

# 2010 PETER MICHAEL FOUNDATION PROSTATE CANCER RESEARCH REPORTS



Memorial Sloan-Kettering  
Cancer Center

## *Imaging of PSMA in Prostate Cancer and Beyond*

### ORIGINAL PROPOSAL

The overall goal of the project is to develop new tools to detect prostate cancer. Using the same imaging agent, we aim furthermore to detect cancer independent of the tumor type by their neovasculature. The objective of this proposal is to gain insights into the function of prostate specific membrane antigen (PSMA) in prostate cancer and tumor neovascularization utilizing novel imaging probes and to exploit these to monitor current and create future therapies. PSMA can provide several valuable advantages over other antigens, because i) the expression on prostate cancer and neovasculature of other tumors (and lack thereof on normal vasculature) makes it an ideal tumor target; ii) dose-dependent internalization of PSMA results in accumulation of the targeted agent in the tumor; and iii) the enzymatic activity can be used to image PSMA function with an activatable probe and to treat tumors with a prodrug.

### PROGRESS REPORT

Dr. Hebert Alberto Vargas has been appointed as the new Pelican Fellow at Memorial Sloan-Kettering Cancer Center (MSKCC) in New York. He joins the prostate imaging group at MSKCC after completing his specialist training in clinical radiology at Cambridge University Hospital (UK). The goal of Dr. Vargas' research is to evaluate pathways for integrating conventional and novel magnetic resonance imaging (MRI) techniques and clinical parameters (such as biopsy, serum or tissue biomarkers), in order to allow less invasive and yet personalized care for patients with prostate cancer, through evidence-driven diagnosis, treatment selection and treatment follow-up. He is committed to the multidisciplinary approach to cancer which is part of the philosophy at MSKCC, and works in close collaboration with other members of the group, which includes physicists, chemists, pathologists, biologists, radiologists and nuclear medicine physicians, urologists, radiation oncologists and medical oncologists.

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**Principal Investigator:**  
**HEDVIG HRICAK, MD, PhD, DrHC**  
Chairman of Department of Radiology  
Carroll and Milton Petrie Chair



**Peter Michael Pelican Postdoctoral Fellow:**  
**HEBERT ALBERTO VARGAS, MD, MS**

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## Dr Vargas' projects can be summarized in 3 main areas:

- 1 Assessment of prostate cancer aggressiveness: Currently the biggest challenge in managing patients with newly diagnosed prostate cancer is shifting from tumor detection alone to distinguishing between patients with aggressive disease who need radical treatment and patients with indolent disease who need no treatment and can be placed on active surveillance. Dr. Vargas is investigating the role of quantitative parameters derived from an MRI technique termed "diffusion-weighted imaging" in risk stratification for patients with prostate cancer. The aim is to "triage" patients at diagnosis, so that patients with more aggressive cancers can be offered radical treatments, while patients with "indolent" cancers can be managed more conservatively. His preliminary findings on this topic have been submitted for publication.
- 2 The role of MRI in active surveillance of prostate cancer: It is proposed that patients with "low-risk" prostate cancer can be managed expectantly, with close observation and follow-up. Several relevant clinical questions remain to be answered, such as which patients should be offered active surveillance and when should a patient on active surveillance be offered treatment. Dr. Vargas' research in this area includes investigating the role of MRI in the selection of patients who are suitable candidates for active surveillance. In addition, in patients with low-risk cancer, he is attempting to determine whether MRI can be used as a noninvasive method to replace transrectal prostate biopsies known as "surveillance biopsies". Those biopsies are at present a standard of care during active surveillance.
- 3 Evaluation following treatment for prostate cancer: Patients with newly diagnosed prostate cancer have many management options, including active surveillance, radiation therapy, surgery and novel focal therapies (e.g. cryotherapy, focused ultrasound, laser). Dr. Vargas is investigating MRI as a potential tool to detect recurrence in patients who have been treated for prostate cancer. Some of his work in this area has already resulted in publications in major peer-reviewed journals and he was awarded a "Certificate of Merit" for his presentation on this topic in the American Roentgen Ray Society 2010 Annual Meeting in San Diego, USA.

