UITC ABSTRACTS 2015

Monday morning

1. TISSUE PARAMETERS 1

1.1 **Quantitative ultrasound as a predictor of tumor response prior to treatment initiation,** Hadi Tadayyon [1,2], Ali Sadeghi-Naini [1,2], Lakshmanan Sannachi [1,2], Mehrdad Gangeh [1,2], Maureen Trudeau[3] and Gregory Czarnota,[1,2,4] *[1]Department of Medical Biophysics, University of Toronto, [2]Physical Sciences, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, [3]Division of Medical and Haematologic Oncology, Department of Medicine, Sunnybrook Health Sciences Centre and [4]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, Canada, Gregory.Czarnota@sunnybrook.ca*

Background & Objectives: Neoadjuvant chemotherapy (NACT) is commonly administered to locally-advanced breast cancer (LABC) patients to help shrink inoperable tumors and enable more breast-conserving surgeries. Currently, response of a patient to NACT is determined at the end of a several-month treatment based on tumor size reduction, resulting in a lost window of opportunity to modify an ineffective treatment regimen. Thus, it would be beneficial to identify patients who are unlikely to respond to a certain NACT before treatment initiation, permitting clinicians to select the optimal treatment strategy for the individual patient. The objective of this study was to determine whether mutliparametric quantitative ultrasound techniques could be used to characterize LABC tumors in terms of their chemo-responsiveness (responsive or nonresponsive) prior to treatment initiation.

Methods: Breast ultrasound RF data were acquired from 56 LABC patients prior to treatment initiation. From tumor ROIs, attenuation coefficients and phantom-calibrated QUS parametric maps of spectral midband-fit, slope, intercept, mean scatterer spacing, average scatterer diameter and average acoustic concentration were computed. From each QUS map, three types of statistical features were extracted: means, textures (contrast, correlation, energy and homogeneity), signal-to-noise ratios (SNRs) and contrast-to-noise ratios (CNRs). Chemo-responsiveness of each patient was predicted using a K-nearest-neighbor classifier trained on the statistical features of the QUS maps. Classification validation was performed using the leave-one-patient out method. Responses determined based on tumor size reduction at the end of treatment were used as true class labels.

Results: Unpaired *t*-tests determined the attenuation coefficient to be the strongest QUS feature which could discriminate chemo-responsive and nonresponsive tumors with *p*-value = 0.02. Multiparametric classification results demonstrated that when the attenuation coefficient plus mean, texture, SNR and CNR features of QUS maps were used in combination, a response prediction rate of 86%, nonresponse prediction rate of 71%, and an overall classification accuracy of 82% could be achieved.

Conclusions: Multiparametric QUS employing KNN classification algorithm is a promising aid for treatment selection of LABC patients. Definitive causes of negative tumor response are not fully understood, although many factors could contribute to this behavior, including cancer type, grade, stage, hormone-receptor status and patient's age and health. Multiparametric QUS may potentially account for these factors. This method could be used in conjunction with biopsy-determined cancer grade, type and stage to assist the health care team in determining the optimal treatment options for LABC patients.

1.2 **Quantitative ultrasound monitoring of tumor cell death responses,** Lakshmanan Sannachi[1, 2], Azza Al-Mahrouki[1,2], William Tyler Tran[1, 2] and Gregory J. Czarnota,[1-3] *[1]Department of Medical Biophysics, University of Toronto, [2]Physical Sciences, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre and [3]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, Canada, Lakshmanan.Sannachi@sunnybrook.ca*

Introduction: Quantitative ultrasound techniques have been shown to be capable of detecting cell death through studies conducted on *in vitro* and *in vivo* models. Recently, hybrid models have been developed based on quantitative ultrasound parameters (QUS) to quantify cell death percentage from treated mice tumor and to distinguish treatment responders and nonresponders from locally-advanced breast cancer patient (LABC) populations early on during chemotherapy. QUS parameters estimations using radiofrequency echoes acquired with clinical ultrasound systems must be independent of the data acquisition set-up. This study was performed to compare this QUS method in monitoring tumor cell death response from animal and human using two different clinical-array systems.

Methods: Radiofrequency data were acquired from xenografted human breast cell line tumors (MDA-MB231) before and after injection (4, 8, 12 and 24 hours) of paclitaxel chemotherapy agent, using low-frequency linear-array transducers L14-5/36 and 9L with frequency range 4- 9 MHz with Ultrasonix and GE-LOGIQ clinical systems, respectively. Similarly, RF data were acquired from LABC patients before and during treatments (week 1, 2, 4 and 8) using the same clinical scanners. QUS parameters, including midband fit (MBF), spectral slope (SS) and 0-MHz intercept (SI) were estimated from tumour regions.

Results: In both mice tumor and LABC treatment monitoring studies, the trends observed in the changes of QUS parameters after treatment using GE and Ultrasonix systems were similar. The mean-squared (RMS) errors between clinical scanners were highly variable. For example, in mouse tumors, the average RMS errors calculated for the MBF parameter before treatment and was 1.5 dB. Tumor-sampling differences yielded up to 28.5 dB RMS error after treatment. In LABC patients, the RMS errors calculated for this QUS parameter before and after treatment were 2.7 dB and 2.3 dB, respectively, with similar volumes sampled.

