

ABSTRACTS

1. Quantitative Ultrasound 1

1.1 Ultrasonic model-based imaging and breast cancer detection. Michael L. Oelze and William D. O'Brien, Jr., *Beckman Institute, University of Illinois at Urbana-Champaign, 405 N Mathews, Urbana, IL 61801, oelze@uiuc.edu* (invited overview).

Early detection and diagnosis of breast cancer through imaging leads to improved prognosis. Quantitative ultrasound (QUS) imaging techniques were explored for classifying tumors in rodent models of breast cancer. The QUS imaging technique was based on estimates of scatterer properties obtained through parameterizing the ultrasonic backscatter from tissues. Initially, parameterization of the ultrasonic backscatter was accomplished through the use of generic models for scattering (i.e., the Gaussian form factor). Initial QUS analysis yielded moderate success for classifying tumors. However, in most cases, the scatterer property estimates did not correspond to the underlying structure that it was intended to model.

A new technique was developed, which allowed models of scattering to be constructed directly from underlying tissue microstructure. The new modeling technique was based on the construction of three-dimensional impedance maps (3DZMs) of histological sections of tissues. Tissue blocks were fixed, stained, sectioned into serial slides and photographed at high magnification. The serial slides were registered to form a three-dimensional block. The 3DZM was constructed by associating pixel intensity values to the characteristic acoustic impedance of tissues. From the 3DZMs, scatterer properties could be estimated using existing models, new models could be deduced and scattering sources identified.

Scatterer properties estimated from the 3DZMs were compared to scatterer property estimates from ultrasonic backscatter. Estimates of the average scatterer diameter using the 3DZM and ultrasonic backscatter were within 10% relative error. 3DZMs were also used to predict scattering sources and create new models for scattering from cells. New models for scattering, the addition of new parameters and more appropriate analysis bandwidths yielded significantly improved classification of different kinds of tumors. Feature analysis plots indicated cancer classification was improved when using multiple parameters over few parameters.

This work was supported by NIH Grants CA111289, CA96419, and EB006741.

1.2 A historical perspective on quantitative ultrasound techniques. Timothy J. Hall, *Department of Medical Physics, University of Wisconsin-Madison, Madison, WI 53706, tjhall@wisc.edu* (invited).

The desire to use ultrasound signal analysis to differentiate between disease types is effectively as old as medical ultrasound imaging itself. There have been a number of 'successes' and 'failures' along the way but a great deal of progress has been made. Although Lord Rayleigh described the basics of acoustics in the late 1800's, actually classifying tissue types with ultrasound requires a great deal more detail in the analysis. Modern methods allow us to now more accurately model the interaction between an acoustic wave and the inhomogeneous medium (tissue) in which it is propagating.

The goals of this presentation are to describe some of the key underlying principles in quantitative ultrasound imaging (QUS; aka 'tissue characterization') with emphasis on the contributions of the Wisconsin (and formerly Kansas) group. One particular failure in QUS – commercial software for liver attenuation estimation – will be used as an example of how the process can 'fail.' A rigorous understanding of both the physics and underlying biology

is essential for maximizing success. Specifically, the motivation for system-independent parameter estimation will be reviewed. The models for the acoustic properties of tissue will be described and the relation between these models and measured acoustic parameters of tissue will be highlighted.

The prospects for the future of QUS are very encouraging. There is a great deal of ultrasound system development that will assist in further advancing these techniques and reducing the variance in estimates of QUS parameters. Some of those developments will also be highlighted.

1.3 Compensating for attenuation when performing ultrasound tissue characterization, Timothy A. Bigelow, *Department of Electrical Engineering, University of North Dakota, Grand Forks, ND 58202, timothybigelow@mail.und.nodak.edu* (invited).

For years, many investigators have attempted to quantify the ultrasound backscatter from tissue by analyzing the power spectrum of the rf echoes to estimate the correlation length of the tissue microstructure. Their goal has been to use the correlation length and possibly the correlation function (i.e., tissue-scattering model) for the tissue to assess the pathology of the imaged region (i.e., QUS imaging). The correlation length is determined from the backscattered power spectrum based on some assumed model for tissue scattering. Therefore, prior to determining the correlation length, all of the changes to the spectrum due to ultrasound propagation must be compensated. For backscattered echoes from weakly-focused sources, the primary changes to the spectrum due to propagation are the frequency-dependent attenuation in the region of interest (i.e., local attenuation) and the frequency-dependent attenuation along the propagation path leading to the region of interest (i.e., total attenuation). Over the bandwidth of most sources, both the local and total attenuation have a linear dependence on frequency. Therefore, it is the slope of this frequency dependence that must be compensated when estimating the correlation length.

The impact of local attenuation slope on the backscattered power spectrum is minimal. Therefore, it is possible to obtain accurate estimates of correlation length even if the local attenuation slope is not known exactly. In a series of computer simulations and theoretical calculations, we determined that the optimal value to assume for local attenuation slope when the local attenuation is not known exactly is given by $a_{loc} \sqrt{(a_{high}^2 + a_{low}^2) / 2}$, where a_{high} and a_{low} are the largest and smallest attenuation slope values expected for the tissue.

While the impact of local attenuation can be minimal, the strong dependence of the estimate for correlation length on the total attenuation slope may be the reason that QUS imaging has had only limited success in clinical practice. Previous approaches to obtain the total attenuation slope have had many deficiencies. Estimates based on changes in backscatter intensity with depth have assumed that the tissue along the propagation path is homogeneous. Estimates obtained by summing multiple estimates of local attenuation to obtain an estimate of total attenuation are highly computationally intensive and prone to errors as the errors can accumulate with increasing tissue depth. Lastly, simultaneously estimating both scatterer size and total attenuation results in poor precision and is highly dependent on an accurate model for the tissue scattering structure prior to obtaining the estimate. Therefore, a new method to obtain total attenuation slope is needed if QUS imaging is to reach its full potential.

Recently, the down shift in center frequency of the backscattered ultrasound echoes compared to echoes obtained in a water bath was calculated to have the form $f_{norm} = mf_o + b$ after normalizing with respect to the source bandwidth, where m depends on correlation length, b depends on total attenuation slope and f_o is the center frequency of the source as measured from a reference echo. Therefore, the total attenuation slope can be determined independent of correlation length by measuring the down shift in center frequency from multiple sources

(i.e., different f_o) and fitting a line versus f_o to the measured shifts after normalizing with respect to bandwidth, f_{norm} . The intercept of the line gives the total attenuation slope along the propagation path. The calculations were verified using computer simulations of five spherically-focused sources with 50% bandwidths and center frequencies of 6, 8, 10, 12 and 14 MHz. The simulated tissue had Gaussian-scattering structures with effective radii of 25 μ m placed at a density of 250/mm³. The attenuation of the tissue was varied from 0.1 to 0.9 dB/cm-MHz. The error in the attenuation along the propagation path ranged from 3.5 \pm 14.7% for a tissue attenuation of 0.1 dB/cm-MHz to 7.0 \pm 3.1% for a tissue attenuation of 0.9 dB/cm-MHz, demonstrating that the attenuation along the propagation path could be accurately determined using backscattered echoes from multiple sources using the derived algorithm.

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1.4 Novel low-frequency ultrasound detection of apoptosis *in vitro* and *in vivo*, Gregory J. Czarnota,^{1,2} Naum Papanicolau,^{1,4} Justin Lee,^{1,2} Behzad Banihashemi,^{1,2} Branislav Debeljevic,^{1,4} Shawn Ranieri,^{1,2} Mohammed Azrif,¹ Raffi Karshafian,^{1,2} Anoja Giles,¹ Alireza Sadeghian⁴ and Michael C. Kolios,^{2,3} ¹*Radiation Oncology and Imaging Research, Sunnybrook Health Sciences Centre and Department of Radiation Oncology, University of Toronto,* ²*Department of Medical Biophysics, University of Toronto,* ³*Department of Physics, Ryerson University and* ⁴*Department of Computer Science, Ryerson University, Canada, gregoryczarnote@gmail.com* (invited).

The aim of many cancer therapies is to induce apoptotic cell death. We demonstrate here for the first time that diagnostic-range conventional-frequency ultrasound imaging may be used to detect apoptotic cell death *in vitro* and *in vivo*.

In vitro experimentations employed a leukemia cell model (AML-3). Apoptosis was induced in cells by exposure to 10 μ g/ml cisplatin for various times (0, 6, 12, 24, 48 and 72 hours). Concentration dependence was also evaluated by preparing samples of 0, 10, 20, 40, 60, 80 and 100% apoptotic cells. Samples were examined by a 10 MHz, 3.8cm conventional transducer using an Ultrasonix-RP ultrasound device and results compared with data collected by a 30 MHz f2 high-frequency transducer coupled to a VisualSonics VS40B ultrasound device. For experiments *in vivo* solid tumors were grown in SCID ($n = 32$) mice using a human prostate cell line (PC-3). Half of the mice received an 8 Gy dose in a single fraction to the tumour with the remaining mice left untreated. Ultrasound analyses were carried out examining spectral parameters and statistical methods. Samples were processed for histopathologic analysis using both hematoxylin and eosin and TUNEL staining for apoptosis.

For experiments conducted with *in vitro* samples, analyses indicated an up to 8-fold increase in ultrasound backscatter intensity (6.2 \pm 1.0 dB) coinciding with maximal apoptosis (48 hours). Detection-limit experiments indicated a statistically-significant difference between viable cells and the 10% apoptotic sample. Results also correlated well with high-frequency ultrasound data with the exception that increases in backscatter at 30 MHz were larger (12.0 \pm 0.8 dB). Increases in spectral slope with cell death were suggestive of a decrease in mean scatterer size consistent with our working model that nuclear compaction and degradation during apoptosis influences ultrasound backscatter.

Data from low-frequency experiments conducted with *in vivo* tumours indicated an increase in backscatter of similar magnitude and correlated with the presence of cell death in histology. Conventional frequency ultrasound backscatter intensity increases (7.1 \pm 0.58 dB) correlated well with high-frequency ultrasound data (6.1 \pm 0.63). Changes in spectral slope and 0-MHz intercept were also consistent with data acquired at high frequency. This

is an indication that techniques developed for high frequency research may be potentially employed at conventional frequencies without degradation of apoptotic cell detection.

In summary, ultrasound analyses using conventional frequency ultrasound with correlational immunohistochemistry and histology indicated that conventional frequency ultrasound could detect apoptosis *in vitro* and *in vivo* in a preclinical prostate cancer model. This opens the avenue for this type of modality to be used to monitor the efficacy of cancer treatments leading to customization and optimization of treatments.

2. Bone 1

2.1 Ultrasonic characterization of cancellous bone, Keith A. Wear, *Food and Drug Administration, Rockville, MD, kaw@cdrh.fda.gov* (invited overview).

Broadband ultrasonic attenuation (BUA) and speed of sound (SOS) are measured clinically in the management of osteoporosis. BUA and SOS exhibit high inter-system variability. A recent clinical trial involving 73 women showed that (1) BUA accuracy can be substantially improved by using phase-insensitive reception rather than conventional phase-insensitive reception, and (2) SOS variability can be substantially improved by correcting estimates for variations in system parameters including bandwidth and transit-time marker location. BUA is the result of absorption and scattering. Scattering consists of both longitudinal-to-longitudinal and longitudinal-shear scattering. Recent analysis of data from 23 human femur samples *in vitro* suggests that the Faran Cylinder Model and the Weak Scattering Model accurately predict the frequency dependence of the backscatter coefficient. Recent analysis of data from 16 human calcaneus samples *in vitro* suggests that backscatter coefficient estimates that are based on phase sensitive attenuation compensation significantly overestimate average magnitude and exponent of frequency dependence of backscatter coefficient.

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2.2 Coupling numerical simulations of wave propagation with three-dimensional bone microstructures is full of answers, Pascal Laugier, *Université Pierre et Marie Curie - Laboratoire d'Imagerie Paramétrique UMR CNRS 7623, Paris France, laugier@lip.bhdc.jussieu.fr* (invited).

Our description of how the ultrasonic wave interacts with bone has up to now been mostly empirical. However, owing to the complexity of bone structure and to its tremendous variability, elucidation of relationships between ultrasonic properties of bone and its material or structural properties is difficult to determine from direct measurements. This uncertainty limits our understanding of the factors that influence quantitative ultrasound parameters, which may include microstructure, mineralization, stiffness and viscous properties. Time-domain finite difference simulations (FDTD) coupled with actual three-dimensional microstructures provide a new way into the interaction between bone and ultrasound. Our results indicate that most of the properties observed empirically are reproduced in numerical simulations. Simulated results such as the findings that: (1) the attenuation varies linearly with frequency, (2) the slope of frequency-dependent attenuation and sound velocity increase quasilinearly with the bone volume fraction, (3) the majority of samples show a negative velocity dispersion and (4) two compression waves may propagate upon certain trabecular orientations agreed well with experimental measurements and impressively demonstrated the power of these computational tools. We conclude on the appropriateness of using FDTD to elucidate physical interaction mechanisms, to assess sensitivity of ultrasonic parameters to

bone properties, to find solutions to inverse problems and to test new experimental configurations.