## Publications Referencing Work Sponsored by the Peter Michael Foundation

Santra S, Kaittanis C, **Grimm J**, Perez, JM. Drug/dye-loaded, multifunctional iron oxide nanoparticles for combined targeted cancer therapy and dual optical/magnetic resonance imaging. *Small*. Aug 2009; 5(16); 1862-1868

Perez JM, **Grimm J**, Josephson L, Weissleder R. Integrated nanosensors to determine levels and functional activity of human telomerase. *Neoplasia*. 2008; Oct 10 (10); 1066-1072

**Grimm J**, Hricak H. Imaging in Urology – Looking Forward. *Current Opinions in Urology*; 2008; 18(1); 61-64

**Vargas HA**, Wassberg C, Akin O, Hricak H. Magnetic resonance imaging of treated prostate cancer. *Radiology* 2011 (In press)

Akin O, Gultekin D, **Vargas HA**, Zheng J, Moskowitz C, Pei X, Sperling D, Schwartz L, Hricak H, Zelefsky M. Functional Multi-parametric MRI Improves Detection of Locally Recurrent Prostate Cancer after Radiation Treatment: Preliminary Results. *European Radiology* 2011 (In press)

**Vargas HA**, Akin O, Hricak H. Residual prostate tissue after radical prostatectomy: acceptable surgical complication or treatment failure? *Urology* 2010. (In press)

**Vargas HA**, Shukla-Dave A, Hricak H. Imaging in prostate cancer. In: *Comprehensive Textbook of Genitourinary Oncology*. Editors: Nicholas J. Vogelzang, MD; Peter T. Scardino, MD; Michael J. Zelefsky, MD et al. Lippincott Williams & Wilkins. 2010 (In press)

**Vargas HA**, Akin O, Franiel T, Mazaheri Y, Zheng J, Moskowitz C, Udo K, Eastham J, Hricak H. Diffusion-Weighted Endorectal MRI at 3-Tesla for Prostate Cancer: Tumor Detection and Assessment of Aggressiveness. Submitted to *Radiology* (Submitted)

**Vargas HA**, Akin O, Franiel T, Udo K, Hricak H. MRI of the normal central zone of the prostate and central zone involvement by prostate cancer: Clinical and Imaging Implications (Submitted)

Mazaheri Y, **Vargas HA**, Akin O, Goldman D, Zheng J, Moskowitz C, Hricak H. Non mono-exponential Analysis of DW-MRI data for the Detection of Prostate Cancer. (Submitted)

Wassberg C, Akin O, Shukla-Dave A, **Vargas HA**, Zhang J, Hricak H. The incremental value of contrast-enhanced MR imaging in the detection of biopsy-proven local recurrence after radical prostatectomy (Submitted)

Franiel T, **Vargas HA**, Akin O, Hamm B, Hricak H, Beyersdorff D. MRI before transrectal-guided prostate biopsies for the detection of prostate cancer. (Submitted)





**DUKE CANCER INSTITUTE**

A National Cancer Institute-designated Comprehensive Cancer Center



**UNC**

ESHELMAN  
SCHOOL OF PHARMACY



**Principal Investigators:**

**DANIEL GEORGE, MD, Duke**

**RUDY JULIANO, PhD, UNC**

**BRUCE SULLENGER, PhD, Duke**

## *Cell-specific Interference Strategies for Prostate Cancer*

### ORIGINAL PROPOSAL

Interference RNA (RNAi) strategies represent a potentially new targeted approach to silencing specific genetic pathways within cancer; however, efficient delivery of small interfering RNA (siRNA) molecules into target cells is a major obstacle to developing this modality into effective therapy. RNA aptamers represent another novel form of RNA therapeutic – one which binds specifically to unique peptide sequences. Using an RNA aptamer for prostate specific membrane antigen (PSMA), we have created a delivery mechanism that not only results in cell specific binding, but also rapid and efficient endocytosis.

