repetition frequency of 10 Hz. A dichroic mirror was used to transmit 1064 nm wavelength light and reflect light at the wavelength of 532 nm. Light at 1064 nm was coupled to the tissue through a bifurcated optical fiber bundle (Ceramoptec GmbH, Germany). The fiber bundle contains 1600 optical fibers (numerical aperture of 0.22) which are bifurcated in the middle to spread equally over two rectangular areas of 4 cm X 0.1 cm. For data acquisition, an FDA approved clinical ultrasound system (E-CUBE 12R, Alpinion, South Korea) was used which can acquire photoacoustic and ultrasound images simultaneously [37]. For data acquisition a clinically compatible linear array ultrasound transducer was used. The L3-12 linear array transducer has 128 elements with a center frequency of 8.5 MHz and 95% fractional bandwidth with an active area of 3.85 X 1 cm. The two output ends of the fiber bundles were fitted into the custom designed fiber holder along with the linear array transducer into the designated slots of the holder to form the handheld probe. The fitting was made in such a fashion to ensure that the fiber to tissue distance was roughly 1 cm; the fiber to transducer distance was approximately 2 cm and a light delivery angle to the tissue at 15’ [38]. Ultrasound gel was used to ensure coupling. Figure 1(a) shows the schematic representation of the experimental set-up.
For combined US and PA imaging the clinical E-CUBE imaging system needs to be operated in the combined US and PA mode. The data acquisition can be done in different formats including radiofrequency (RF), beamformed, scan converted, or I.Q. data. This along with other parameters like depth of imaging, number of frames to be saved etc. can be specified through programming in python script. For all experiments the data was saved and stored in beam-formed datatype. The imaging system has only 64 parallel DAQ hardware, i.e., data can be collected only from 64 channels for every laser pulse fired. Data acquisition gets initiated only when the trigger from the laser is given for each laser pulse to the E-CUBE system. Therefore, in order to collect data from all the 128 channels two laser pulses are required. The system then combines the data obtained from the 128 channels to form on B-scan image. Therefore, the effective frame rate was 5 frames per second.

![Diagram of the combined ultrasound photoacoustic imaging system](image)

Figure 1: (a) Schematic representation of the combined ultrasound photoacoustic imaging system, (b) Rat body showing the sagittal and the transverse imaging planes.

Animal experiments were performed in accordance with the approved guidelines and regulations, and were approved by the Institutional Animal Care and Use Committee of Nanyang Technological University, Singapore (Animal Protocol Number ARF-SBS/NIE-A0263). Healthy adult male Sprague Dawley rats of weight 225±25 g (aged 8-10 weeks) were obtained from InVivos Pte. Ltd., Singapore. The rats were anesthetized using a mixture containing Ketamine and Xylazine of dosage of 85 and 15 mg/kg, respectively before experimentation. 0.2 mL of the mixture per 100 g of the rat body weight was administered by an intraperitoneal injection. Hair from the abdominal area of the rat was removed using commercial depilatory cream before imaging. A 23G urinary bladder catheter was inserted through the urethra and was secured with tissue glue to prevent leakage. During experiments, animal was maintained under anaesthesia with 0.75% of isoflurane gas (Medical Plus Pte Ltd, Singapore) along with oxygen (1.2 L/min). The mixture was delivered through a nose cone wrapped with a breathing mask covering the mouth and nose of the animal completely. Peripheral oxygen saturation and the heart rate of the animal was monitored continuously throughout the experiments using a pulse oximeter (Medtronic, PM10N with veterinary sensor, Minneapolis, Minnesota, USA). The animal was placed in a supine position and the combined US + PA images were recorded with the handheld probe. After image acquisition, the animal was euthanized with an overdose of pentobarbital. The planes of imaging (sagittal and transverse) are shown in Fig. 1(b).

A rectangular chicken tissue slice of approximately 1 cm in thickness was placed upon the abdominal region of the rat. The PA contrast agent that was used for the experiments was black ink, and saline was used as control. Combined US-PA image of the bladder was taken before and after injection of dye or saline in both the transverse and the sagittal plane. 0.8 mL of black ink or saline was injected into the bladder through the catheter. After 10 minutes, the bladder was imaged in both the planes. Once, the PA signal from the bladder was clearly visible, depth imaging was performed. For depth, imaging chicken tissue slices of 0.5 cm thickness was stacked one by one until a depth of 2 cm. Combined US and PA images were obtained and stored in both the transverse and sagittal plane.
The laser coupling efficiency of the fiber at the wavelength of 1064 nm was ~50%. The energy of light falling on the surface was ~45 mJ per pulse. The total area of illumination was ~5 cm². Therefore, the fluence was calculated to be approximately as 9 mJ/cm² on the surface. The maximum permissible energy (MPE) on skin at 1064 nm is 100 mJ/cm² according to the American National Standards Institute (ANSI) [39]. Therefore, all studies were done well within the prescribed safety limit.

