Cancer Imaging

Simultaneous transrectal ultrasound and photoacoustic human prostate imaging

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Imaging technologies that simultaneously provide anatomical, functional, and molecular information are emerging as an attractive choice for disease screening and management. Since the 1980s, transrectal ultrasound (TRUS) has been routinely used to visualize prostatic anatomy and guide needle biopsy, despite limited specificity. Photoacoustic imaging (PAI) provides functional and molecular information at ultrasonic resolution based on optical absorption. Combining the strengths of TRUS and PAI approaches, we report the development and bench-to-bedside translation of an integrated TRUS and photoacoustic (TRUSPA) device. TRUSPA uses a miniaturized capacitive micromachined ultrasonic transducer array for simultaneous imaging of anatomical and molecular optical contrasts [intrinsic: hemoglobin; extrinsic: intravenous indocyanine green (ICG)] of the human prostate. Hemoglobin absorption mapped vascularity of the prostate and surroundings, whereas ICG absorption enhanced the intraprostatic photoacoustic contrast. Future work using the TRUSPA device for biomarker-specific molecular imaging may enable a fundamentally new approach to prostate cancer diagnosis, prognosis, and therapeutic monitoring.

Introduction

Prostate cancer (PCa) is the most common non-skin cancer among men. More than 1.2 million cases are diagnosed worldwide each year, most often using the standard diagnostic approach in which an abnormal digital rectal exam or elevated prostate-specific antigen (PSA) in the blood prompts a transrectal ultrasound (TRUS)–guided prostate biopsy, where needles are placed blindly into the prostate because of an inability to reliably image PCa on US (1, 2). This approach leads to over-detection of indolent tumors of little clinical relevance and under-detection of some aggressive cancers (2). To address this clinical need, emerging in vitro diagnostic as well as in vivo imaging technologies have focused on detecting reliable biomarkers of PCa with high sensitivity and specificity (3–6), including three-dimensional (3D) TRUS-based imaging strategies for differentiating malignant prostate tissue using elastography (6) and angiography (7, 8). Among these, magnetic resonance imaging (MRI)–guided targeted TRUS biopsies tended to provide higher detection rate for clinically relevant PCa (9). Molecular imaging could further improve PCa care by allowing more accurate biopsies, and better assessment of tumor grade and aggressiveness, and help choose optimal management option (active surveillance, surgery, focal, or radiation therapy) for both clinically relevant and insignificant cases. Toward this goal, molecular imaging techniques such as hyperpolarized 13C MRI for mapping metabolic changes of PCa (10) and positron emission tomography (PET) radiotracers for targeting PCa biomarkers (prostate-specific membrane antigen) (11) are being translated and evaluated in the clinic. However, MRI and PET are not suitable for frequent screening, monitoring, or real-time biopsy guidance due to their limited availability, high cost, and use of ionizing radiation in PET.

TRUS is nonionizing, inexpensive, portable, and widely available and is the current gold standard for guiding prostate biopsy. Although TRUS alone is not sufficient for reliable imaging of PCa, it is an ideal platform to integrate relevant molecular imaging strategies that could improve PCa visibility. Photoacoustic imaging (PAI) is a quintessential nonionizing method to pair with TRUS because they both share the same detection platform, and PAI provides complementary functional and molecular optical contrasts of deep tissue (up to 12 cm) with a submillimeter ultrasonic spatial resolution (12, 13). Hemoglobin absorption enabled high-contrast PAI of blood vasculature, associated angiogenesis, oxygen saturation, and total hemoglobin concentration (13–16); moreover, PAI molecular imaging strategies that specifically target cancer biomarkers have been demonstrated to improve both diagnostic sensitivity and specificity in preclinical cancer models (17, 18). Over the past decade, PAI has evolved as a multiscale imaging technology, enabling in vivo imaging of structures ranging from organelles to organs (13), and has been translated to clinical studies by adapting existing clinical US devices for breast (19–21) and ovaries (22) to simultaneously enable PAI by attaching light guides to these devices. PAI studies on prostate had long been limited to animal imaging (23–25), such as imaging of implanted brachytherapy seeds inside the canine prostate (25); these were recently extended to clinical prostate imaging, wherein a single-wavelength (756 nm) PAI was performed for identifying a neurovascular bundle during invasive radical prostatectomy (26) and for imaging angiogenesis of prostate tumors during transrectal imaging of three patients with PCa (27, 28). Although these studies are encouraging, developing a transrectal device that compactly integrates...
Both US and optical components for in vivo deep-tissue molecular-specific multispectral PAI of the prostate is a key challenge.