2.3 Ultrasonic assessment of the radius *in vitro*. Jonathan J. Kaufman,^{1,2} Gangming Luo,² Vincent LeFloch² and Robert S. Siffert,¹ *¹The Mount Sinai School of Medicine, New York, NY 10029 and ²CyberLogic, Inc., New York, NY 10012, jkkaufman@cyberlogic.org* (invited).

The overall objective of this research is to develop an ultrasonic system for noninvasive assessment of the distal radius. The specific objective of this study was to examine the relationship between cortical bone mass and ultrasound measurements *in vitro*. Nineteen human radii of unknown origin were measured in through transmission in a water bath. A 3.5 MHz rectangular (1 cm x 4.8 cm) single element transducer served as the source and a 3.5 MHz rectangular (1 cm x 4.8 cm) linear array transducer served as the receiver. The linear array consisted of 64 elements with a pitch of 0.75 mm. Ultrasound measurements were carried out at the '1/3' location of each radius and two net time delay parameters, t_{NetDW} and t_{NetCW} , associated with a direct wave (DW) and a circumferential wave (CW), respectively, were evaluated. The cortical thickness (CT), medullar thickness (MT) and cross-sectional area (CSA) of each radius was also evaluated based on a digital image of the cross-section at the 1/3rd location. The linear correlations between CT and t_{NetDW} was $r = 0.91$ ($p < 0.001$) and between MT and t_{NetDW} t_{NetCW} was $r = 0.63$ ($p < 0.05$). The linear correlation between CSA and a nonlinear combination of the two net time delays, t_{NetDW} and t_{NetCW} , was $r = 0.95$ ($p < 0.001$). The study shows that ultrasound measurements can be used to noninvasively assess cortical bone mass as represented by cortical thickness and cross-sectional area. A tabletop device that can be used to assess radial mass at the 1/3rd location is presently being fabricated and will be tested clinically in the coming months.

2.4 Experimental demonstration of negative dispersion arising from multiple wave interference: a potential source of negative dispersion in bone, Adam Q. Bauer, Karen R. Marutyan, Mark R. Holland and James G. Miller, *Washington University in St. Louis, Washington University, St. Louis, MO, abauer@hbar.wustl.edu*.

Background: Ultrasound-based methods designed to assess the status of cancellous bone as a diagnostic screening approach for identifying early-stage osteoporosis and potentially for monitoring the effects of pharmacological therapy rely upon measurements of intrinsic ultrasonic velocity and attenuation properties. Although there is a general consensus regarding the frequency dependence of the attenuation coefficient, published results demonstrate considerable variation in the measured frequency dependence of phase velocity. On average, many laboratories report that the phase velocity of ultrasonic waves propagating through cancellous bone decreases with increasing frequency. This negative dispersion does not appear to be causally consistent with the ultrasonic Kramers-Kronig relations that relate the attenuation coefficient to dispersion. A better understanding of the source of this inconsistency might aid in the development of enhanced methods that could potentially increase sensitivity and specificity of diagnoses.

Objective: The goal of this study was to investigate the potential role of interference in phase-sensitive measurements of the attenuation coefficient and dispersion (frequency dependence of phase velocity) properties of a phantom in which two temporally-overlapping signals are detected – a situation analogous to that of the simultaneous propagation of fast and slow waves in cancellous bone.

Methods: The phantom consisted of a flat and parallel Plexiglas™ plate into which a step discontinuity was milled. This plate was secured to a motion controller and translated through the azimuthal plane of the transmitted field. Two sets of measurements of the appar-

ent attenuation and apparent phase velocity were obtained for specific spatial locations of the plate: one set with the transmitting transducer excited with a broadband pulse centered at 5 MHz and the other set using select narrowband signals from 3 MHz to 7 MHz. The broadband data were analyzed using standard magnitude and phase spectroscopy techniques. The measured attenuation coefficient and phase velocity of the narrowband data were calculated using log-spectral subtraction and time-domain rf correlation, respectively.

Results: As the interrogating ultrasonic field is translated across the step discontinuity of the plate, the observed frequency dependences of the phase velocity and attenuation coefficient exhibit significant changes. Negative dispersion is observed at specific spatial locations of the plate at which the attenuation coefficient rises approximately linearly with frequency, a behavior analogous to that of bone measurements reported in the literature. For all sites investigated, broadband and narrowband data demonstrate excellent consistency over the experimental bandwidth.

Conclusion: Results of this study suggest that the interference between the two signals simultaneously reaching a phase sensitive piezoelectric receiver may be one source of the measured apparent negative dispersion. Because the detected signals were comprised of two separate signals yet analyzed as though only one signal was present, the true ultrasonic properties of the phantom were obscured using standard magnitude and phase spectroscopy analysis. This observation may provide insights into some aspects of the nature of the reported variations in ultrasonic characterization of cancellous bone. Understanding the mechanisms responsible for the observed negative dispersion could aid in determining the true material properties of cancellous bone, as opposed to the apparent properties measured using conventional data analysis techniques.

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3. Review, Priorities and Funding of NIH Programs

3.1 **Peer review for imaging technology at NIH**, Antonio Sastre, *Scientific Review Administrator, Innovative Ultrasound and Imaging, Center for Scientific Review, NIH, sastrea@csr.nih.gov* (invited)

3.2 **Latest developments and funding opportunities in the National Cancer Institute**, Houston Baker, *Program Director, Cancer Imaging Program, National Cancer Institute, NIH, bakerhou@mail.nih.gov* (invited).

3.3 **Latest developments and funding opportunities in the National Institute for Biomedical Imaging and Bioengineering**, Hector Lopez, *Program Director, Division of Applied Science and Technology, National Institute for Biomedical Imaging and Bioengineering, NIH, lopezh@mail.nih.gov* (invited).

4. ARFI/ELASTICITY 1

4.1 **Acoustic radiation force impulse imaging: optimization for clinical applications**, Kathryn R. Nightingale, Mark L. Palmeri, Liang Zhai, Michael Wang, Ned Rouze, Kristin Frinkley, David Bradway, Jeremy Dahl, Stephen Hsu, Doug Dumont and Gregg Trahey, *Department of Biomedical Engineering, Duke University, Durham, NC 27708-0281, kathy.nightingale@duke.edu* (invited overview).

Acoustic radiation force based imaging methods comprise a subset of elastographic imaging methods that utilize acoustic radiation force to mechanically excite tissue and then monitor the tissue response with ultrasonic methods. Acoustic Radiation Force Impulse (ARFI) imaging is a method in which the displacement response within the region of excitation is monitored and differences in displacement magnitude and timing are reflective of differences in tissue viscoelastic properties. As with conventional ultrasonic imaging, the optimum beam sequences, acoustic parameters and data postprocessing methods vary as a function of clinical application. ARFI imaging sequences are implemented on a modified Siemens Antares scanner, using commercially-available transducers. Typically, sequences are implemented with pushing pulses at a lower frequency and displacement monitoring pulses at a higher frequency within the transducer bandwidth. Displacement monitoring is performed using correlation-based methods. ARFI images provide interesting structural information that is well correlated with matched B-mode images. In many instances, ARFI images demonstrate improved contrast over conventional ultrasound images. For lesion visualization, single frame, multi-focal zone implementations provide maximum contrast and boundary discrimination. For screening applications, tradeoffs can be made between frame-rate and contrast that facilitate repeated tissue interrogations and more extensive fields of view. Methods for quantifying tissue stiffness through monitoring radiation force induced shear wave propagation, as originally proposed by Sarvazyan, are also under investigation. Results from ongoing clinical studies using these methods in a variety of organs (e.g. liver, prostate, breast and heart) will be presented.

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4.2 Can laser-induced microbubbles be used to assess the viscoelasticity of the surrounding tissue? Stanislav Emelianov, Andrei Karpiouk, Salavat Aglyamov, *Department of Biomedical Engineering, University of Texas at Austin, Austin TX 78712, emelian@mail.utexas.edu* (invited).

The interaction of tissue with nanosecond to femtosecond pulsed laser light is used in several biomedical and clinical applications ranging from diagnosis to therapy. In microsurgery, for example, one or a sequence of short, intense laser pulses produces a localized surgical effect through the process of laser-induced optical breakdown where precise photodisruption of soft tissue in the focal zone is produced. However, to insure successful presurgical planning, surgical procedure and postoperative stages of pathology treatment, the primary tissue must be analyzed before and after selective laser intervention.

During laser-induced optical breakdown, the shock wave is emitted from the site of laser-tissue interaction as highly-confined and fast-expanding plasma is created and a cavity is then transformed into a microbubble. We have developed an ultrasound method both to characterize laser-tissue interaction in a turbid medium and to assess mechanical properties of tissue utilizing passive (i.e., natural) and active (i.e., externally-induced) dynamics of the gas microbubble. Indeed, high temporal and spatial resolution, real-time measurements of the size and location of the cavity and translation and deformation and oscillations of the gas microbubble are possible using high-frequency ultrasound detection of shock waves and active pulse-echo probing of the site. In addition, the analytical and numerical models of gas bubble behavior in a viscoelastic medium were derived to assess mechanical properties of the medium immediately surrounding the microbubble.

The experiments were conducted in water and gelatin samples of various concentrations to simulate a tissue environment. The samples were irradiated using laser pulses of different levels of laser fluence. To provide external excitation of the bubble, a 1.5 MHz focused ultrasound transducer was used. The passive oscillations of the laser-induced cavity and ac-

tive translation and vibration of the gas bubble were measured using either 25 MHz or 48 MHz focused ultrasound transducers.

The results of our theoretical, numerical and experimental studies of gas bubble dynamics in a viscoelastic medium demonstrate that measurements of gas bubble dynamics can be used to assess the mechanical properties of the tissue. Furthermore, using the developed technique, bubble behavior in a viscoelastic medium and bubble response to internal or external excitation can be studied. Finally, the physics of laser-tissue interaction at micrometer/microsecond scale can be studied using high-frequency ultrasound.

4.3 Challenges in development of a clinical vibro-acoustography system, Mostafa Fatemi, *Mayo Clinic College of Medicine, Rochester, MN, fatemi@mayo.edu* (invited).

Vibro-acoustography is an imaging method based on vibroacoustic response of tissue. Studies of human and animal subjects as well as excised tissues specimens have produced promising results. Organ- and disease-specific studies, such as imaging breast and prostate for detection of various lesions, have demonstrated the potential of this technology for clinical applications. These studies have generally been conducted using various 'experimental' implementations of vibro-acoustography. Translation of this technology to the clinic requires the development of a clinical-grade vibro-acoustography system and testing its performance under clinical settings.

This paper focuses on the development of a clinical vibro-acoustography system. The new vibro-acoustography system utilizes a clinical (B-mode) ultrasound system as the base platform. The ultrasound system provides the two intersecting ultrasound beams necessary for vibro-acoustography. The vibroacoustic response of tissue (i.e, the acoustic signal) is detected by an audio hydrophone and processed by a separate unit to produce the image. This approach has two advantages: (1) it takes advantage of the existing beam forming and transducer technologies available in modern clinical ultrasound systems; and (2) it provides the flexibility of performing vibro-acoustography and traditional B-mode in a single dual-modality imaging system. This approach, although advantageous in some aspects, introduces new challenges. For example, vibro-acoustography requires transmission of two ultrasound tone-bursts at two different frequencies. For this purpose, one needs to generate two independent, but co-focused, beams using the same transducer. Other challenges include generation of sufficient ultrasound power and achieving the desired resolution. This paper discusses problems and solutions in developing a clinical vibro-acoustography system, and presents design strategies in the context of clinical applications.

This work is supported by Grants EB 00535, CA 91956 and CA 127235 from the National Institute of Health. Mandatory disclosure of conflict of interest: Some of the techniques presented here are patented by Mayo Clinic and the author.

4.4 Carotid plaque morphology and composition using a combined ARFI/B-mode/Doppler imaging system, Jeremy Dahl,¹ Douglas Dumont,¹ Brett Byram,¹ Jason Allen,² Elizabeth Miller,² and Gregg E. Trahey,^{1,3} ¹*Department of Biomedical Engineering, Duke University, Durham, NC,* ²*Department of Medicine, Duke University, Durham, NC* and ³*Department of Radiology, Duke University, Durham, NC, jjd@duke.edu.*

Atherosclerotic plaque in the carotid artery is considered a primary cause of ischemic stroke. Evidence suggests that ischemic stroke is associated less with calcified and fibrous plaques than with those containing softer tissue such as lipid pools, macrophages, foam cells and debris from intraplaque hemorrhage. The soft tissue is often surrounded by a fibrous cap, which is prone to rupture if it is thin. Unfortunately, the definition of a vulnerable plaque remains somewhat unclear, because the cap thickness defining vulnerability varies in the literature from 65 μ m to up to 700 μ m.