Therefore we propose to develop a novel RNA chimera, combining our PSMA aptamer with an AR-specific siRNA. We hypothesize that a PSMA aptamer-AR (androgen receptor) siRNA chimera will demonstrate cell-specific inhibition of AR that is more potent than known AR antagonists. The specific aims of the research are to: Create and demonstrate the in vitro specificity and efficacy of the PSMA aptamer-AR siRNA Chimera. Demonstrate in vivo activity of the PSMA aptamer-AR siRNA Chimera.

### PROGRESS REPORT

RNA aptamers represent a novel form of RNA therapeutic delivery – one which can specifically bind a unique peptide sequences on a cell's surface in order to achieve cell specific delivery of a therapeutic agent. Dr. Bruce Sullenger PhD is a world authority in this field of research. Using an RNA aptamer specific for prostate specific membrane antigen (PSMA), our team has created a delivery mechanism that results in prostate cancer cell-specific delivery of a therapeutic agent. This targeted delivery approach is flexible and can be combined with numerous different therapies in order to specifically deliver them to the cells of interest.

Although we are interested in pursuing multiple avenues, our team is currently focused on utilizing this technology towards the delivery of novel agents that elicit androgen receptor (AR) inhibition. AR expression is conserved in most prostate cancers and is frequently increased with more advanced, “so-called” hormone-refractory tumors. By being able to target and modulate AR activity, it could be possible to develop new treatments for prostate cancer. Unfortunately, there are relatively few mechanisms and agents currently available that can directly alter AR activity clinically, with only relatively weak partial antagonists, such as bicalutamide, available.

We have strategized that by coupling our PSMA aptamer with an AR-specific splice oligo will create variants in the AR gene in order to silence its expression. Dr. Rudy Juliano, PhD at the UNC School of Pharmacy is a world expert in splice oligos, small nucleic acids that can specifically alter the DNA transcription of genes to create shorter, non-functioning forms. Together, we have collaborated on the concept of developing a chimeric molecule that will allow specific delivery of the therapeutic payload to prostate cancer cells and subsequent shut-down of the AR expression. Ultimately such a targeted strategy could allow us to one day inhibit this hormonal pathway in cancer cells while sparing the patient from the harmful and debilitating side effects.

# PROGRESS REPORTS



Dr. Jenny Freeman, PhD

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We are thrilled to announce that we have identified Dr. Jenny Freeman, PhD, an accomplished molecular genetics researcher, as the Peter Michael Research Associate to lead this effort. We are in negotiations with both Universities to finalize her appointments.

Finally, we have leveraged our support from the Peter Michael Foundation to initiate a collaboration with colleagues at John Hopkins Medical Institute. Working with Drs. Theodore DeWeese, MD, Chairman of Radiation Oncology and Shawn Leopold, PhD an Assistant Professor of Cell Biology, we have identified a target gene for radiation sensitivity and linked this to another PSMA aptamer to create a novel radiation sensitizer for prostate cancer. Through the Duke Translational Research Institute we will create a GMP-quality RNA therapeutic of this agent for IND development and clinical testing. Together these collaborations create a critical mass of research in the field of RNA therapeutics, focused on prostate cancer, through a unique network of collaborators and Universities.