Here, we report an integrated spectroscopic TRUS and PA (TRUSPA) device built using a relatively new class of miniaturized capacitive micromachined ultrasonic transducer (CMUT) arrays. We fully characterized the instrument and validated using tissue-mimicking phantoms, in vivo mouse models of PCa, ex vivo intact human prostates, and in vivo human prostate transrectal imaging \( n = 20 \), including first-in-man contrast-enhanced prostate imaging using intravenous administration of the U.S. Food and Drug Administration (FDA)–approved indocyanine green (ICG) contrast agent \( n = 10 \). Compared to the wide use of piezoelectric transducers in conventional US imaging, our CMUTs are designed and fabricated in-house using microelectromechanical systems \( 29, 30 \), and offer advantages such as wide bandwidth, improved signal-to-noise ratio (SNR) due to direct or proximal bonding with application-specific integrated circuits (ASICs), ease of fabricating large 1D (linear) as well as 2D arrays with 500 \( \mu \)m thickness \( 31–36 \), and high PA depth sensitivity \( 37 \).

**RESULTS**

We developed the TRUSPA device by tightly integrating a custom-made fused silica-silica fiber optic light guide and a custom-designed printed circuit board (PCB) that bonds a linear CMUT array to four ASICs (Fig. 1 and figs. S1 and S2). Design, description, and characterization of the CMUT array \( 64 \) elements, 5-MHz center frequency) are presented in table S1 and figs. S3 and S4. A gray-colored polydimethylsiloxane (PDMS) lens coating on the CMUT array provides electrical insulation, mechanical stability, and elevation focusing (fig. S5) \( 38 \). As shown in fig. S1, a tunable nanosecond laser (Opotek Inc., 10-Hz pulse repetition rate, 5-ns pulse width, 680- to 950-nm wavelength range) was coupled to the fiber optic bundle of the TRUSPA device to deliver light deep into the prostate from different angles \( 39 \). A PC-based US imaging platform (Verasonics Inc.) was synchronized with the laser firing for an interleaved US and PA data acquisition and reconstruction using delay-and-sum beamforming (fig. S6). The TRUSPA system displays B-mode US (grayscale), PA (red color scale), and co-registered US and PA images in real time at 10 frames per second (fps) (movie S1).

**Evaluation of the integrated TRUSPA system**

Analysis on pulse-echo measurements from the PDMS-air interface for all 64 CMUT elements demonstrated that 6 elements lost wirebonding contact during the PDMS encapsulation process, and that there was \( \leq 1\% \) variation in the PDMS thickness across all CMUT elements (fig. S5). We characterized the US field of the TRUSPA device using both Field II simulations \( 40 \) and a calibrated hydrophone (Onda HNP-0400) and measured \( \approx 2.5 \) MPa output focal pressure at the optimal bias voltage settings of 90-V DC and 30-V AC (figs. S7 and S8). Analysis of pulse-echo reflections from a flat metal target in immersion demonstrated that the TRUSPA device has a center frequency of 5 MHz and a 6-dB fractional bandwidth of about 80% (fig. SSD). The TRUSPA device provided optical fluence of \( \approx 10 \) mJ/cm\(^2\) on the tissue surface, which is well within the American National Standards Institute (ANSI) safety limit of 30 mJ/cm\(^2\) at 800-nm wavelength \( 41 \). We evaluated the deep-tissue imaging capabilities of the TRUSPA device using a variety of biological tissue environments, including surgically removed human prostates. First, we demonstrated high geometric uniformity and co-registration accuracy of US as well as PA modes of the TRUSPA device by imaging a custom-made structural phantom (Fig. 2). The 10% intralipid, 1% agar, and 0.1% India ink–based phantom consisted of nine fishing wire targets (0.3 mm diameter) placed on and off axis at different depths and orientations inside the homogeneous background mimicking optical properties of prostate tissue with absorption and reduced scattering coefficients, respectively \( \mu_a = 0.1 \) cm\(^{-1}\) and \( \mu_s' = 10 \) cm\(^{-1}\) (Fig. 2, A and B) \( 42 \). All wires generated US signal due to acoustic impedance mismatch with respect to the background, although some wires that were painted black absorbed photons and generated a PA signal (Fig. 2, C to E). We then imaged the phantom through a \( \approx 2.5 \)-cm-thick porcine tissue (boneless pork loin), to further increase scattering and heterogeneity, and demonstrated that all wire targets could still be imaged (Fig. 2, F to H). PA
data analysis on wire targets 1 and 9, at depths of 37 and 52 mm, respectively, from the surface of the porcine tissue, demonstrated SNRs of ~22 and 13 dB, axial resolutions of 0.32 and 0.34 mm, and lateral resolutions of 0.8 and 1.1 mm, respectively (Fig. 2, I to L).