Acoustic Radiation Force Impulse (ARFI) imaging is an ultrasonic imaging method developed for imaging the mechanical properties of tissue. The technique uses commercially-available ultrasound scanners to generate short duration acoustic radiation forces that cause localized displacements in tissue of approximately 1-10 μm . The response of the tissue to the radiation force is observed using conventional B-mode imaging pulses and images are formed from the displacements generated.

We have combined ARFI imaging with B-mode and Doppler imaging to construct 2D and 3D imaging technique for observing the morphology and composition of atherosclerotic plaques in the carotid artery. We have created high-resolution 2D and 3D ARFI images in 10 healthy and 15 diseased patients displaying the capabilities of this system. Images in healthy patients show smooth arterial walls with little variance in the vascular stiffness. Images of carotid plaques in diseased patients show large, homogeneously-stiff or heterogeneous-soft/stiff occlusions with irregular borders. For some patients, a fibrous cap was visible over a soft tissue core.

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4.5 Measurement of arterial wall thickness using acoustic radiation force impulse imaging, Jeremy Dahl,¹ Douglas Dumont,¹ Jason Allen,² Elizabeth Miller² and Gregg E. Trahey^{1,3} ¹*Department of Biomedical Engineering, Duke University, Durham, NC,* ²*Department of Cardiology, Duke University, Durham, NC* and ³*Department of Radiology, Duke University, Durham, NC, dmd@duke.edu.*

Carotid intima-media thickening (C-IMT) has been shown previously to be an important risk marker for cardiovascular disease and events. Typically, the intima-media is visualized as a double-line pattern with ultrasound, appearing as a long-parallel structure bounded by the leading edges of the lumen-intima and adventitia-media interfaces. While the IMT can be, and is, often measured at both walls, preference is generally given to far-wall measurements as resolution of the double-line pattern necessary for accurate IMT measurement can be difficult in the near wall due to acoustic clutter and other confounding factors.

Acoustic radiation force impulse (ARFI) imaging is a technique that measures the local displacement of tissue to an applied radiation force. ARFI has previously been shown to be well-suited for visualizing both diseased and healthy vascular tissue. ARFI displacement images typically visualize the entire vascular wall (adventitia and intima-media), with vascular tissue generally displacing less than surrounding muscle, fat and fascia. In this work, we present the results from a study comparing IMT measurements with ARFI-derived wall thickness in the common carotid from twenty-four volunteers. Volunteers were divided into both a high-risk and low-risk group for cardiac disease according to their ARIC percentile.^(1,2) Proximal and distal wall IMT was found to be statistically larger in the high-risk group than the low-risk group ($p = 0.007$ proximal wall, $p = 0.008$ distal wall). Proximal and distal ARFI wall-thickness was also found to be statistically larger in the high-risk group than the low-risk group ($p = 0.008$ and $p = 0.003$). Adventitia thickness (ARFI-derived arterial wall thickness - IMT) was not different between groups for the proximal or distal walls ($p = 0.74$ and $p = 0.78$, respectively). Our results suggest that ARFI-derived wall thickness may be a viable companion to IMT, especially in difficult-to-image patients in which the double-line pattern may not be easily observed by conventional ultrasound.

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(1) *Stroke* 9, 1297–1304 (1993).

(2) *Am J Epidemiol* 5, 478–487 (2000).

5. ARFI/Elasticity 2

5.1 Spatially-modulated acoustic radiation force: theory and initial applications, Stephen McAleavey, *Department of Biomedical Engineering, University of Rochester, Rochester, NY, stephenm@bme.rochester.edu* (invited).

Quantitative, noninvasive methods for estimating tissue shear modulus are potentially useful in applications from detection of diffuse diseases, e.g., liver fibrosis, to monitoring of mechanical properties of engineered tissues *in vitro* and *in situ*. Recently, we have developed an acoustic radiation force based method for tissue modulus estimation called Spatially-Modulated Ultrasound Radiation Force (SMURF). Short bursts of ultrasound with a deliberate spatial variation in intensity are used to generate shear waves of known wavelength. The propagation of this shear wave is measured using ultrasound tracking methods and the temporal frequency of the shear wave estimated. Given the known wavelength and material density and the measured estimate of temporal frequency, the shear modulus at the point of excitation may be calculated easily from the relationship $G = (\rho f)^2$.

We will present current results of our studies of this method. We have programmed a Siemens Antares scanner to generate spatially-varying pushing beams. The methods for push beam generation will be described. Techniques for image formation using SMURF as well as images of shear modulus in phantoms of known geometry will be presented. We are currently investigating the mechanical properties of collagen gels containing cells. Changes in mechanical properties of these gels in response to varying degrees of extracellular matrix development is detectable with SMURF and will be presented.

5.2 Signal processing to reduce decorrelation in ultrasound motion estimation, W.F. Walker,^{1,2} F.W. Mauldin¹ and F. Viola,¹ *Department of Biomedical Engineering, University of Virginia and ²Department of Electrical and Computer Engineering, University of Virginia, bwalker@virginia.edu* (invited).

Ultrasound motion estimation is a foundational component of clinical and research techniques including Color Flow Doppler, Spectral Doppler, Radiation Force Imaging, and Sonorheometry. In each of these applications, motion estimates are corrupted by signal decorrelation resulting from nonuniform target motion across the acoustic beam. In blood flow imaging, nonuniform motion results from cross beam flow and from blood shear. In radiation force imaging and sensing, nonuniform motion results from the variation in applied radiation force across the beam profile. In both applications, resultant decorrelation has been believed to place a fundamental limit on the performance of these techniques.

In this paper, we present a novel signal processing approach that dramatically reduces decorrelation in blood flow estimation and radiation force imaging and sensing. The proposed method is easily implemented in modern hardware. Initial simulation results show an 87.6% reduction in the sum squared error of time delay estimates under reasonable imaging conditions. The method was tested on experimental data acquired from the common carotid artery of a healthy 25 year old male volunteer. Raw radiofrequency echo data was acquired using an Ultrasonix Sonix RP system under custom software control. In this experiment, the correlation level was increased from 0.90 to 0.9997 and the standard deviation of peak blood velocity estimates were reduced by 59.9%. Additional experiments were performed using our custom radiation force sensing system. The system was used to quantify the clotting properties of fresh whole blood acquired from a healthy 39 year old male volunteer. Application of the novel processing method improved signal correlation from approximately 0.995 to 0.999994. The standard deviation of peak displacement estimates was reduced by an average of 51.3%. Time to clot estimates obtained over 10 trials showed a 16.5% reduction in standard deviation.

The described method is readily implemented and has the potential to dramatically improve the performance of blood velocity estimation and radiation force imaging and sensing applications.

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5.3 Supersonic shear imaging: quantitative imaging of tissues viscoelasticity, Mickaël Tanter,¹ Jeremy Bercoff,² Thomas Deffieux,¹ Jean-Luc Gennisson,¹ Gabriel Montaldo¹ and Mathias Fink,¹ ¹*Laboratoire Ondes et Acoustique, ESPCI, CNRS, INSERM, Université Paris VII, Paris, France* and ²*Supersonic Imagine, France, mickael.tanter@espci.fr* (invited).

This paper presents a review of the supersonic shear imaging modality and initial clinical evaluations for breast lesions imaging. This technique is based on the combination of a radiation force induced in tissue by an ultrasonic beam and ultrafast imaging sequence capable of catching in real-time the propagation of the resulting shear waves. The local shear wave velocity is recovered using a time-of-flight technique and enables two-dimensional (2D) mapping of shear elasticity. This imaging modality is implemented on a conventional linear probe driven by a dedicated ultrafast echographic device. Consequently, it can be performed during a standard echographic exam. *In vivo* assessment of dispersion affecting the propagation of viscoelastic waves in soft tissues will be described as it is key to understanding the rheological behavior of human tissues. The estimation of dispersion curves is local and can be performed separately in different regions of the organ. This signal processing approach based on the supersonic shear imaging modality introduces a new concept of *in vivo* shear wave spectroscopy that could potentially become a great tool in tissue characterization and medical diagnosis. The *in vivo* ability of this Shear Wave Spectroscopy to quantify local shear elasticity and viscosity will be illustrated.

5.4 Comparison of methods to measure the speed of shear waves generated by acoustic radiation force, Ned C. Rouze, Mark L. Palmeri and Kathryn R. Nightingale, *Department of Biomedical Engineering, Duke University, Durham, NC 27708-0281, ned.rouze@duke.edu*.

Background: Acoustic radiation force can generate shear waves at remote positions within tissue. Tracking these waves gives a measure of the shear wave speed and, thus, tissue stiffness, that may be used to assess tissue health. Typically, shear wave tracking is performed by measuring tissue displacement through time at positions laterally offset from the radiation force excitation. Characteristic features of the wave are identified and the times for these features to reach fixed lateral positions are estimated. The shear wave speed is found by assuming a linear relation between time vs. position data.

In this report, we compare seven methods used to identify characteristic features of displacement vs. time profiles and evaluate these methods in terms of accuracy, sensitivity to jitter and computational efficiency. The seven methods identify the following: (a) time of peak displacement as described by Palmeri et al⁽¹⁾ (b) time for peak displacement of a quadratic function fit to displacement data, (c) time for the leading edge of the wave to reach the half-maximum displacement, (d) time for peak displacement of a Gaussian function fit to the leading edge, (e) time of the leading edge from the Gaussian fit, (f) time delay from cross correlation of displacement profiles relative to a reference profile⁽²⁾ and (g) time delay from cross correlation between adjacent displacement profiles.

Methods: The accuracy of each method was evaluated using simulated data. Finite-element methods⁽³⁾ were used to model the response of materials with known properties following radiation force excitation. Elastic materials with Young's moduli in the range 1.0 – 48.0 kPa, a Poisson's ratio of 0.499 and shear wave speeds in the range 0.5 – 4.0 m/s were simulated. For each material, 20 realizations with randomly-distributed scatterers were

generated, and simulation of ultrasonic imaging of these displacement fields was performed using Field-II.⁽⁴⁾ Experimental validation was performed with data acquired using a Siemens SONOLINE Antares scanner and a PH4-1 transducer in homogeneous phantoms with shear wave speeds of approximately 1.3 m/s. Ten acquisitions were performed at different positions within each phantom to evaluate the reproducibility of the measurements. Computational efficiency was evaluated by comparing the run times required to determine the shear wave speed from experimental phantom data.

Results: Each of the methods gives systematic deviations from the theoretically-predicted shear wave speeds that are less than 0.08 m/s. Methods (c) and (e), which identify the leading edge, overestimate the speed while methods (a), (b), and (d), which identify the peak displacement, underestimate the speed. The cross correlation methods (f) and (g) give the best performance with typical deviations on the order of 0.01 m/s. SNR (defined as shear wave speed/standard deviation) determined from the 20 scatterer realizations were typically on the order of 100. For the experimental measurements, SNR values were typically on the order of 25. The Gaussian fit methods (d) and (e) and cross correlation methods (f) and (g) gave the best results with SNR values typically on the order of 40. All of the methods had approximately equal computational efficiency except for the Gaussian fit methods (d) and (e), which required roughly 40 times greater computational effort.

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(1) *Ultrasound Med Biol* 34 (2008).

(2) *Inv Prob* 22, 681-706 (2006).

(3) *IEEE Trans Ultrason Ferroelec Freq Contr* 52, 1699-1712 (2005).

(4) *IEEE Trans Ultrason Ferroelec Freq Contr* 39, 262-267 (1992).

5.5 Advanced pulse sequences for ARFI imaging, Richard Bouchard,¹ Stephen Hsu,¹ Jeremy Dahl,¹ Chen W. Ong¹ and Gregg E. Trahey,^{1,2} ¹*Department of Biomedical Engineering, Duke University, Durham, NC* and ²*Department of Radiology, Duke University Medical Center, Durham, NC, rrb@duke.edu.*

The real-time application of Acoustic Radiation Force Impulse (ARFI) imaging *in vivo* requires short acquisition times for a single ARFI image, repeated acquisition of these frames and an effective motion filter to reduce physiologic motion. Due to the high energy of pulses required to generate appreciable radiation force, however, repeated acquisitions could result in substantial transducer face and tissue heating. We describe and evaluate several beam sequencing schemes that are designed to reduce acquisition time and heating. These techniques reduce the total number of radiation force impulses needed to generate an image and minimize the time between successive impulses. Additionally, we describe novel beam sequencing schemes that allow for more robust and effective motion filters. We have implemented these sequences on a commercially-available diagnostic ultrasound scanner and present qualitative and quantitative analyses of the trade-offs in image quality resulting from these acquisition schemes in a tissue-mimicking phantom. Results indicate that these techniques yield a significant improvement in frame rate with only moderate decreases in image quality. Tissue and transducer face heating resulting from these schemes is assessed through finite element method (FEM) modeling and thermocouple measurements.