Morris Cancer Center at Duke Cancer Institute



Memorial Sloan-Kettering Cancer Center, NY

Photograph by David Sundberg | Esto



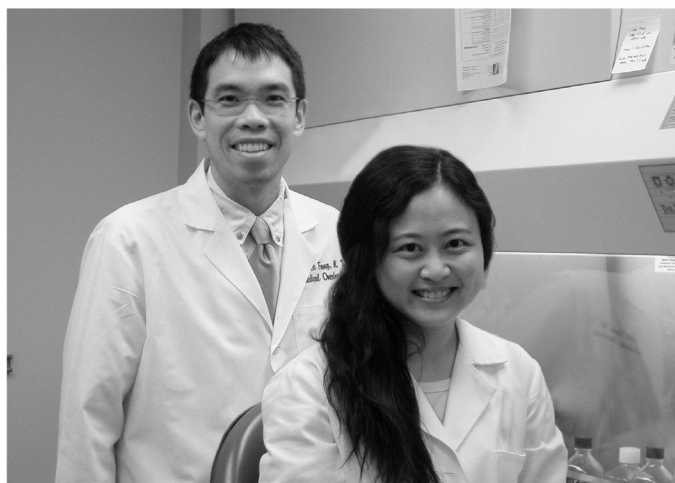
UCSF Helen Diller Family Comprehensive Cancer Center



University of North Carolina School of Pharmacy



## UCSF Helen Diller Family Comprehensive Cancer Center



**Principal Investigator:**

**LAWRENCE FONG, MD**

Associate Professor, Department of Medicine,  
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**Peter Michael Pelican Postdoctoral Fellow:**

**SERENA KWEK, PhD**

### *Next Generation Therapeutic Cancer Vaccines for Prostate Cancer*

#### ORIGINAL PROPOSAL

We have discovered that the immune systems in some prostate cancer patients can recognize their own cancer. Tumors, however, can produce different substances and create an environment within the host to dampen these immune responses. By introducing a treatment that can stimulate the immune system, we can drive the immune system to overcome this immunosuppressive milieu, thereby inducing anti-tumor responses. One such approach involves treating prostate cancer patients with both a bone marrow growth factor, GM-CSF, as well as an anti-CTLA4 antibody. The former drug serves to expand the number the cells that serve to educate the immune system, instructing

it on what to target. The latter drug releases the brakes on the immune system by blocking one of the crucial immune system checkpoints.

We propose to define the immune targets (antigens) to which patients receiving CTLA4 blockade treatment are responding. Because some patients have dramatic clinical responses and others do not, we can determine whether immune responses to particular antigens are associated with clinical responses or, alternatively, side effects. Moreover, we can determine whether preexisting immune responses to particular antigens could predict who will respond to this treatment. These results could help guide us to select the patients who would derive a clinical benefit from this treatment. Rather than identifying these antigens in animal models, the approach by which most immunotherapies are developed, our proposal focuses on the antigens relevant for prostate cancer patients. As a result, the antigens that we discover should be immediately relevant for humans.

#### PROGRESS REPORT

During this first year of support, we have analyzed the immune responses of patients receiving treatment with anti-CTLA4 antibody (ipilimumab) for prostate cancer. We have identified new target proteins, or antigens, to which the immune system may be targeting to kill cancer cells by using spotted protein arrays to screen blood from treated patients for immune responses. We are now in the process of validating these different antigens by looking at the expression of these antigens in prostate cancer cells. We are also taking these observations from cancer patients and going into mouse models to immunize the mice to these different antigens, and see preliminary signs that these antigens can help protect mice from prostate tumors. This work lays the groundwork for developing more potent vaccines for prostate cancer.

**Dr. Fong** was invited as a plenary speaker at the 25th Anniversary Meeting for the International Society for Biologic Therapy of Cancer (ISBTc) on 10/2/10. This represents the annual meeting for one of the longest existing societies that has focused on immunotherapies for cancer and is attended by researchers from around the world. There, Dr. Fong presented his work on the immunotherapy of prostate cancer, including work focusing on identifying new vaccine targets for prostate cancer that is supported by the Peter Michael Foundation.