Next, using fiducial tubes of blood or ICG placed in the mid (through the urethral opening) and anterior regions of excised human prostates, we demonstrated that the TRUSPA device can generate high-contrast and high-resolution PA images of hemoglobin and ICG molecules in the human prostate background, whereas US images displayed poor contrast of these fiducial tubes (Fig. 3). Table S2 summarizes the key TRUSPA imaging parameters quantified from US and PA signals in Figs. 2 and 3. In Fig. 3 (K to N), we present an example of ex vivo imaging of PCa on surgically removed human prostate obtained from a patient who underwent a radical prostatectomy as a routine standard of care. As shown in Fig. 3M, this patient had a fair amount of disease (1.1 cm, PIRADS 4) in the right lateral peripheral zone (PZ). This prostate was imaged using the TRUSPA device, and the malignant region was specifically targeted during the scanning. As shown in Fig. 3L, the co-registered PA/US image at 800 nm showed the boundary of the tumor mass (white circled region) in the right lateral PZ on the US image (grayscale) and strong PA contrast of ~20 dB, which was not present when scanned through other regions of the prostate. We imaged 20 surgically removed prostates to identify distinct PA features of PCa. Because there was a lot of blood loss from the prostatectomy, it was difficult to distinguish the malignant region in excised human prostates based on intrinsic PA contrast alone unless the tumor(s) was preidentified on MRI and appeared hypoechoic on US at that location, as in Fig. 3L. To further evaluate the TRUSPA system SNR as a function of imaging depth and laser wavelength, we imaged an Eppendorf tube of 8 mm diameter filled with ICG solution (1 mg/ml) at different depths (up to 5 cm) inside chicken breast tissue (fig. S9). These results demonstrated a linear decrease (~10 dB/cm) in the system SNR, ~34 dB at 18 mm to ~13 dB at 47 mm. The ICG spectral profiles from three different depths (18, 32, and 47 mm) demonstrated a decaying PA signal after 800 nm, similar to the standard ICG molar extinction spectrum. In future, a model-based fluence correction that accounts for depth and wavelength dependence will likely help in achieving higher spectral accuracies at deeper depths from the surface (beyond 30 mm) needed for robustly quantifying the ICG distribution and perfusion. In addition, the PA contrast beneath the ICG tube in fig. S9 (I and L) was most likely due to blood absorption in the chicken tissue that shows similar PA intensity levels as the ICG tube, because of the higher optical fluence present at lower depths than at the depth of the ICG tube.

In the next step, we studied the in vivo detection of ICG by administering ICG solution (50 μl at 2.5 mg/ml) intravenously into mice (n = 5) bearing subcutaneous PCa (PC3 cells) tumors and performed a simultaneous US and spectroscopic (multiwavelength) PAI.
using the TRUSPA device. US images showed the tumor boundary and other anatomical structures of the mouse, whereas the PA images (co-registered with the US) showed uptake of ICG dye in the tumor vasculature and inside the tumor region (Fig. 4). Spectral plots of pre- and post-ICG imaging, obtained from quantified mean PA contrast as a function of wavelength for five regions of interests (ROIs; R1, R2, R3, R4, and R5 as defined in Fig. 4O) on multiwavelength pre- and post-ICG PA images (fig. S10), showed distinct trends, similar to a standard venous blood absorption and a mixture of blood and ICG spectra, respectively (Fig. 4, F to I). Furthermore, spectral unmixing on the multiwavelength PA data acquired during the pre- and the post-ICG injection periods (fig. S10) distinguished...
PA contrast originating from Hb, HbO₂, and the ICG molecules (Fig. 4, J to O). This difference in the trends between pre-ICG and post-ICG was used as one of the benchmarks to evaluate the presence of intravenously administered ICG during the TRUSPA imaging of human prostate. Although the tumor region (encircled region) was found to be hypoechoic on US, all PA contrast observed before ICG injection was likely due to absorption of hemoglobin present in the blood within vasculature of the tumor and surrounding regions. Comparison
of image contrast in the encircled tumor region in all unmixed images (Fig. 4, J to O) shows that ICG dye was taken up and retained inside the tumor region and surrounding vasculature, with a fourfold improvement in the PA contrast after ICG injection. The PA contrast and spectral profiles of pre- and post-ICG injection observed at deeper regions (ROI R5 at 35-mm depth shown in fig. S10, A and B) demonstrates the high optical sensitivity and spectral profiling capabilities of the TRUSPA device.