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5.6 Acoustic radiation force impulse imaging of myocardial stiffness propagation, S.J. Hsu, J.L. Hubert, B.C. Byram, P.D. Wolf and G.E. Trahey, *Duke University, Department of Biomedical Engineering, Durham, NC 27708, sjh6@duke.edu.*

Acoustic radiation force impulse (ARFI) imaging has been demonstrated to be capable of measuring local myocardial stiffness changes of heart through the cardiac cycle. Myocardial stiffening occurs during muscle contraction, which is stimulated by myocardial depolarization. As a result, the propagation of stiffness within the heart should follow the propagation of the electrical action potential. We have generated high temporal and spatial resolution ARFI imaging sequences that utilize ECG gating and multi-beat synthesis to measure the propagation of mechanical stiffness waves through a heartbeat.

ARFI images of the left ventricular free wall of an exposed *canine* heart were formed. These images were acquired while the heart was paced externally from the epicardial surface by one of two electrodes positioned on either side of the imaging plane. Two-line M-mode ARFI images were acquired at a sampling frequency of 120 Hz while the heart was being paced from either stimulating electrode. Two-dimensional ARFI images that were also triggered off the stimulating electrodes were formed with multi-beat synthesis across seven heartbeats at a frame rates of 65 Hz. The images were inspected and analyzed to determine a direction and velocity of stiffness propagation. Passive ARFI images, where the radiation force pulse amplitude was set to zero, also were acquired to determine the levels of physiological motion artifact within the ARFI images.

ARFI imaging was able to determine a direction and velocity of the propagation of mechanical stiffness through the myocardium. This stiffness wave followed the expected electrical activation propagation during external pacing and the measured velocities of myocardial stiffness propagation are comparable to typical values of other previously measured electrical conduction velocities. From these results, we believe ARFI imaging to be a promising imaging modality to measure electromechanical wave propagation and determine local myocardial function.

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5.7 ARFI ultrasound for monitoring hemostasis at femoral artery puncture *in vivo*, Russell H. Behler,¹ Timothy C. Nichols,² Melrose W. Fisher,² Melissa C. Caughey² and Caterina M. Gallippi,¹ ¹*Joint Department of Biomedical Engineering¹ and ²Division of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27519, cmgallip@bme.unc.edu.*

Background: The reported incidence of severe vessel complications (including hematomas, pseudoaneurysms, fistulas and groin infections) associated with femoral artery puncture for angiography or intervention is as high as 26%. *In vivo* imaging of hemostasis at the arteriotomy could lower vascular complication rates, facilitate timely ambulation, reduce cost and evaluate the relevance of vascular closure devices relative to manual compression.

Methods: ARFI imaging was performed in a pilot study of 15 (9F, 6M) patient volunteers following routine cardiac catheterization. Serial ARFI images of the arterial puncture site and surrounding soft tissue were collected during manual compression with or without the pGlcNAc fiber-based patch (Marine Polymer Technologies, Danvers MA) until hemostasis was achieved.

Results: ARFI imaging revealed progressive decreases in bleeding rate and distinguished time to hemostasis by two factors: (1) ARFI-induced streaming of blood pooled in soft tissue above the arteriotomy and (2) relative stiffness of the arterial puncture site. Smaller times to hemostasis were observed when the pGlcNAc fiber-based patch was employed in this small pilot study.

Conclusion: This work demonstrates the feasibility of noninvasive ARFI imaging for monitoring hemostasis at femoral artery puncture, *in vivo*.

6. Heart

6.1 Measurements of the magnitude of cyclic variation of backscatter in the interventricular septum of normal fetal human hearts at mid-gestation, Mark R. Holland, Allyson A. Gibson, Carol A. Kirschner, Deborah Hicks, Achiau Ludomirsky and Gautam K. Singh, *Washington University in St. Louis, St. Louis, MO, mrh@wuphys.wustl.edu*.

Background: The interventricular septum is one of the earliest segments of the heart to exhibit rapid growth during fetal development and contributes to the overall growth of both ventricles. Observations suggest the septum is affected early by growth perturbances that consequently result in a hypoplastic right or left ventricle. In fetuses that exhibit cardiac hypertrophy, such as in some of the offspring of diabetic mothers or in those with hypertrophic cardiomyopathy, the septum is often the earliest segment to hypertrophy. Thus, logically and morphogenetically, characterization of the septum may provide an early indicator of the perturbation in the growth and development of the fetal heart. Measurements of the systematic variation of backscattered ultrasound from the myocardium over the heart cycle (i.e., cyclic variation of backscatter) may represent a useful approach for characterizing the interventricular septum during fetal heart development.

Objective: The goal of this investigation was to measure the magnitude of cyclic variation from the interventricular septum of hearts of normal human fetuses at mid-gestation and compare these values with those obtained from the left and right ventricular free walls. This study represents a further step in establishing regional differences in measurable echocardiography-based features that may be useful in developing enhanced diagnostic methods to assess altered heart development.

Methods: Echocardiographic images of 11 fetuses (20 to 28 weeks gestation) with structurally normal hearts were obtained using an imaging system configured to give a linear relationship between the displayed grayscale value and the level of ultrasonic backscatter expressed in decibels. Long-axis images of the fetal hearts were analyzed by placing regions-of-interest in the interventricular septum and the walls of the left and right ventricles. Cyclic variation data were generated by measuring the mean backscatter level within each region-of-interest for each of the acquired image frames over several heart cycles.

Results: The measured mean magnitude of the cyclic variation backscatter from the interventricular septum was found to be 2.8 ± 1.6 dB compared with values of 4.5 ± 1.2 dB and 2.4 ± 1.0 dB (mean \pm SD) for the left and right ventricular free walls, respectively.

Conclusion: Results show that the measured magnitude of the cyclic variation of backscatter from the interventricular septum was similar to that observed from the right ventricular free wall but significantly less than that observed for the left ventricular free wall ($p < 0.01$). This result would not have been anticipated based solely upon measured regional differences in the level of backscatter from the excised hearts of fetal pigs using a model relating the overall backscatter level and the predicted magnitude of cyclic variation. Thus, these measurements suggest the observed cyclic variation from the developing septum may reflect the influence of, and is morphogenetically consistent with, the complex origin and development of the septum and not from the exclusive contribution of the developing left ventricle. This observation is consistent with previous findings of the septum exhibiting characteristics of a morphologically and functionally-bilayered structure.

(1) *J Am Soc of Echocardiogr* 17, 1131-1137 (2004).

(2) *Ultrasound Med Biol* 33, 1955-1962 (2007).

6.2 Measurement of the cyclic variation of ultrasonic integrated backscatter from mouse hearts, Joseph J. Hoffman, Attila Kovacs, James G. Miller and Mark R. Holland, *Washington University in St. Louis, St. Louis, MO, hoffman@wustl.edu.*

Background: Measurements of the systematic variation of backscattered ultrasonic energy from the myocardium during the heart cycle (cyclic variation of backscatter) have been successfully used to characterize a wide spectrum of cardiac pathologies in large animal models and human subjects. Cyclic variation has also been estimated in mice using gray-scale analysis of 2D echocardiographic images acquired with clinical imaging systems.⁽¹⁾ Measurement of cyclic variation from mice based on analyses of high-frequency backscattered rf data will enhance the capability to characterize specific features of backscattered ultrasound arising from altered myocardial properties in transgenic mouse models.

Objective: The purpose of this study was to evaluate the feasibility measuring the cyclic variation of backscatter in mice using M-mode integrated backscatter images generated from high-frequency radiofrequency (rf) data.

Methods: Backscattered rf data were acquired from the hearts of mice under light anesthesia using a dedicated mouse research imaging system (VisualSonics Vevo 770). For each mouse interrogated, a series of backscattered rf A-lines was obtained from the parasternal long-axis view over several heart cycles using a single-element broadband transducer with nominal center frequency of 30 MHz (Probe Model 707B). The acquired backscattered rf data were converted into integrated backscatter M-mode images by applying a boxcar averaging filter to the received backscatter intensity signal. Cyclic variation measurements were obtained by determining the average value of the integrated backscatter within a region-of-interest placed in the midmyocardium of the posterior wall at each time step over several heart cycles. This cyclic variation data was characterized in terms of its average magnitude over the heart cycle.

Results: Preliminary measurements demonstrate an observable cyclic variation of integrated backscatter from the myocardium of all mice investigated at the high frequencies used. The nature of the observed cyclic variation of backscatter and estimates of its magnitude obtained with the high frequency rf-based integrated backscatter method appear similar to that of previous 2D-based studies at lower frequencies.

Conclusion: This study suggests that measurements of cyclic variation of integrated backscatter can be obtained at relatively high frequencies in mouse models. Preliminary results demonstrate a measured magnitude comparable to that observed in animal models and human subjects at lower frequencies. These results suggest that measurements of the cyclic variation of integrated backscatter may provide an approach for the longitudinal assessment of changes in myocardial properties in genetically manipulated mouse models of specific cardiac pathologies.

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(1) *J Am Soc of Echocardiogr* 17, 883-892 (2004)

6.3 Bayes classification and ROC analysis of the magnitude and time delay of cyclic variation of myocardial backscatter from asymptomatic type 2 diabetes mellitus subjects, Allyson A. Gibson,¹ Robert F. Wagner,² Jean E. Schaffer,¹ Linda R. Peterson,¹ Karla M. Robert,¹ Troy A. Haider,¹ Kyle R. Bilhorn,¹ Mark R. Holland¹ and James G. Miller,¹ *Washington University in St. Louis, Missouri, St. Louis, MO and*² *Food and Drug Administration, Silver Spring, MD, agibson@hbar.wustl.edu.*

Background: Type 2 diabetes mellitus is a growing concern in populations in which obesity is on the rise. Evidence is emerging that hyperlipidemia plays a central role in the pathogenesis of heart failure in diabetic patients, independent of atherosclerosis. Early de-

tection of diabetic patients at high risk for developing diabetic cardiomyopathy might permit effective intervention. The long-term goal of this exploratory study is to determine whether myocardial tissue characterization based on measurements of the magnitude and time delay of cyclic variation of myocardial backscatter might be a useful indicator of hearts at potentially higher risk for developing diabetic cardiomyopathy. Ultimately, a longitudinal study over many years would be required to answer this significant but challenging question.

Objective: In this preliminary investigation, we focused on the very modest goal of determining how well measurements of the magnitude and time delay of cyclic variation of myocardial backscatter, individually and in combination, can be used to segregate subgroups of individuals based on plasma markers.

Methods: In this study, two dimensional parasternal long axis echocardiographic images of 116 patients and normal volunteers, ages 30-55 years old, were acquired using a GE Vivid 7 ultrasonic imaging system utilizing a M3S probe. Images were acquired in the harmonic imaging mode, with the system configured to provide a linear relationship between backscatter intensity expressed in decibels and displayed grayscale value. Cyclic variation data were produced by measuring the mean backscatter level within a region-of-interest in the posterior wall of the heart for each recorded image frame over five heart cycles. The resultant cyclic variation data were characterized in terms of the magnitude and time delay relative to the systolic interval. Biochemical assays were performed on blood samples drawn from subjects after overnight fasting. ROC analyses were employed on the measured magnitude and time delay of cyclic variation, individually and in combination. The magnitude and time delay were combined using Bayes classification and then processed with ROC analysis to illustrate the relative effectiveness of using one or two features to segregate subgroups of individuals. In one study, the subjects were segregated based on those identified as having diabetes ($n = 78$) versus those identified as controls ($n = 38$). In another study, the same subject population was grouped based on the ratio of triglyceride to high-density lipoprotein cholesterol for each subject. This ratio is a modest surrogate for insulin resistance, exhibiting an area under the ROC curve of 0.75 for determining a subject's resistance to insulin.⁽¹⁾ (Kannel et al.,) *Subjects with the highest lipid ratio (fourth quartile, N=29) were classified as relatively insulin resistant compared to subjects with the lowest lipid ratio (first quartile, N=29).*

Results: For the study in which the subjects were grouped according to frank diabetes versus controls, ROC analysis of magnitude of cyclic variation yielded an area under the curve of only 0.56. Time delay of cyclic variation resulted in an area of 0.72. The combination of magnitude and time delay generated a ROC area of 0.74. For the study in which the subjects were segregated according to the ratio of triglycerides to high-density lipoprotein cholesterol, the magnitude of cyclic variation alone resulted in an area of 0.62 and time delay of cyclic variation yielded an area of 0.68. Combining the information from magnitude and time delay of cyclic variation resulted in an area under the ROC curve of 0.75.

Conclusion: Results indicate that by combining information from magnitude and time delay of cyclic variation using Bayes classification a larger area under the ROC curve is measured than when each feature is analyzed individually. Although the areas under the ROC curves obtained are quite modest, they are comparable to the areas obtained when the ratio of triglyceride to high-density lipoprotein cholesterol is used as a surrogate for insulin resistance. In the long view, these results suggest that monitoring the hearts of patients with type 2 diabetes using the combination of magnitude and time delay of cyclic variation of backscatter might permit observation of changes associated with potential lipotoxicity that could underlie the development of diabetic cardiomyopathy.

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(1) *Am J Cardiol* 101, 497-501 (2008).

7. Machine Learning

7.1 Machine learning for tissue characterization, Mark J. Rondeau, *The Margaret M. Dyson Vision Research Institute, New York, NY 10065, mark.rondeau@cornell.edu* (invited overview).