3. RESULTS AND DISCUSSION

Non-invasive urinary bladder (UB) imaging is very critical and is very useful for different applications. Here, we show the structural bladder imaging with combined US and PA imaging. Black ink was used as a photoacoustic contrast agent. It was chosen because of its strong optical absorbance in the near infrared wavelength region. For experiments, 1064 nm was chosen as the imaging wavelength. Due to weak absorption of light and lower scattering, the penetration depth of light is high at this wavelength. For bladder imaging, higher imaging (penetration) depth is needed especially in humans, as the bladder is located below the abdominal muscle which is of few centimetres thickness. Saline was used as control as it does not have any photoacoustic contrast at 1064 nm. Figures 2(a) and 2(b) show the combined US-PA image of the urinary bladder before and after injection of the black ink in the transverse plane of the rat respectively. Figures 2(c) and 2(d) show the combined US-PA image of urinary bladder before and after injection of black ink in the sagittal plane, respectively. There was no change observed in the PA signal before and after injection of saline in both the planes. After injection, a strong PA signal can be observed from the top part of the bladder wall due to black ink. The PA signal from the bladder is highlighted in red. As the signal from the top part of the bladder is very strong the signal from the bottom part of the bladder gets masked. The grayscale image represents the US image and the colour is a representative of PA signal. Structural information will help in identifying bladder deformity, which can indicate to other bladder disorders.

![Combined US-PA image of the rat bladder before and after black ink injection.](image)

Figure 2: (a) Combined US-PA image of the rat bladder before black ink injection in the transverse plane, (b) Combined US-PA image of the rat bladder after black ink injection in the transverse plane, (c) Combined US-PA image of the rat bladder before black ink injection in the sagittal plane, (D) Combined US-PA image of the rat bladder after black ink injection in the sagittal plane.

In rats the bladder is located superficially (very close to the skin). However in humans, the bladder is located much deeper depending on the muscular and fat content of the person, typically 2-3 cm. Therefore, combined US-PA imaging of bladder at different depths using chicken tissue slices of different thickness is shown in both sagittal and transverse planes. Figures 3(a-d) shows combined US-PA images of the bladder at different depths of 0.5 cm, 1 cm, 1.5 cm, and 2 cm in the transverse plane. From the images, it can be noticed that the PA signal is very clear at the imaging depths of 0.5 cm and 1 cm. After 1 cm, with increase in depth the PA signal decreases. However, there is enough PA signal for imaging the bladder even at higher depths. From the stored
beam-formed images, SNR of the PA signal from the bladder was calculated at the various depths. SNR is given by SNR=(V/n, where V is the PA signal amplitude and n is the standard deviation of the background noise. 20 frames were averaged and the SNR was calculated from the averaged image. The SNR values for various depths of 0.5 cm, 1 cm, 1.5 cm, and 2 cm were 86, 95, 54 and 19 respectively. Even at 2 cm imaging depth the SNR value is approximately 20, which is sufficient for imaging. It is also to be noted that the fluence of imaging is much smaller than MPE advised by ANSI for 1064 nm. Therefore, the imaging depth can be further enhanced by increasing the laser power, and thereby increasing the fluence or by using extrinsic contrast agent with higher light absorption at 1064 nm.

Figure 3: Combined US-PA images of the rat urinary bladder at depths of 0.5 cm, 1 cm, 1.5 cm, 2 cm respectively in the transverse plane.

Here, we have discussed the prospect of using an FDA approved clinical US-PA imaging system for bladder imaging. PA imaging structural imaging of the bladder. There are a few limitations with the current format of clinical PA imaging. One is the fiber based light delivery; there is major energy loss as the coupling efficiency of the fiber is very less. Hence, there is a lot of limitation in operating the system at the maximum energy possible within the ANSI permissible limit. Higher laser energy yields better images and improved penetration depth. Better light delivery solutions are needed. Another limitation for the current imaging system is 3D imaging is not possible with a handheld system. A better 3D imaging platform may provide better information regarding the structure and function of the bladder.

4. CONCLUSION

Combined ultrasound photoacoustic clinical imaging systems are tested for various biological applications. Handheld imaging system leads to more clinically translatability. Structural urinary bladder imaging in small animals is shown with the handheld combined ultrasound and photoacoustic imaging system. Depth imaging of up to 2 cm is also demonstrated shown. Imaging of the urinary bladder can be used for imaging bladder disorders like vesicoureteral reflux, urinary incontinence, bladder cancer, etc.

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REFERENCES


K. Sivasubramanian, and M. Pramanik, “High frame rate photoacoustic imaging at 7000 frames per second using clinical ultrasound system,” Biomedical Optics Express, 7(2), 312-323 (2016).