**Pilot clinical TRUSPA imaging studies**

With previous approval from the Stanford Institutional Review Board (IRB), we collected in vivo transrectal images of human prostates ($n = 20$) in US and PA modes of the TRUSPA device, including 10 subjects who received intravenous ICG during the TRUSPA imaging of the prostate. In vivo results demonstrated the capability of the device to simultaneously display anatomical information on the US, functional and molecular information on PA, and co-registered US + PA images of the prostate in real time at 10 fps (movie S1). The US images from the TRUSPA provided the sagittal anatomy of the prostate and surrounding tissue to a ~6-cm depth from the rectal wall with the ±20° field of view (FOV) (Fig. 5). From the PA images alone, the origin of optical contrast within the prostate remained unclear. However, the co-registered US + PA images, with overlaid anatomical and PA contrasts of prostate, demonstrated high PA contrast from dense vasculature adjacent to several prostatic structures. Neurovascular bundles in the posterior region (Fig. 5A), the dorsal venous complex that spans the prostate capsule, PZ, transitional zone (TZ), the anterior zones (AZs) of the prostate (Fig. 5B), the seminal vesicles (Fig. 5B), and regions of suspicious hypoechoic mass (Fig. 5C) were identified. In Fig. 6, we present in vivo TRUSPA imaging results from a patient with proven PCa as evidenced on preoperative PET-MRI, using 68-Ga-labeled PET tracer targeting of bombesin on the PCa cells (43), and followed by PET-MRI contrast-targeted biopsy (with MRI-TRUS fusion) of the prostate using the conventional TRUS device. In agreement with the PET-MRI results, the TRUSPA device displayed a distinct PA contrast from the right peripheral base of the prostate, which was not present when scanned through other prostatic regions of this patient (movie S1).

The final imaging experiments involved contrast-enhanced TRUSPA imaging of human prostates using intravenous ICG (2.5 mg/ml) in the dose range of 5 to 75 mg ($n = 10$; table S3). In Fig. 7, we present in vivo TRUSPA prostate imaging results after intravenous ICG (25 mg dose) in a patient with biopsy-proven cancer in the left peripheral base of the prostate. To better evaluate the nature of ICG time activity, we applied principal components analysis (PCA) on the mean PA values of the 60-grid ROIs (fig. S11B) defined on each 800-nm PA image acquired during pre- and post-ICG injection period. The coefficient of the first PCA that accounts for 83% of the total variance in PA contrast as a function of time (relatively to ICG injection) showed the time-activity curve with an average ICG arrival time of about 2.5 min and washout time of about 6 min after ICG injection, except from the left base of the prostate (Fig. 7G and fig. S11J). These plots also show that the time activity is relatively higher in the left base of the prostate (having malignant region covered by ROIs 13 to 18 and 24 to 26) with 20% increase in the intraprostatic PA contrast after ICG injection. In addition, the spectral plots of mean PA contrast versus wavelength (Fig. 7H), using multiwavelength (750 to 950 nm, 25-nm step size) PA data acquired before and after intravenous ICG injection (fig. S12), showed different trends for pre- and post-ICG imaging, consistent with intravenous ICG results on mice models of PCa (Fig. 4). To further validate the presence of ICG within the prostate, we performed spectral unmixing of ICG from the Hb and HbO2 using the multiwavelength PA data (fig. S12). The unmixed post-ICG image showed a distinct PA contrast with ~10% contrast enhancement from the left base of the prostate at peak ICG arrival time, with respect to the unmixed pre-ICG image (Fig. 7, I and J). A null hypothesis test on the contrast change between post- and pre-ICG unmixed images, defined as the ICG slope, of seven patients who underwent intravenous ICG (25 to 75 mg) resulted in statistically significant changes ($P = 0.009$) (Table 1). The ICG slope trend is like that of unmixed 800-nm image slope (the peak absorption wavelength of ICG) with $P = 0.578$, but not to that of unmixed 950-nm slope with
P = 0.110 due to negligible absorption of ICG at 900-nm wavelength. Average percentage increases of 36 ± 22 for the 800-nm measurement and 9 ± 6.5 for unmixed ICG measurements were calculated (n = 7 patients). Furthermore, we observed a greater ICG contrast for patients with higher ICG doses of up to 75 mg (fig. S13). For the patient with the 5-mg dose, we observed negligible time activity of ICG and contrast improvement in the unmixed ICG image (fig. S14).