Machine learning is a broad, new academic field that sits somewhere near the corner of computer science and statistics. Recent advances in algorithms and theories, particularly in terms of supervised learning (classification problems), makes machine learning techniques a viable alternate to standard statistical approaches for many applications, including data mining and speech recognition. In this talk, we will examine the current state of a variety of machine-learning approaches that are appropriate to classification problems typically seen in diagnostic imaging and tissue characterization. These will include tree-based classifiers, as well as kernel-based methods and advanced variants of artificial neural networks, such as Deep Learning. Standard software environments, including WEKA, libsvm, SVMlight will be discussed and worked examples in ultrasound tissue characterization using the Cornell-Iowa-Chicago uveal melanoma database and the Riverside Research Institute prostate database will be presented.

8. Bone 2

8.1 Ultrasonic bone assessment with apparent backscatter, Brent K. Hoffmeister, David P. Johnson, John A. Janeski, Daniel A. Keedy, Brian W. Steinert, Ann M. Viano and Sue C. Kaste, *Department of Physics, Rhodes College, Memphis, TN, hoffmeister@rhodes.edu* (invited).

Ultrasonic bone assessment is a term commonly used to refer to the ultrasonic characterization of bone tissue. A wide variety of ultrasonic techniques are being developed to detect and monitor changes in bone tissue produced by osteoporosis and other degenerative bone diseases. Many techniques use separate transmitting and receiving transducers to propagate ultrasonic pulses either through or along regions of bone. However, certain clinically-interesting sites such as the hip and spine are not easily accessible to such measurements. To address this limitation, we are developing single transducer techniques based on backscatter measurements of bone. The procedure involves propagating broadband ultrasonic pulses through regions of cancellous bone and analyzing the apparent backscattered power as a function of frequency. The term 'apparent' means that backscattered signals are not compensated for the effects of attenuation and diffraction. We have performed *in vitro* measurements of human cancellous bone using single element broadband transducers with center frequencies of 1, 2.25, 5, 7.5 and 10 MHz. We observe that the apparent backscattered power generally decreases with frequency over the bandwidth of each transducer. In addition, we find that parameters based on frequency analyses of the apparent backscattered power correlate with the density and mechanical properties of cancellous bone. These results suggest that apparent backscatter may be useful for bone-assessment purposes.

8.2 Noninvasive characterization of trabecular bone quality in humans using scanning quantitative ultrasound imaging, Yi-Xian Qin,¹ T. Peng,¹ Y. Xia,¹ W. Lin,¹ B. Gruber² and C. Rubin,¹ *¹Department of Biomedical Engineering and ²Department of Medicine, Stony Brook University, SUNY, Stony Brook, NY, Yi-Xian.Qin@sunysb.edu* (invited).

Introduction: Microgravity and aging-induced bone loss is a critical skeleton complication occurring particularly in the weight-supporting skeleton, leading to osteoporosis and fracture. Bone integrity is dependent not only on the mineral density but also on the quality of bone, which includes strength and structural parameters. Advances in quantitative ultrasound (QUS) provide a unique method for evaluating both bone strength and density. Using a newly-developed confocal scanning ultrasound diagnostic system (SCAD), the goals of this work were to (1) noninvasively measure bone quality at critical skeleton sites, e.g., the proximal femur and (2) longitudinally monitor the effects of calcaneus bone loss and recovery in a 90-day bedrest.

Methods: QUS scanning performed at the proximal femur (cadaver) and calcaneus (bed-rest subjects) regions converged the ultrasound energy in the trabeculae. QUS images of $80 \times 80 \text{ mm}^2$ for hip and $40 \times 40 \text{ mm}^2$ for calcaneus were obtained, both with 0.5 mm resolution. QUS was processed to calculate the ultrasound attenuation (ATT; dB), wave ultrasound velocity (UV), and the broadband ultrasound attenuation (BUA; dB/MHz). An automatic region-of-interest (ROI) in bone was selected for total of 500 pixels.

Hip measurement: 22 human proximal femurs were measured with the SCAD. QUS data were correlated to bone volume fraction using microCT ($\sim 20 \text{ }\mu\text{m}$ resolution), DXA and mechanical strength tests.

Bed-rest measurement: The longitudinal evaluation of bone quality were performed in a total 17 human subjects during a 90-day continuous bedrest (8 disuse and 9 disuse plus treated, using 30Hz, 0.3g vibration) using SCAD and DXA in day 0 (baseline), day 60 and day 90. Interrelationships between QUS parameters and DXA-determined BMD were evaluated through multiple correlations, with a student T-test and the significance level was set at $p < 0.05$.

Results: Hip bone assessment: QUS parameters, combined UV and BUA prediction, are highly correlated to microCT BV/TV ($r^2 = 0.8$), Tb.Sp ($r^2 = 0.78$), Tb.N ($r^2 = 0.82$) and Tb.Th ($r^2 = 0.64$). In addition, similar relationship between the QUS parameters and bone mechanical properties were observed, such as the stiffness ($r^2 = 0.75$) and ultimate strength ($r^2 = 0.78$).

Bed-resting bone quality prediction: QUS indicated that disuse alone induced $1.2 \pm 0.4\%$ bone loss via UV while disuse plus treatment increase bone mass at $1.3 \pm 0.4\%$ with baseline. Longitudinal subtle changes were predicted by the UV and BUA comparing disuse plus treatment and disuse alone, i.e., 2.2% at 60 days and 3.3% at 90 days for UV, and 6.5% (60 days) and 19.2% (90 days). In disuse alone, a strong correlation was observed between BUA at the heel and pooled whole body (WB) BMD ($r^2 = 0.84$), and between UV and BMD at the calcaneus ($r^2 = 0.79$), WB ($r^2 = 0.85$), hips ($r^2 \sim 0.9$) and pelvis ($r^2 = 0.9$). However, no significant correlation was found between heel QUS and whole body BMD, suggesting the treatment and bone turnover may be localized.

Discussion: These results demonstrated that QUS measurement has the capability to predict bone BMD, microstructure and mechanical properties in human bone. QUS has demonstrated sensitivity to progressive change of bone quality, particularly in the trabecular bone region where remodeling occurs initially. Calcaneus QUS can be a good predictor for predicting longitudinal bone loss and recovery at particular treatment sites in the hip and pelvis, which can be used for instant and follow-up measurement of bone demineralization occurring during long-term space flights, and, ultimately, provide a portable, noninvasive device for bone loss assessment in space and on Earth.

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8.3 Experimental determination of Young's modulus of cortical bone tissue using high-resolution scanning acoustic microscopy, Fabienne Rupin,¹ Amena Saïed,¹ Davy

Dalmas,² Françoise Peyrin,³ Kay Raum,⁴ Etienne Barthel,² Georges Boivin and ⁵ Pascal Laugier,^{1,1} *Université Pierre et Marie Curie-Paris6, Laboratoire d'Imagerie Paramétrique, Paris, F-75005 France; CNRS, LIP, Paris, F-75006 France, ²Unité Mixte CNRS/Saint-Gobain "Surface du Verre et Interface" UMR 125, Saint-Gobain Recherche, F-93303, Aubervilliers, France, ³ESRF/CREATIS, 38043 Grenoble, France, ⁴Q-BAM Group, Dept. of Orthopedics, Martin Luther University of Halle-Wittenberg, 06097 Halle, Germany and ⁵INSERM Unité 831, Faculté de Médecine Laennec, Université de Lyon, 69372 Lyon, France, laugier@lip.bhdc.jussieu.fr, rupin@lip.bhdc.jussieu.fr.*

The assessment of the microscopic mechanical properties of bone tissue is important for understanding the pathogenesis of many metabolic skeletal diseases. Knowledge about these properties provides data sets to the developed computational models aiming at analyzing the biomechanical behaviour of the whole organ. The micron-level mechanical properties of bone are classically determined using nanoindentation. Despite the improvements in the technique accuracy, data are obtained in a limited number of points and the test is inherently destructive (plastic deformation of the tissue at the contact area). As a noncontact technique, Scanning Acoustic Microscopy (SAM) provides, nondestructively, images directly related to bone density and elastic stiffness. The current work aimed at determining the micron-level elastic stiffness (Ea) of bone generated from SAM-based acoustic impedance (Z) for the dual purpose of comparing, on site matched regions, Ea to nanoindentation elastic modulus (En) and correlating the two modulus to the degree of mineralization of bone (DMB). A 200 MHz SAM-based acoustic impedance (8 μm spatial resolution) was combined with synchrotron radiation microtomography (for bone mineral density at 10 μm resolution level) to map the distribution of near surface Ea of two embedded cortical bone transverse sections taken from femoral mid-diaphysis of two women cadavers (78 and 85 years of age). Nanoindentation measurements were done in line scans across the cortical radial direction (in the peripheral, middle and inner layers) of each anatomical quadrant ($n = 8$). Within a single quadrant, regions of osteonal and interstitial tissue were tested (2 line scans, 30 indents each at 30- μm interval with a 2- μm depth, totalling 240 indents per transverse section). The position of the indents within the samples (produced before synchrotron microtomography) were visualized using SAM imaging. Indent images, Ea and DMB type maps were digitally matched using a custom-developed image fusion and analysis software in order to correlate the local two modulus and mineral density with mechanical properties. Comparison between Ea and En was first performed on three homogeneous materials (aluminium, PMMA and polycarbonate) of known Poisson ratio (ν). In these materials, the mean values of Ea and En differed by less than 1%. In bone, DMB varied between 0.6 and 1.2 g/cm^3 and Z ranged between 5 and 13 Mrayl, resulting in an elastic modulus Ea varying between 10 and 65 GPa for $\nu = 0.3$. En showed a lower range (between 11 and 27 GPa). A good linear correlation was found between Z and En ($R^2 = 0.57, p < 0.001$) as well as between Ea and En ($R^2 = 0.59, p < 0.001$). The DMB variability appeared to have low significant effect on Ea ($R^2 = 0.30, p < 0.001$) and En ($R^2 = 0.26, p < 0.001$) variabilities. The discrepancy between Ea and En may likely be due to the uncertainty in determining the value, which is known to vary between 0.2 and 0.4 in bone. Analysis of spatial variation in the stiffness of bone throughout bone tissue is currently underway to determine the relationships between the histology and mechanical properties of bone samples. This preliminary work has demonstrated the value of SAM estimates of Young's modulus. Combined with nanoindentation, SAM may be used to derive the local Poisson's ratio.

8.4 Development of bone as a hierarchical material/structural composite, J. Lawrence Katz, Bioengineering Research Center (BERC), University of Kansas, Lawrence, KS 66045, jaelkaye@ku.edu.

Beginning in 1968, I began using ultrasonic wave propagation (UWP) techniques to study the elastic properties of both hydroxyapatite (HAp) and human compact cortical bone. The first paper on modeling the single crystal elastic constants of HAp and Bone as transverse isotropic solids was published in 1971. Using the UWP data on HAp and collagen properties in the literature, I was able to model bone as a simple two-phase composite material, also published in 1971. In that paper, I indicated that such a simple model would not work, so I began to realize that the actual hierarchical structure of bone had to be considered in both the experiments and the modeling as a composite. I first published a paper outlining this concept in 1971 in the *Israel J. Med. Sci.* This was followed by a series of UWP studies of human compact cortical bone's anisotropic elastic properties, published in 1976. During this same period, I had my students use SEM and x-ray diffraction studies of single osteons and osteon lamellae, the microstructural elements of cortical bone. The data resulting from these studies allowed me to calculate the elastic properties of bone as a hierarchical material/structural composite, published in a series of papers in various journals, proceedings, and books from 1976-1979. These calculations culminated in my 1980 publication in *Nature* where I adapted the Hashin-Rosen model for hollow fiber reinforced composites, using the osteonic microstructural organization of bone in my adaptation. Descriptions of the impact of this early proposal of mine can be found in John Currey's books as well as in the Steve Cowin edited *Bone Mechanics* volume. This initial description has since been picked-up and advanced by a number of other experimentalists and modelers. In the years since this early work, my UWP measurements and modeling have continued with studies of trabecular bone properties as well as with increased understanding of the influence of anisotropy on bone's elastic properties.