## STANFORD CANCER CENTER

Stanford University Medical Center



**Principal Investigator:**  
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Director of Canary Center at  
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Nuclear Medicine Professor in Departments  
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**Hamalainen Peter Michael Postdoctoral Fellow:**  
**SRI-RAJASEKHAR KOTHAPALLI, PhD, MS,**  
**MTech, MSc**

*We intend to  
**revolutionize prostate  
cancer management** based on  
**Theranostics** – the combination and consequent  
synergy of state-of-the-art, multimodality molecular  
imaging for diagnosis with high specificity  
**molecular-targeted** therapeutics.*

### ORIGINAL PROPOSAL

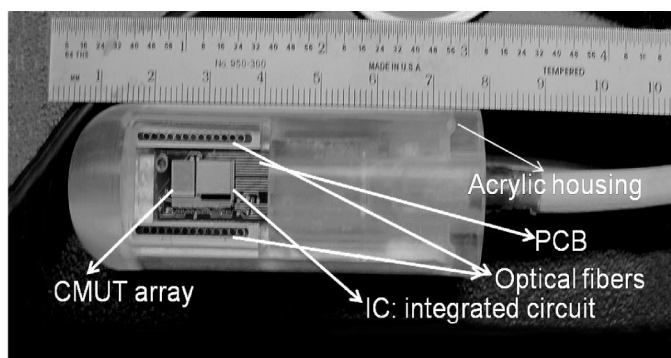
Photoacoustic molecular imaging is an emerging technology that overcomes, to a great extent, the spatial resolution and depth limitations of whole-body optical imaging. Photoacoustic imaging is capable of monitoring molecular

levels of cancer-specific proteins in a living subject. In this technique, a conventional photoacoustic imaging instrument is coupled with an imaging agent (contrast agent) targeted to a cancer-specific protein to achieve a specific imaging signal. The concept is that light goes into the body and sound comes out. Nanoengineering is employed to develop the imaging agent – nanoparticles specifically designed to seek and identify prostate cancer at the cellular level. The imaging agent is introduced into the body and the nanotubes attach to prostate cancer cells wherever they may be, in the gland or out if the cancer has metastasized. The laser light is passed over the subject causing the nanotubes to emit an ultrasound that is captured and imaged outside of the body. The next step would be to design the nanotubes to carry a pay load. Then an audio sound could be sent back into the body that triggers the nanotubes to destroy the cancerous cells only.

### PROGRESS REPORT

Prostate cancer is the most common non-skin cancer in American men. The American Cancer Society estimates that during 2010, approximately 220,000 new cases of prostate cancer will be diagnosed, and approximately 32,050 men will die of this cancer in the United States. Standard screening methods for prostate cancer - such as blood screening for prostate specific antigen (PSA), digital rectal examination (DRE), and transrectal ultrasound (TRUS) guided prostate biopsy – have limited ability (sensitivity and specificity) in early diagnosis of prostate cancer. Physicians routinely

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**1** Dual modality transrectal ultrasound and photoacoustic probe now constructed. IC and CMUT are flip-chip bonded and placed on PCB (printed circuit board). PCB is rested in between two parallel fiber optic light guides that focus light 0.5 inch above the CMUT surface. The entire PCB-light guide assembly is housed in an acrylic probe. The probe has similar dimensions (1 inch thick and 60 mm in length) as conventional transrectal ultrasound probe.