**DISCUSSION**

We report the development and the initial clinical use of an integrated TRUSPA device capable of simultaneously imaging US-based prostatic anatomy and PA-based functional and molecular optical contrasts in human subjects. Compact bonding of the CMUT array to the ASICs not only improved the noise floor but also provided enough room around the array to optimally distribute fiber optic cables for delivering light deep into the prostate from different angles. High receive sensitivity and near 100% fractional bandwidth of the CMUTs (31–36) further contributed to the high PA sensitivity observed in our TRUSPA experiments. A compact TRUSPA architecture that tightly integrates both optical and ultrasonic components is required given the space constraints for tranrectal imaging of the prostate. Deep prostate PAI is not easily achieved by attaching a fiber optic light guide to the existing clinical TRUS devices that use lead zirconate titanate (PZT) arrays. Our approach overcame challenges such as lower depth and molecular sensitivity of previous PAI studies (26–28) and demonstrated multispectral imaging of intrinsic and extrinsic molecular PA contrast in the range of 3 to 4 cm inside the human prostate. Wider FOV and finer structural details seen on clinical TRUS devices (due to large aperture size, higher number of elements, and higher center frequency of curvilinear PZT array) can be extended to future TRUSPA designs by using a linear/curvilinear CMUT array of comparable parameters (31–33). Furthermore, highly sensitive and large 2D (matrix) CMUT arrays could be implemented for real-time 3D imaging of prostate, covering both sagittal and axial views at the same time.

In pilot clinical studies involving several patients, the real-time co-registration of US and PA images allowed simultaneous mapping of local optical absorption of hemoglobin contrast in PA images to several prostatic structures identified on US images, such as seminal vesicles, neurovascular bundles, dorsal venous complex, and prostate capsule. Although contrast-enhanced ultrasonic techniques such as Doppler US are capable of indirectly mapping blood vessels, these techniques based on blood flow velocity have poor spatial resolution and lower sensitivity compared to PAI, which has demonstrated high sensitivity to detect single blood cells (44). For one patient with advanced PCa, TRUSPA demonstrated a distinct intrinsic PA contrast from the malignant region of the prostate, confirmed by simultaneous PET-MRI and subsequent targeted biopsy results. We also studied ICG contrast-enhanced prostate imaging as the best possible first clinical step toward understanding the extrinsic molecular PA sensitivity of TRUSPA in humans. Using plots of time activity (PA contrast at 800 nm versus time), multispectral analysis (PA contrast versus wavelength), and spectrally unmixed ICG imaging, we observed dose-dependent (up to 75 mg total at 2.5 mg/ml) PA contrast enhancement of prostate. Comparison of spectrally unmixed images of Hb, HbO2, and ICG in clinical and preclinical studies showed that the origin of the PA contrast in ICG images can be attributed to vascular space, because ICG rapidly binds to plasma proteins in the vasculature (45–47). Although the ICG activity and contrast in the tumor region (left base) is distinct compared to other regions (apex and anterior) for the patient case presented in Fig. 7, because of increased blood flow to prostate tumors than surroundings (48), future studies on a large number of patients with different types and grades of PCa would further help to elucidate the differential uptake of ICG in the malignant region. The maximum (75 mg) dose of ICG used in this study is well below the clinically permissible (2 mg/kg) and the highest dose previously injected (5 mg/kg) with no toxicity in humans (49–52). For further comparison, this dose is also quite lower than the average dose (10 mg/kg) of MRI contrast agents in humans (10, 53). Although increasing the ICG dose would further enhance the intraprostatic contrast and spectral ICG results, PA molecular imaging approaches that specifically target PCa biomarkers such as bombesin and prostate-specific membrane antigen (PSMA) (11, 18, 43), when integrated into the TRUSPA device, could improve the specificity of targeted biopsy and the ability to predict the prognosis and help induce focal therapy. There is a high potential for further improving the molecular sensitivity and depth of penetration.
(beyond 5 cm) of TRUSPA using a suitable combination of the following approaches: clinically translatable small molecules or nanoparticles with higher PA contrast than ICG (54), a model-based fluence correction for better spectral accuracy (55, 56), using more sensitive and larger CMUT (or other ultrasonic) arrays, advanced beamforming techniques such as spatial compounding (57), increasing light throughput from the current fluence of ~10 mJ/cm² to the ANSI safety allowing fluence of 30 mJ/cm² at 800 nm or 100 mJ/cm² at 1064 nm (41), and delivering light through the urethra (58). The effect of motion artifacts on the PA and US image quantitation using our TRUSPA probe will be also characterized in future studies.