9. Quantitative Ultrasound 2

9.1 High-frequency quantitative ultrasound imaging of human lymph nodes, Jonathan Mamou,¹ Alain Coron,² Masaki Hata,³ Junji Machi,³ Eugene Yanagihara,³ Pascal Laugier² and Ernest J. Feleppa,¹ *¹Riverside Research Institute, New York, NY, ²CNRS and Université Pierre et Marie Curie-Paris 6, Paris, France and ³University of Hawaii and Kuakini Medical Center, Honolulu, HI, mamou@rrinyc.org.*

High-frequency ultrasound offers a means of investigating biological tissue at the microscopic level. We developed high-frequency quantitative ultrasound (QUS) methods to evaluate freshly-dissected lymph nodes of cancer patients and to test our hypothesis that QUS can differentiate and detect nodal metastases. Three-dimensional (3D) radiofrequency echo-signal data were acquired from lymph nodes using a 25.6-MHz center-frequency transducer. Each node was inked prior to 3D histological fixation to enable proper spatial orientation of ultrasonic images and QUS results with respect to histological sections. Echo signals were processed to yield two QUS estimates associated with tissue microstructure: scatterer size and acoustic concentration. The QUS estimates were obtained following established methods using a Gaussian-scattering model. QUS images were generated by expressing QUS estimates as color-encoded pixels and overlaying them on conventional gray-scale B-mode images. Four lymph nodes from a patient with stage-3 colon cancer were compared using these methods; histology showed that one of the four nodes contained metastatic cancer and the remaining three were cancer free. The metastatic node had an average estimated scatterer size that was significantly larger than the average estimates of scatterer size in the cancer-free nodes and the statistics of both QUS estimates in the metastatic node were less uniform than the QUS statistics in the other nodes. These initial results demonstrate the validity of our QUS methods and suggest a potential tool for identifying meta-

static foci in lymph nodes including micrometastases that might not be detected by current standard pathology procedures. High-frequency echo-signal data from additional lymph nodes, including axillary sentinel lymph nodes of breast-cancer patients, as well as those from colon and rectal cancer patients, currently are being investigated using this approach.

9.2 Quantitative ultrasound assessing tumor responses to radiotherapy in cancer mouse models, Roxana M. Vlad,^{1,2} Sebastian Brand,³ Anoja Giles,² Michael C. Kolios,^{1,4} and Gregory J. Czarnota,^{1,2,5} ¹*Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, M4N 3M5,* ²*Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, M4N 3M5,* ³*Martin-Luther-University of Halle-Wittenberg, Orthopaedic Clinic, Magdeburger St. 22,06112 Halle, Germany,* ⁴*Department of Physics, Ryerson University, Toronto, Ontario, Canada, M5B 2K3* and ⁵*Department of Radiation Oncology, University of Toronto, Toronto, Ontario, Canada, M5G 2M9, roxana.vlad@sunnybrook.ca.*

Purpose: Currently, there is no routine imaging modality to assess tumour responses to cancer treatment within hours to days after delivery of radiation treatment. In this study, we demonstrate the application of ultrasound imaging and quantitative ultrasound to characterize tumor responses to cancer radiotherapy *in vivo* as early as 24 hours after treatment delivery.

Methods: Two mouse models (FaDu and C666-1) of head and neck cancer were exposed to different radiation doses of 2, 4 and 8 Gy. Data were collected with an ultrasound scanner using frequencies of 10-30 MHz (VisualSonics VS40B). Parameters calculated from normalized power spectra were used to form colour-coded parametric images. These were constructed by superimposing estimates of the ultrasonic parameters on top of the ultrasonic images and were used as indicators of response.

Results: Tumors exhibited large variable hyperechoic areas at 24 h after radiotherapy indicating heterogeneous tumor responses. Measured ultrasound integrated backscatter increased by 6.5-8.2 dB ($p < 0.001$) and spectral slopes increased by 20 to 40% ($p < 0.05$) in these regions compared with untreated tumours and areas in a tumor that remained isoechoic even after treatment.

The hyperechoic areas in the ultrasonic images were found to correspond to areas that stained TUNEL positive for apoptosis. These were characterized by pyknotic nuclei, nuclear condensation and fragmentation, the morphological characteristics of cell death by apoptosis. Parametric images computed from the estimates of ultrasound integrated backscatter and spectral intercept were able to differentiate such regions of the tumor that responded to treatment from those that did not.

Conclusions: The results indicated that the cell structural changes following radiotherapy may have a profound influence on ultrasonic spectral parameters. This provides a foundation for future investigations regarding the use of ultrasound in cancer patients to individualize treatments noninvasively based on their responses to specific interventions.

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9.3 Changes measured in the backscattered ultrasound signals during cell death can be potentially explained by an increase in cell size variance, Roxana M. Vlad,^{1,2} Veronika Orlova,³ John W. Hunt,¹ Michael C. Kolios^{1,3} and Gregory J. Czarnota,^{1,2,4} ¹*Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, M4N 3M5,* ²*Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, M4N 3M5,* ³*Department of Physics, Ryerson University, Toronto, Ontario, Canada, M5B 2K3* and ⁴*Depart-*

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Rationale: Ultrasound backscatter power measured from cell samples undergoing different types of cell death (apoptosis, mitotic arrest and a mixture of both) has been demonstrated to increase by 4-7 dB. Apoptosis and mitotic arrest are characterized by disparate cell structural changes. During apoptosis cell shrink, nuclei condense and fragment, whereas in mitotic arrest cells and nuclei can enlarge. Here we explore the mechanism by which cell structural changes during the sequence of cell death lead to the increases measured in ultrasonic signals. Our hypothesis is that cell death induces changes in cell size distributions towards increasing their randomization. Large changes in backscatter intensities have been predicted, in the past, for collections of scatterers, with the same number and properties, but different degrees of randomization.⁽¹⁾

Methods: Simulations of ultrasound scattering were compared to experimental results, in which four different cell lines (FaDu, AML, Hep2, C666) were exposed to radiation therapy and a chemotherapeutic drug, in order to produce different types of cell death. Ultrasound data were collected with an ultrasound scanner using frequencies of 10-30 MHz (Visual-Sonics VS40B). Attenuation-corrected ultrasonic integrated backscatter (UIB), cell size distributions and quantitative measurements of cell death were determined for all treatment conditions.

Results: The UIB increased in all cell lines, except C666-1, by 4-7 dB ($p < 0.001$) following exposure to therapy. The variance of cell sizes increased more than two fold for all cell lines, except C666-1. The UIB measured from viable cells and those exposed to therapy, correlated well with the variance of cell sizes ($r = 0.79$ and $p = 0.006$) and did not present a significant correlation with the average cell size. The changes measured in UIB with increasing variance of cell sizes were in good agreement with the simulations of ultrasound scattering with increasing randomization. These predicted no increase in ultrasound scattering for randomizations higher than a certain threshold consistent with the observed result for C666.

Conclusion: The increases measured in the UIB could be in part explained by the changes in the spatial organization of cells following the sequence of cell death. The rest of the contribution to the UIB increase can be potentially attributed to other changes (size, acoustic properties) in cells and nuclei following cell death.

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9.4 Quantitative ultrasound evaluation of the human cervix, Helen Feltovich,¹ Kibo Nam,² Maritza A Hobson,² Josephine M Harter,³ Mark A Kliewer⁴ and Timothy J Hall,²
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Objective: Despite 100 years of research, preterm delivery rates are increasing. The financial and emotional costs are staggering. Interventions that treat intrauterine infection, reduce inflammation, inhibit uterine contractions or stitch closed the uterine cervix do not prevent preterm birth. Studies of cervical biochemistry, molecular biology and tensile strength suggest that collagen is responsible for cervical strength. The cervix is composed of an extracellular matrix (predominantly collagen with elastin and proteoglycans) and a cellular portion (smooth muscle, fibroblasts, epithelium, and blood vessels). Organized, crosslinked collagen resists degradation. A long, slow process of cervical softening associated with col-

lagen fibril reorganization occurs well before active ripening in preparation for labor (preterm or term). Ripening involves synthesis of proteins, further reorganization and cervical shortening and is irreversible prior to delivery. Evaluation of cervical microstructure is critical to understanding tissue dysfunction like preterm cervical weakening (cervical insufficiency). Electron microscopy shows that cervical tensile strength is associated with collagen fibril reorganization in animals. However, lack of noninvasive technology sophisticated enough to detect microstructural changes has compromised evaluation in pregnant women.

Methods: Hysterectomy specimens ($n = 5$) were scanned with two linear array transducers (Siemens Antares VFX9-4 and VFX13-5). Radiofrequency (rf) echo data were acquired with the endocervical canal in the image plane and parallel to the transducer face. The angle between the acoustic beam and tissue was used to assess anisotropic acoustic propagation by electronic control of transmit/receive angles from -14 to $+14^\circ$. A region of interest (ROI) was selected and the power spectrum of the rf signals computed for each angle. The locations were varied to assure result consistency.

Results: The nonnormalized power spectra of the backscattered rf signals from the cervix was about 3-4 dB higher for normal incidence than for those steered 14° regardless of ROI position. Visual inspection of the normalized spectra for normal incidence and steered beams showed that the spectra were effectively identical. This strongly suggests that there was no additional frequency-dependent absorption or scattering effect. For comparison, the nonnormalized power spectra for normal incidence versus 14° steered beams differed by only about 1 dB for equivalent data acquired from a phantom with randomly-distributed spherical scatterers. The differences in the incident versus steered beams in the cervix are consistent with scattering from an aligned (anisotropic) scattering structure. The lack of a frequency-dependent scattering effect with beam steering suggests that the dominant scatterers are much smaller than the 150 μm wavelength.

Conclusions: This novel approach identifies a component of cervical microstructure that is anisotropic (aligned), and too small to be muscle. Thus, it is overwhelmingly likely that we are detecting collagen. Furthermore, our data suggest that this component is reliably and noninvasively assessable in the human cervix. Ongoing studies will definitively identify this component and then quantify and track its changes throughout human pregnancy. If the cervix behaves as predicted from animal models, it will convert from an aligned (anisotropic) to a random (isotropic) scattering well before labor. Detecting early changes in cervical microstructure that occur prior to gross changes seen later (such as cervical shortening and dilation) may provide opportunity to develop earlier, more specific interventions for cervical insufficiency.

9.5 Development of deformation compounding: a novel technique for noise reduction in ultrasound elastography. Maria-Teresa Herd, Timothy J. Hall and James A. Zagzebski, *Department of Medical Physics, University of Wisconsin-Madison, Madison, WI 53706, mherd@wisc.edu.*

Many quantitative ultrasound (QUS) techniques are based on estimates of the radiofrequency (rf) echo signal power spectrum. Historically, low-noise spectral estimates required spatial averaging over large regions-of-interest. Spatial-compounding techniques have been used to obtain robust spectral estimates with smaller regions of interest. A new technique, 'deformation compounding,' is another method for providing robust spectral estimates with small regions-of-interest. Motion-tracking software is used to follow a ROI during strain imaging. The deformation spatially reorganizes the scatters so that the resulting echo signal is decorrelated. The rf signal power spectrum in the ROI is then averaged over several frames, thus undergoing deformation compounding. For small strains, the tis-

sue properties do not change. However, spectral estimates are uncorrelated which allows us to reduce noise for measurements of tissue properties.

Before the ROI can be averaged over any frames it must be established that the rf signal between the frames are uncorrelated. The amount of strain on tissue mimicking phantoms required to reduce the correlation between frames to near 0.1 was tested and found to be near 2% or less.

In this study, deformation compounding is used to improve measurements of attenuation and backscatter coefficients (BSC). The viability of deformation compounding has been studied using phantoms with known attenuation and BSC. These tests show deformation compounding to be a promising method to improve signal-to-noise in measurements of these tissue properties and is now being applied to clinical research in breast QUS.

9.6 Ultrasonic Doppler-mode detection of cyclic rigid-body motion of prostate brachytherapy seeds *ex vivo*, Robert Muratore,¹ Andrew Kalisz,¹ S. Kaisar Alam,¹ Geet K. Bhatt,¹ Frank N. Muratore² and Ernest J. Feleppa,¹ *Riverside Research Institute, New York, NY and ²FNM Productions, East Islip, NY, muratore@rrinyc.org.*

Optimum placement of permanent brachytherapy seeds in the prostate ensures adequate treatment of likely cancerous regions and sparing of other regions, e.g., the neurovascular bundle. Transrectal ultrasound (TRUS) is used to guide implantation. However, the seeds reflect ultrasound specularly, impeding their visualization at some angles relative to the ultrasound beam. We hypothesize that at resonance frequencies, cyclic rigid body motion (i.e., shaking) of the seeds will be detectable with Doppler-mode ultrasound.

Single and multiple TheraSeeds (Theragenics Corp., Buford, GA USA) were inserted in an Aquaflex gel pad (Parker Laboratories, Inc., Fairfield, NJ USA) or beef muscle. The gel and beef with the seeds were shaken at frequencies ranging from 10 Hz through 10 kHz with an electrodynamic transducer (model ET-132, Labworks Inc., Costa Mesa, CA USA) or a loudspeaker voice coil. In one experiment, a 10-MHz linear array transducer (model EUP-L34T, Hitachi Medical Corp., Tokyo Japan) and a commercial ultrasound scanner (model EUB-525, Hitachi) in B-mode and power-Doppler-mode were used to detect seeds. In another experiment, a mechanically-scanned, 10-MHz, A-mode transducer (model Panametrics V312, Olympus NDT Inc., Waltham, MA USA) and a custom Doppler cross-correlation algorithm were used to detect seeds. Adjustable parameters included vibration amplitude and frequency, receiver gain, and relative angles of shaker, seed, and ultrasound transducer.