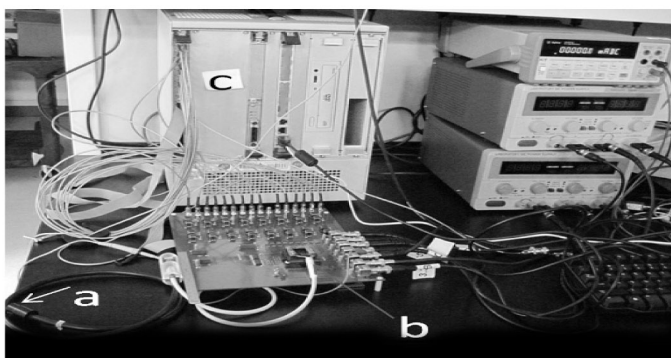
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employ a combination of these methods to perform better diagnosis but unfortunately this strategy still lacks overall diagnostic accuracy. Other emerging imaging modalities for prostate diagnosis, such as magnetic resonance imaging (MRI), X-Ray CT (computed tomography), PET (positron emission tomography) suffer from some of the following disadvantages such as employing ionizing radiation (X-Ray CT and PET), limited soft tissue contrast (CT), limited sensitivity (CT and MRI), limited spatial resolution (PET) and high cost (MRI, PET, and CT). TRUS, however, employs non-ionizing radiation to obtain anatomical features of the prostate, is very safe, and relatively cheap. These anatomical features are used to identify/estimate several diagnostic features such as volume of the prostate, prostate specific antigen (PSA) density, isoechoic, hypoechoic, and hyperechoic areas of prostate. Therefore TRUS continues (despite lack of sensitivity and specificity) to be the most successful imaging technique in the prostate screening stage. Novel functional and molecular imaging approaches built upon the above strengths of TRUS are required to achieve high sensitivity and specificity, and thereby reduce widespread metastasis of the cancer.

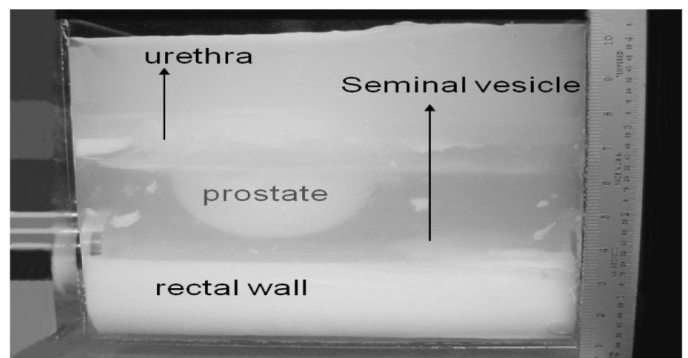
To improve the diagnostic accuracy of transrectal ultrasound (TRUS) in prostate cancer screening, we have now developed a dual modality, transrectal photoacoustic (TRPA) and TRUS, probe that integrates a fiber optic light guide and a state of art 16x16 two dimensional (2D) capacitive micromachined ultrasound transducer (CMUT) array, with a center frequency of 5.5 MHz, connected to the front-end

electronics. To provide a means of integrating the CMUT array with electronics, each element of the array is connected to a flip-chip bond pad on the back side of the array via a through-wafer interconnects. The array is flip-chip bonded to a custom-designed integrated circuit (IC) that comprises the front-end circuitry (pulser and preamplifier) for the transducer elements. The flip-chip bonded device (CMUT array plus IC) is then glued, using a non-conductive epoxy, to the custom made printed circuit board (PCB) cable. As shown in **Figure 1** the fiber optic light guide, flip-chip bonded device, and the PCB cable are all housed in a transrectal probe made of acrylic. As shown in **Figure 2**, the cable is connected to a (field programmable grid array) FPGA-based data acquisition system that can acquire volumetric (B-mode) imaging data in real time. As shown in **Figure 3**, we also developed a transrectal prostate phantom that mimics both ultrasound and photoacoustic properties of prostate. In a few weeks we will validate and optimize the performance of the dual modality probe using transrectal prostate phantom experiments. This dual modality probe facilitates the marriage of the rich optical contrast based functional and molecular photoacoustic imaging methods with grey-scale TRUS methods that display anatomical features of the prostate. Please note that in our previous progress report, using optimized ultrasound parameters (5.5 MHz center frequency), light properties (800 nm to 1064nm), and image reconstruction algorithms, we demonstrated that light absorbing objects placed as deep as 5 cm inside prostate tissue mimicking phantoms are imaged with good contrast. We will next start detailed testing of our imaging probe in the constructed tissue phantom.