Our present pilot clinical study has some limitations. First, we imaged a small number of patients (n = 20; 10 without and 10 with intravenous ICG). More than 50% of cases had unknown cancer status, as their first TRUS-guided biopsy was performed after the TRUSPA imaging. Future clinical studies will image a large cohort of patient subjects with clinically relevant and insignificant PCa to further understand the diagnostic usefulness of TRUSPA measurements using robust statistical analysis. Second, the pathology results and the corresponding TRUSPA images of a patient might not be completely matched. However, given that the major pathological findings did not change from specimen to specimen within the tumor...
The objectives of this prospective study were to develop, validate, and then clinically translate the TRUSPA device that uses miniaturized CMUTs for human prostate imaging, and primarily investigate the intraprostatic PA contrast without and with the use of intravenous ICG injection in humans. The first set of validations studies was designed to test the deep-tissue imaging capabilities of the device using different tissue-mimicking phantoms as well as surgically removed intact human prostates \((n = 20)\) from patients who underwent radical prostatectomy as a routine standard of care. In the next step, using mouse models of PCa \((n = 5)\), the intravenous ICG imaging capabilities of the device were tested. These mouse studies were also designed to help test our quantitative spectral plotting and spectral unmixing methods using pre- and post-ICG injection multispectral PA data. The pilot clinical studies involving patients with PCa \((n = 20)\); in which \(n = 10\) with intravenous ICG) were designed to test the ability of the device to simultaneously image anatomical and vascular (including vascular perfusion) contrasts of the prostate in the US and PA modes of the device, respectively. The results of our initial trial are presented here as a feasibility study. The other study designs, along with methods, are provided in the following sections. All experiments involving humans and animals were approved by the IRB and Administrative Panel on Laboratory Animal Care, respectively, of the Stanford University. Informed consent was obtained from all participating patients.

### Mouse PCa models

About 5 million PC3 cells [American Type Culture Collection (ATCC)] transfected with a lentivirus expressing both a green fluorescent protein (eGFP) and bioluminescent reporter gene Luciferase (Luc2) were implanted subcutaneously in the lower back of nude male mice \((n = 5)\). Tumors were allowed to grow to a size of 50 to 1000 mm\(^2\) over about 3 weeks. Tumor growth and activity were monitored using bioluminescence imaging acquired by an IVIS 200 after intraperitoneal administration of substrate \(D\)-luciferin at a dose of 150 mg/kg (Biosynth).

### In vivo transrectal imaging of human prostate without and with intravenous ICG

Patients with PCa with elevated serum PSA were first imaged with the TRUSPA device immediately before routine TRUS-guided biopsy procedure. Among the 20 patients we imaged, 10 without ICG and 10 with intravenous ICG (IC-Green, Akorn), only 5 patients had biopsy-proven PCa before the TRUSPA imaging. Hence, more than 50% of the patients we imaged had their first prostate biopsy after our TRUSPA studies, and their cancer status was not known during the TRUSPA study. As in conventional TRUS, the TRUSPA device was inserted into each patient’s rectum and the prostate was visualized first in the US mode before starting the laser illumination for subsequent PA image acquisition. The device was linearly moved and/or rotated to visualize different regions of the prostate and surrounding structures such as the bladder at the urologist’s discretion. Frame grabber software (Camtasia) recorded the video of time-lapse TRUSPA frames (each frame consisting of a US, PA, and co-registered US + PA image) displayed on the computer screen. Raw US and PA data were saved for ROI identification by the urologist and reconstructed using the delay-and-sum beamforming \((59)\). Among the 10 ICG patients, the ICG dose was gradually increased from 5 mg (1 patient), 25 mg (2 patients), 50 mg (2 patients), to 75 mg (5 patients). The TRUSPA device was directed toward an ROI in the prostate, based on patient’s preoperative MRI results, and was held still throughout the pre- and post-ICG imaging. To study the time activity of ICG (arrival and washing out) inside the prostate,