Seeds were detected in Doppler mode at shaking frequencies near 400 Hz and 800 Hz; the peaks were broad. In gel, and with reduced contrast-to-noise, seeds were also detected near 1,200 Hz. From the harmonic relationship of the signal peaks, we conclude that a single dominant vibration mechanism exists but is not adequately described by a single-point-mass Kelvin-Voigt vibration model. The breadth of the peaks suggests that damping is important. Seeds were clearly detectable across a wide range of angles; from this, we conclude that the vibrations were not constrained to a single direction.

We anticipate that shaking of seeds will be simple to implement clinically and has the potential to locate seeds robustly.

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9.7 Detection of brachytherapy seeds using a singular-spectrum-analysis algorithm, Sarayu Ramachandran, Jonathan Mamou and Ernest J. Feleppa, *Riverside Research Institute, New York, NY, sarayu@rrinyc.org.*

Brachytherapy is a commonly-used technique to treat cancer. In prostate brachytherapy, small, titanium-shelled radioactive seeds are inserted transperineally into the prostate. Treatment planning and seed insertion are carried out under the guidance of transrectal ultrasound (TRUS). Motion of the prostate gland during seed insertion can result in misplacement of the seeds and consequently can cause dosimetric errors. An ability to visualize the seeds in the TRUS image during the implantation process would enable immediate dose-correcting implantations and negate the need for post-operative radiotherapy.

A singular-spectrum-analysis (SSA) algorithm previously was shown to be capable of detecting seeds inserted orthogonal to the ultrasound beam. The SSA algorithm chooses pairs of eigenvalues from the autocorrelation matrix of envelope-detected seed echo signals and uses these pairs to derive P -values that indicate the relative likelihood of the presence of a seed. We now extend the algorithm to detect seeds at various angles to the beam and to test the algorithm on ultrasound images of seeds from different manufacturers.

The algorithm was used to analyze ultrasound scans of seeds inserted in an acoustically-transparent gel pad and in a piece of beef. Scan data were acquired with the seed initially orthogonal to the beam of a 5-MHz ultrasound transducer. The angle of the seed was then varied, in 1° steps, up to 23° from orthogonal, with echo-signal data collected at each step. The performance of the algorithm was assessed using a score computed from the P -values, with similar scores being computed from the B-mode data as a comparison. The SSA algorithm was found to be successful in detecting seeds accurately up to a 10° angle from orthogonal to the ultrasound beam. The scores also showed that the SSA algorithm generally was superior to conventional B-mode images in detecting seeds.

9.8 Dependence of ultrasound backscatter on mixtures of suspended-scatterer sizes, Shreedevi Dasgupta, Ernest J. Feleppa and Jonathan Mamou, *Riverside Research Institute, New York, NY, dasgupta@rrinyc.org*.

Theoretically, ultrasonic backscatter is related to several scatterer properties including scatterer shape, concentration, relative acoustic impedance and size. Theoretical frameworks exist that relate frequency-dependent backscatter to scatterer, signal-processing and transducer properties. For example, if concentration and impedance properties remain the same, larger scatterers are predicted to backscatter a larger portion of the incident ultrasound than smaller scatterers backscatter; however, few studies have been performed to quantify the manner in which frequency-dependent ultrasonic backscatter varies with mixtures of scatterers having different sizes. Therefore, we initiated studies to investigate backscatter behavior when the scattering environment contains scatterers of different sizes.

Backscattered radiofrequency (rf) data were acquired from different concentrations of nominal 10- μm and 20- μm styrene beads in suspension. Rf data then were acquired from mixtures of scatterers of these two sizes. The mixtures were created by adding known concentrations of 20- μm beads to a constant concentration of 10- μm beads in suspension. Scatterer acoustic impedance was estimated as the product of published scatterer density and acoustic propagation values. Spectral parameters were computed from acquired rf data using standard Fourier methods, and compared to the spectral parameters predicted by the theory based on the known values of scatterer sizes, concentrations and acoustic impedance.

The spectral parameters obtained for suspensions of 10- μm and 20- μm beads did not vary linearly with concentration. As expected, the slope value predicted by the theoretical framework was dominated by the larger scatterers in the suspension. A better understanding of the contributions to the total ultrasonic backscatter by different-sized scatterers will expand the theoretical framework for application to cases where scatterer sizes are not uniform, as is typical of biological tissues.

10. High-Frequency/Small-Animal Imaging

10.1 Micro-ultrasound in preclinical biomedical research, F. Stuart Foster, *Department of Medical Biophysics, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Canada, stuart.foster@sunnybrook.ca* (invited overview).

This year, the Nobel prize for medicine was given to three scientists for the discovery of genetic knock-out techniques for the mouse. The next major project following the sequencing of the genome is the coordinated and systematic knocking out of each of the mouse's ~30,000 genes and the discovery of the phenotypes associated with these mutations. The National Institutes of Health and other international organizations are betting hundreds of millions of dollars that this will lead to critical discoveries needed along the path to better healthcare. Imaging will play a major role in this enterprise. This presentation will focus on what high frequency micro-ultrasound brings to the table. The principles of this technology will be described and applications in the area of cardiovascular and cancer research will be outlined. The recent introduction of high-frequency contrast agents has further expanded potential for micro-ultrasound to perform functional and targeted molecular imaging in disease models and interventional studies. The current state of the art in high-frequency contrast imaging of the mouse will be examined. Examples of functional imaging of cardiovascular disease and tumour microcirculation will be used to illustrate the potential and limitations of the current technology. The principles of molecular imaging with high-frequency ultrasound will be described and their application to relevant targets for angiogenesis and inflammation will be described. The next generations of high-frequency imaging systems will provide improved performance based on optimization of the microbubbles themselves, a better understanding of microbubble interactions at high frequencies in both the bound and unbound state and improved capabilities for nonlinear excitation. Preliminary results for subharmonic imaging and radial modulation imaging will be presented.

10.2 Serial 3D high frequency ultrasound evaluation of left ventricular contractile dyssynchrony in mice after myocardial infarction, Yinbo Li,¹ Christopher D. Garson,¹ Yaqin Xu,¹ Patrick, A. Helm,³ Brent A. French¹⁻³ and John A. Hossack,¹ *Departments of¹Biomedical Engineering, ²Medicine and ³Radiology, University of Virginia, Charlottesville, VA 22908, jh7fj@virginia.edu* (invited).

Heart failure (HF) continues to be one of the most costly and prevalent medical problems in modern society. One major obstacle to effective treatment of HF is our limited understanding of the heart's response to injury. For example, the time-dependent alterations in the left ventricular (LV) contractile function in response to myocardial infarction (MI) are not fully understood. Therefore, there is a sustained interest in tracking progression following MI towards HF, especially in the entire extent of the LV in three dimensions (3D). Thus, there is a need for noninvasive methods to conduct serial, *in vivo*, cardiac assessments that minimize disturbance to cardiac physiology yet possess the high temporal and spatial resolution necessary for characterization of cardiac function.

We used high frequency ultrasound (VisualSonics Vevo770) for the serial assessment of LV contractile dysfunction in murine hearts after surgically-induced MI. High-frequency ultrasound provides the necessary noninvasive, real-time and high-resolution imaging characteristics. Ultrasound was performed on 8 C57Bl/6 mice before and at 1, 3, 5, 7, 14, 21, 28 and 105 days after induced MI. At each time point, B-mode image sequences at multiple short-axis and long-axis cross-sections were acquired to encompass the entire LV. Myocardial motion was measured from the image sequences using speckle-tracking based on a Minimum Sum of Absolute Value (MSAD) search algorithm. Subpixel tracking resolution was obtained via parabolic interpolation. Cardiac analysis of the 3D LV was achieved by repeat-

ing motion tracking on all cross-sections spanning the entire heart. Contractile dysfunction was evaluated by two measures: regional motion deficit and motion delay, which were assessed, respectively, as the reduction in regional peak displacement and the delay in time to peak radial strain (T_{peak}) over different myocardial regions. A finite-element 4D (3D + time) model was constructed based on the dynamics of 3D LV geometry along the cardiac cycle, and cardiac function parameters were mapped onto the surface of the 4D model. Two global motion parameters, mean strain magnitude and contraction dyssynchrony, which were defined as the area-weighted regional peak strain and the standard deviation of regional T_{peak} over the 3D LV, respectively, were computed at each MI stage to evaluate the contractile function of the entire heart. For histological validation, the size of dysfunctional myocardial area determined *in vivo* by ultrasound was compared with the infarct size measured by post-mortem tissue staining, and a good correlation was obtained ($R = 0.91$).

Therefore, this study demonstrated that high frequency ultrasound may serve as a reliable tool for serial, *in vivo*, 3D assessment of murine cardiac function during the progression of LV remodeling after MI.

10.3 High-frequency detection of cell death: assessment of chemotherapy, radiotherapy, photodynamic therapy and novel microbubble-therapy effects, Gregory J. Czarnota, Justin Lee, Raffi Karshafian, Behzad Banihashemi, William Chu, Charles Cho and Michael C. Kolios, *Radiation Oncology and Imaging Research, Sunnybrook Health Sciences Centre and Department of Radiation Oncology, University of Toronto, Toronto, Canada, gregoryczarnota@gmail.com* (invited)

Our objective was to test the ability of high-frequency spectroscopic ultrasound to noninvasively monitor apoptotic response using four *in vivo* tumour xenograft models. In one, melanoma-bearing mice were treated with photodynamic therapy, in a second, lymphoma-bearing mice were treated with CHOP chemotherapy, whereas in the third system a number of tumour models were treated with radiation. In the last of our systems, prostate-bearing mice were treated with a new form of microbubble-enhancement of radiation response.

Solid tumours were grown in SCID mice using a malignant human melanoma ($n = 26$, HTB-67) and lymphoma ($n = 36$, CRL-2261) cell line. Mice also had PC3 tumours ($n = 50$, and $n = 72$) grown for radiation and microbubble treatments. Melanoma tumours were treated with 110 J/cm^2 of 633 nm laser light, 24 hours following exposure to 10 mg/kg of Photofrin given *i.p.* as a photosensitizer. Lymphoma-bearing animals were treated with CHOP (cyclophosphamide, adriamycin, vincristine and prednisone) chemotherapy. Prostate-bearing mice received radiotherapy alone and in combination with novel microbubble radiosensitizers in various doses.

Tumours were examined by 30 to 40 MHz high-frequency ultrasound prior to treatment and at different times after treatments. Ultrasound data collection consisted of acquiring tumour images in addition to spectroscopic data for quantitative analyses of backscattered ultrasound. Animals were sacrificed immediately after analysis and tumours excised for histopathologic analysis. Histological sections were examined by haematoxylin and eosin staining in addition to TUNEL for apoptosis. Ultrasound images and corresponding spectroscopic data were analyzed and tested for correlation with histopathological findings.

In terms of results, we observed a time-dependent increase in ultrasound backscatter after treatment. For photodynamic therapy, treatments increases in backscatter findings correlated with morphological findings indicating increases in apoptotic cell death, which peaked at 24 h after PDT. We observed 0%, 1.48%, 8.25%, 26.83%, 52.52% and 2.12% apoptosis in terms of cross-sectional tumour area from histopathology after 1 h, 3 h, 6 h, 12 h, 24 h and 48 h of PDT, respectively which corresponded to 1.5%, 4%, 11%, 25.33%,

31.5% and 25% increases in the logarithmically-uncorrected mean intensity in ultrasonograms obtained at 30 MHz.

A different time-dependent increase in backscatter followed treatment with CHOP chemotherapy. At 5 h, 12 h, 24 h, 48 h and 72 h, and the measured high intensity (10 dB increase) patch areas on high-frequency ultrasound were (mm^2 (SD)); 1.76 (1.0), 0.27 (0.19), 2.03 (0.74), 2.93 (2.1), and 0.12 (0.11) respectively. Representative mid-band fits changed from -51 dBr to 44 dBr after treatment and was maximal at 24 h. Spectral slope was invariant at 0.66 dBr/MHz prior to chemotherapy and -0.60 dBr/MHz 48hrs after CHOP chemotherapy. Image analysis demonstrated a correlation between the size of high intensity patches on high-frequency ultrasound and immunohistochemical TUNEL staining of apoptotic areas. Animals treated with radiotherapy and novel microbubble radiosensitization exhibited a dose dependent response in terms of mid-band fit and 0-MHz intercept with increasing radiation doses and microbubble-radiosensitizer doses. Ultrasound estimates of apoptosis correlated well with histopathological estimates.

In conclusion, spectroscopic analysis can detect time-dependent apoptosis in *in vivo* melanoma and non-Hodgkin's tumor models treated with photodynamic therapy and CHOP chemotherapy, respectively. Radiotherapy and novel radiosensitization experiments in prostate and other cancer models, which were monitored with similar methods, also indicated a similar correlation between frequency-dependent signal changes and histological changes.

In our study, we observed a close correlation between therapy-induced apoptotic cell death and changes in mean backscatter intensity and other spectral parameters. The results indicate that this method can be used as an accurate predictor of therapy response.

10.4 Nonlinear responses of polymer-shelled contrast agents to high-frequency ultrasound, Sarayu Ramachandran, Jonathan Mamou and Jeffrey A. Ketterling, *Riverside Research Institute, New York, NY, sarayu@rrinyc.org* (invited).