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**2** Data Acquisition System. PCB cable of the probe connected (arrow indicating "b") to the FPGA based 16 channel data acquisition system. A commercial FPGA-based signal-processing system (Lyrtch signal processing, Quebec City, Canada), labeled as "c", is used for analog-to-digital (ADC) conversion and image reconstruction. The system has 16-channels of 100-MSPS, 14-bit ADC's. Data from these ADC's flow into a Xilinx Virtex II 6000 FPGA. The FPGA controls the transmit/receive sequence, element addressing, and real-time image reconstruction. The input face of fiber optic light guide, arrow indicating "a", will be connected to a nano-second pulsed tunable (670 to 1064nm) laser with a repetition rate of 10 Hz. The light power density on the rectal wall will be adjusted to the ANSI safety limit of 20 mJ/cm<sup>2</sup>.



**3** Transrectal prostate phantom that mimics photoacoustic and ultrasound properties of prostate tissue. The prostate along with structures simulating the rectal wall, seminal vesicles and urethra is contained within an 11.5 cm X 7.0 cm X 9.5 cm clear acrylic container. Dimensions of prostate tissue are 5 cm X 4.5 cm X 4 cm and volume is 53 cc. Rectal wall dimensions are 6 cm X 11 cm X 0.5 cm. Rectal wall and urethra are made of 1% Lyposyn (20% Intralipid emulsion) and 10% agar mixture and have reduced scattering coefficient of 10 cm<sup>-1</sup>. Prostate is made of 1.5% Intralipid and 10% agar mixture and has reduced scattering coefficient of about 15 cm<sup>-1</sup>. Ultrasound speed is 1500 cm-1. Attenuation 0.5 dB/cm/MHz.



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Stanford Cancer Center

## IMAGE RECONSTRUCTION

The ultrasound and photoacoustic data acquired by the FPGA system is reconstructed using the classical synthetic aperture (SA) focusing technique along with a coherence factor (CF) weighting. Prior to image reconstruction, the A-scan from each element in the transducer array is filtered to eliminate out-of-band noise. These A-scans are then appropriately delayed and summed to form the image. The delays are calculated based upon the distance from the transducer element to the reconstructed point on the beam. Envelope detection is performed in the end, after the image reconstruction. The beam formed image is then multiplied by a coherence factor weighting. This weighting reduces focusing errors resulting from sound velocity inhomogeneities and other possible phase errors. The final photoacoustic and acoustic images are logarithmically compressed according to the dynamic range desired before being co-registered and displayed on the monitor.

In ultrasound mode, like in TRUS, the CMUT array transmits ultrasound waves into the prostate tissue and detects echos that are reflected from the prostate tissue. This

provides anatomical features of the prostate. In photoacoustic mode, however, the probe transmits laser light pulses (from the fiber optic light guide) into the prostate tissue. These light pulses when absorbed by endogenous chromophores (such as oxy/de-oxy hemoglobin and water) and exogenous molecule targeted agents create wide band ultrasound waves. These waves are then detected by CMUT array.

## PUBLICATIONS

J. Levi, S.R. Kothapalli, T.J. Ma, K. Hartman, B.T. Khuri-Yakub, S.S. Gambhir. Design, synthesis, and imaging of an activatable photoacoustic probe. *Journal of the American Chemical Society*, 132(32): 11264-9, 2010.

## ABSTRACTS PRESENTED AT INTERNATIONAL MEETINGS

S.R. Kothapalli, T.J. Ma, S. Vaithilingam, O. Oralkan, B. Khuri-Yakub, S.S. Gambhir. Transrectal Photoacoustic Imaging of the Prostate using Capacitive Micromachined Ultrasound Transducers. World Molecular Imaging Congress, 0381B. Kyoto, Japan, September, 2010.

If you know of someone who you believe would be interested in learning more about the Peter Michael Foundation, our events and our work, please email us.

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