### MATERIALS AND METHODS

#### Study design

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we acquired TRUSPA frames of 800 nm (the peak absorption wavelength of the ICG molecule) at multiple time points during the pre- and post-ICG sessions. To further study the presence of ICG inside the prostate—using the spectral plots and the spectral unmixing of oxyhemoglobin, deoxyhemoglobin, and ICG—we acquired multi-wavelength PA images from 750 to 950 nm in steps of 25 nm during the pre- and post-ICG time periods. The complete procedure took about 10 min.

**PA data thresholding, time gain compensation, and spectral unmixing**

**Thresholding**

The TRUSPA system SNR was studied under multiple imaging conditions including with laser ON and laser OFF conditions to understand inherent system-level noise. Our calculations showed that PA signal from a typical target in the laser ON image is more than 10 dB than the noise in the laser OFF image. We then studied the amount of noise in all our imaging conditions (phantoms, ex vivo tissue specimens, mice, and human studies) and applied thresholding on all PA images to bring them to an acceptable SNR of greater than 3 dB.

**Time gain compensation settings**

Depth-dependent time gain compensation (TGC) settings were applied for both US and PAI to compensate for the decrease in respective signal strengths with an increase in tissue depth.

**Spectral unmixing**

A nonparametric constraint-based linear spectral unmixing approach (60) was applied on multiwavelength PAI data that is compensated for both US and PAI to compensate for the decrease in respective signal difference, and two patients with high movement during the imaging.

**Statistical analysis of ICG patients**

Intraprostatic ICG activity was analyzed using pre- and post-ICG PA measurements in 7 of 10 patients. Three patients were excluded: one patient with 5-ng ICG dose with negligible pre- and post-ICG difference, and two patients with high movement during the imaging. The five mean PA measurements of prostate used for the statistical analysis were as follows: (i) 800 nm at which ICG absorption is maximum; (ii) 950 nm with minimal absorption of ICG; and unmixed images of (iii) ICG, (iv) oxyhemoglobin (HbO2), and (v) deoxyhemoglobin (Hb). The statistical analysis was performed on the difference (slope) between these respective pre- and post-injection measurements. Table 1 presents slopes (positive if increase, negative if decrease) of 800 nm, 950 nm, and unmixed ICG measurements. A total of n = 70 measurements adjusted for dose and clustering within patients (n = 7) were used in the analysis of Table 1. Two null hypotheses were tested. One null hypothesis, "is slope = 0?", provides the P value for no change in signal difference between respective pre- and post-meaures; the other null hypothesis, "is slope = ICG slope?", provides the P value for the slope of a certain measurement (800 or 950 nm) following the ICG slope.

**SUPPLEMENTARY MATERIALS**

stm.sciencemag.org/cgi/content/full/11/507/eaa2169/DC1 Materials and Methods
Fig. S1. Description of the TRUSPA imaging system next to the patient bed in the urology clinic. Fig. S2. Schematics and images that describe the TRUSPA device, operating principle, and its data acquisition. Fig. S3. Images of the CMUT array and ASIC. Fig. S4. Simulated output pressure of a CMUT cell and experimental impedance measurements of a single CMUT element.

**REFERENCES AND NOTES**

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Simultaneous transrectal ultrasound and photoacoustic human prostate imaging


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Probing the prostate

Molecular imaging can help improve detection of cancer, but some modalities require ionizing radiation, making them nonideal for repeated imaging. Kothapalli et al. developed a probe that could perform ultrasound and photoacoustic imaging simultaneously, two modalities that do not require ionizing radiation. Testing the transrectal ultrasound and photoacoustic device in vitro, in mouse models, and using excised human prostates demonstrated the ability to view anatomical features within tissue and vascular contrast within tumors. Administering a dye improved photoacoustic contrast when imaging the prostates of human subjects with cancer. This device allows for real-time anatomical, functional, and molecular imaging of the human prostate and could be easily adopted into clinical workflows.