Acoustic contrast agents have been studied and used at low-MHz frequencies but their high-frequency (>15 MHz) responses also may yield clinically useful information. High-frequency ultrasound (HFU) has the advantage of finer spatial resolution and is ideal for ophthalmic and small-animal imaging. The combination of contrast agents and HFU could result in improved microvasculature imaging and targeted imaging. This study examines the subharmonic content of backscatter from polymer-shelled contrast agents excited with 40-MHz bursts of 10, 15 and 20 cycles. The three polycaprolactone-shelled agents (POINT Biomedical, San Carlos, CA) studied here had mean diameters of 0.56, 1.1 and 3.4 μm . The agents were heavily diluted and passed through a flow phantom that allowed single-bubble events to be imaged at various peak-negative pressures, from 0.75 to 5 MPa. At each exposure setting, the backscatter data from 1,000 separate single-bubble events were digitized and analyzed for subharmonic content. All three contrast agents displayed subharmonic activity. The results showed that the likelihood of subharmonic content in the backscattered signal increased as the number of cycles was increased. For the 1.1 μm agent, the highest likelihood of a subharmonic event was around 2 MPa. Tone bursts with less than 10 cycles and greater than 20 cycles were also studied to better understand the thresholds for subharmonic activity. The results display the potential for polymer-shelled agents to be used for nonlinear HFU imaging, with the acquired data suggesting possible conditions for obtaining a nonlinear response from each size distribution of contrast agent.

11. Imaging

11.1 Dual apodization with cross-correlation, Jesse Yen and Chi Hyung Seo, *USC Viterbi School of Engineering, Los Angeles, CA 90089, jesseyen@usc.edu*.

We propose a new method to use dual apertures or dual apodization functions with cross-correlation (DAX) to reduce side lobes and clutter in ultrasound imaging. Using a uniformly-apodized transmit aperture and two different receive apodization functions, we can distinguish between main lobe and side lobe dominated signals using normalized cross-correlation of rf data segments from the two receive apodization functions. Rf data segments are 2-3 wavelengths long. The cross-correlation are calculated for every sample in the image and serve as a weighting matrix to pass main lobe dominated signals and suppress side lobe or clutter dominated signals. Signals with a comparable mixture of main lobe and clutter are partially suppressed. For cross-correlation coefficients greater than a threshold θ , an amplitude weighting equal to the cross-correlation coefficient is used. The amplitude weighting is set to the threshold $-\theta$ for negative cross-correlation coefficients.

Four different pairs of receive apodization functions have been developed and evaluated using a point target and an anechoic cyst. These apodization functions include a Uniform/Hanning apodization, two translated receive apertures with a common midpoint, two complementary random apodizations and two alternating apodizations. Using a 5-MHz 128-element linear array, contrast-to-noise ratio (CNR) improvements exceed 100% in both simulation and experiments using a tissue mimicking phantom containing a 3 mm diameter anechoic cyst at 30 mm depth. The Uniform/Hanning apodization pair showed the greatest CNR improvement in simulation but proved to be susceptible to 40 MHz quantization in experiments. Experimentally the two alternating apodization functions showed the highest CNR. When a 5% sound speed error was introduced in simulation, the DAX algorithm using the alternating apodization functions showed a 53% CNR improvement in the same anechoic cyst.

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11.2 A dual-layer transducer for 3-D near-field imaging, Jesse Yen, Jong Jeong, Samer Awad and Chi Hyung Seo, *USC Viterbi School of Engineering, Los Angeles, CA 90089, jesseyen@usc.edu*.

The difficulty in fabricating and connecting large numbers of 2-D array elements has limited the development of fully-sampled 2-D transducer arrays with more than 5,000 elements. Instead, we propose a dual-layer transducer array design that uses elongated perpendicular 1-D arrays for 3-D imaging. This transducer design reduces the complexity of fabrication and the number of channels. To demonstrate feasibility, we constructed a 4 cm \times 4 cm prototype dual-layer transducer array that consists of 256 elements for each layer operating in the 5 MHz range. The backing is a tungsten-epoxy backing with an acoustic impedance of 9.3 MRays. The transmit layer is PZT-5H where each element is 0.125 mm \times 40 mm \times 0.3 mm thick with. The element pitch is 0.145 mm. The top layer is P[VDF-TrFE] copolymer and used in receive only and also acts as a matching layer. A 0.025 mm thick polyimide flex circuit was used to define the receive elements. No dicing was done to ease fabrication constraints.

Pulse-echo measurements show a 6 dB fractional bandwidth of 70 % at a center frequency of 4.7 MHz. Impedance measurements showed an electrical impedance near 75 Ω at 5 MHz for the transmit layer, and an electrical impedance of 1.3 k Ω for the receive layer. The measured crosstalk of the undiced P[VDF-TrFE] layer was 24 dB in the frequency range of 3-10 MHz. Preliminary images of a wire target phantom were also obtained.

11.3 Towards photoacoustic imaging of the eye, Ronald H. Silverman,^{1,2} Harriet O. Lloyd,¹ Tatiana Raevski,¹ Mark J. Rondeau,¹ Ying-Chih Chen,³ Fanting Kong³ and D. Jackson Coleman,¹ ¹Weill Cornell Medical College, New York, NY 10065, ²Riverside Research Institute, New York, NY 10038 and ³Hunter College, CUNY, New York, NY 10065, *rhsilverman@optonline.net*.

Ultrasound has been a major technique for diagnostic imaging of the eye for close to a half century. New developments continue to bring improvements in imaging and characterization of anterior- and posterior-segment anatomy and pathology in the eye. The eye, however, is anatomically unique in providing an optically-transparent window to its interior. New fine-resolution techniques such as optical coherence tomography (OCT) have displaced ultrasound to a great extent for evaluating the retina and optic-nerve head.

We are exploring the potential of combining ultrasound with optics to provide unique information regarding ocular tissues that cannot be obtained by ultrasound or optics alone. Photoacoustic imaging is based on the generation of ultrasound waves by tissues through thermoelastic expansion in response to absorption of a short light pulse. We are developing a platform for photoacoustic imaging that uses an 808-nm diode laser to pump a miniature laser that emits at 1,064 nm. With this system, 5-ns light pulses are produced at 250 Hz. The miniature laser is focused to a point coincident with the focus of a 35-MHz ultrasound transducer that has an aperture of 7 mm and a focal length of 12.8 mm. We have conducted experiments with polyvinyl alcohol tissue phantoms including 25- μ m sephadex particles and inclusions of 9- μ m graphite particles as well as other test targets. The photoacoustic assembly is mounted on a scan platform and triggered to allow B-mode scanning.

Photoacoustic images detect light absorption, which is independent of the light detected by OCT or the acoustic backscatter of pulse/echo ultrasound. This photoacoustic system naturally allows acquisition of spatially-coincident conventional pulse/echo ultrasound and photoacoustic data. We are exploring the potential of improving lateral resolution by focusing the laser spot size to less than the ultrasound beam width and are investigating the possibility of varying light wavelength to characterize tissues based on their wavelength-dependent light-absorbing characteristics.

We plan on using this device to image the anterior segment of the eye, first using *ex vivo* pig eyes and later using *in vivo* rabbit eyes. We also plan on conducting photoacoustic imaging of the retina. The reduction in attenuation due to the one-way propagation of ultrasound may allow use of frequencies higher than the 20 MHz now used for high-definition imaging of the posterior segment of the eye.

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11.4 A USB ultrasound probe for vascular access, William D. Richard,¹ David M. Zar,¹ Roman Solek² and Roger Edens,³ ¹Z&R Technologies, L.L.C., ²Interson Corporation and ³Escalon Medical Corporation, *wdr@zandrtech.com*.

Millions of IVs are started each year. By adapting our USB ultrasound probe technology for vascular access, we have developed a platform that provides improved patient outcomes, increased success rates, reduction of complications and greater accuracy and efficiency. Our commercial platform is based on a custom USB ultrasound probe coupled with a touch-screen-based tablet PC. This highly-portable system implements a state-of-the-art vascular access system. We describe the system architecture and user interface and provide several example images illustrating the advantages of the system.

11.5 Ultrasonic clutter: magnitude, impact on lesion detection, effect of harmonic imaging and characterization of origins, Muyinatu Lediju, Michael Pihl, Stephen Hsu, Jeremy Dahl and Gregg Trahey, *Duke University, Department of Biomedical Engineering, Durham, NC, muyinatu.lediju@duke.edu.*

Clutter is a noise artifact in ultrasound images that degrades image quality and obscures diagnostic information. It is most easily observed in anechoic or hypoechoic regions and appears as a diffuse haze overlying structures or signals of interest. We have derived an analytical expression for the extent to which ultrasonic clutter degrades lesion contrast and compared analytical predictions to the measured contrast loss due to clutter in cyst phantoms.

The *in-vivo* bladder is an ideal organ for characterizing abdominal clutter because it contains a hypoechoic fluid (urine), such that echo signals detected within it are considered to be clutter noise. Using the bladder as an experimental model, we quantify physiological abdominal clutter levels in fundamental and harmonic images of five volunteers. Abdominal clutter levels are shown to range from -15 dB to nearly 0 dB, relative to the mean signal of the bladder wall. For this range of abdominal clutter, the analytical expressions predict a range of 0-45 dB in contrast loss, where the exact value depends on the contrast that would exist if clutter were not present; the reported loss considers clutter-free contrasts of 6-48 dB. The fundamental and harmonic images of one volunteer were directly compared to each other with respect to clutter reduction and the maximum signal reduction in the harmonic image was 21 dB, which is consistent with existent literature. Experiments were also conducted to distinguish between the several sources of clutter in bladder images.

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11.6 Pulse wave imaging of normal and aneurysmal abdominal aortas *in vivo*, Jianwen Luo,¹ Kana Fujikura,¹ Leslie S. Tyrie,² M. David Tilson III,^{2, 3} Ioannis K Zervantonakis,¹ Jonathan Vappou¹ and Elisa E. Konofagou,^{1, 4} ¹*Department of Biomedical Engineering, Columbia University, New York, NY,* ²*St. Luke's-Roosevelt Hospital Center, New York, NY,* ³*Department of Surgery, Columbia University, New York, NY* and ⁴*Department of Radiology, Columbia University, New York, NY, jianwen.luo@gmail.com.*

The abdominal aortic aneurysm (AAA) is a common vascular disease, with a prevalence rate of 1.3%-8.9% in men and 1.0%-2.2% in women. The current clinical criterion for treating AAA's is an increased diameter above a critical value. However, the maximum diameter does not correlate well with aortic rupture, the main cause of death from AAA disease. AAA disease leads to changes in the aortic wall mechanical properties. The pulse-wave velocity (PWV) may indicate such a change. Because of limitations in temporal and spatial resolution, the widely used foot-to-foot method measures the global, instead of regional, PWV between two points at a certain distance in the circulation. However, mechanical properties are nonuniform along the normal and pathological (e.g., the AAA and atherosclerosis) arteries; thus, such changes are typically regional. Pulse-Wave Imaging (PWI) has been developed by our group to map the pulse-wave propagation along the abdominal aorta in mice *in vivo*. By using a retrospective electrocardiogram (ECG) gating technique, the radiofrequency (rf) signals over one cardiac cycle were obtained in murine aortas at the extremely high frame rate of 8 kHz and with a field-of-view of 12 × 12 mm². The velocities of the aortic wall were estimated using an rf-based speckle tracking method. An Angiotensin II (AngII) infusion-based AAA model was used to simulate the human AAA case. The sequences of wall velocities noninvasively and visually map the propagation of the pulse wave along the aortic wall. In the normal and sham (with infusion of saline) aortas, the propagation of the pulse

wave was relatively uniform while in the AngII-treated aortas, the propagation of the pulse wave was nonuniform. There was no significant difference in the PWV between sham ($n = 5$) and AngII-treated ($n = 17$) aortas (4.67 ± 1.15 m/s vs. 4.34 ± 1.48 m/s, $p > 0.05$). The correlation coefficient of the linear fitting on the pulse-wave propagation in the sham ($n = 5$) aortas was significantly higher in the AngII-treated ($n = 17$) ones (0.89 ± 0.03 vs. 0.61 ± 0.15 , $p < 0.005$). The wall velocities induced by the pulse wave were smaller and the pulse wave moved nonuniformly along the AngII-treated aorta ($p < 0.005$), with the lowest velocities at the aneurysmal regions. The discrepancy of regional wall velocity and the nonuniform pulse-wave propagation indicated the inhomogeneities in aortic wall properties and the reduced wall velocities suggested the change in aortic wall stiffness. This novel PWI technique may thus constitute an early detection tool of vascular degeneration as well as serve as a suitable predictor of AAA rupture. Finite-element simulation of coupling of the aortic wall motion and the flow dynamics using a fluid-solid interaction (FSI) model will be also shown to investigate the role of wall stiffness, aortic geometry and flow pattern for a parametric analysis of the PWI findings.