Conclusions: The histological analysis showed increases in MDA mice tumor heterogeneity after treatment. This increase in heterogeneity within the tumor accounted for QUS parameter variations from slice to slice. This results in variations between scanners due to limitations in acquiring identical planes. The discrepancy in the RMS error for changes in QUS parameters after treatment between MDA mice tumor and LABC is likely due to the difference in transducers characteristics used in this study. This technical advance shows the potential for QUS technology to function with difference imaging platforms.

1.3 **Early prediction of breast tumor response to chemotherapy using multiparametric quantitative ultrasound,** Hadi Tadayyon[1, 2], Lakshmanan Sannachi [1, 2], Mehrdad Gangeh [1, 2], Ali Sadeghi-Naini [1, 2], , Maureen Trudeau [3] and Gregory Czarnota, [1, 2, 4] *[1]Department of Medical Biophysics, University of Toronto, 2Physical Sciences, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, 3Division of Medical and Haematologic Oncology, Department of Medicine, Sunnybrook Health Sciences Centre and [4]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, Canada, hadi.tadayyon@gmail.com*

Background: Locally-advanced breast cancer (LABC) is an aggressive subtype of breast cancer whose response to chemotherapy treatment varies from patient to patient. Thus, frequent monitoring of LABC tumors makes early detection of refractory patients and switching to a more aggressive regimen possible. In this study, a quantitative ultrasound (QUS) imaging method was used to characterize treatment response of breast tumors in LABC patients receiving preoperative chemotherapy.

Methods: Radiofrequency ultrasound data were collected from 58 breast cancer patients prior to treatment and at 1 week, 4 weeks and 8 weeks after the onset of their several-month treatment, using a clinical ultrasound scanner operating a ~7 MHz linear-array probe. Quantitative ultrasound parameters including spectral midband fit (MBF), spectral slope (SS), spectral intercept (SI), spacing among scatterers (SAS), attenuation coefficient estimate (ACE), average scatterer diameter (ASD) and average acoustic concentration (AAC) were computed from gated RF regions of interest inside the tumor and the results were compared with the patient's true response determined based on established clinical guidelines of tumor size reduction. Finally, the recurrence-free survival of the patients classified using the QUS technique was assessed.

Results: MBF, SI and ACE parameters increased statistically-significantly in patients with treatment-responding tumors compared to nonresponding patients as early as 1 week after the start of the treatment. Discriminant analysis using the *K* nearest-neighbor classifier demonstrated that the best classification performance could be achieved using the combination of MBF, SS and SAS, with an accuracy of 60 ± 10

% at week 1, 77 ± 8 % at week 4 and 75 ± 6 % at week 8. Furthermore, when the QUS measurements at each time (week) were combined with the pre-treatment (week 0) QUS information, the classification accuracies improved (70 ± 9 % at week 1, 80 ± 5 % at week 4 and 81 ± 6 % at week 8). Finally, the QUS classification model demonstrated a significant difference in survival rates of responding and nonresponding patients at weeks 1 and 4 (p=0.035 and 0.027, respectively).

Conclusion: A leave-one-out classifier evaluation was performed for the first time for classification of patients' tumor responses to chemotherapy. Inclusion of the patient's pretreatment ultrasound backscatter parameters in the classification model improved the classification performance. Attenuation, which was neglected in previous studies, was demonstrated to be an important parameter in tumor-response monitoring. This work demonstrated the potential of quantitative ultrasound and machine-learning methods for predicting the response of breast tumors to chemotherapy early and guiding the treatment planning of refractory patients.

1.4 **Quantitative ultrasound analysis of paclitaxel-induced mitotic catastrophe in breast-cancer cells,** Maurice Pasternak[1, 2], Anoja Giles[3] and Gregory Czarnota,[2, 3] [1]*Department of Laboratory Medicine and Pathobiology, University of Toronto,* [2]*Physical Sciences, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre and* [3]*Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada, gregory.czarnota@gmail.com*

Background & Objectives: Chemotherapy is a common treatment modality of malignant breast cancers, to which cancerous cells exhibit vast changes to their structure as they undergo cell-death processes. High-frequency ultrasound (HFUS) has shown promise of detecting tumor cell death on the basis of these structural alterations, reflected through differences in the ultrasound backscatter. However, the studies that had shown this were limited to ultrasound B-mode and immunohistochemical image analysis over extended periods of time (≥24 hours). The objective of this study has been to determine whether these observations hold a quantitative relationship to the degree of cell death in an *in vitro* cell pellet system serving to mimic breast tumor tissue.

Methods: Ultrasound radiofrequency (RF) data was acquired from 1μM paclitaxel-treated cell pellets at 6, 12 and 24-hour time points in addition to untreated control pellets. The RF data was analyzed according to previous methods to extract acoustic parameters, including the attenuation coefficient, speed of sound, spectral midband-fit (MBF), slope, intercept, estimated scatterer diameter and estimated scatterer concentration. Additionally, pellets were processed for haemotixylin & eosin (H&E) and *in-situ* end labelling stains, in addition to transmission-electron microscopy (TEM) to ascertain the identity of subcellular scatterers accounting for observations in RF-derived parameters. Parallel cell populations were analyzed by flow cytometry for cell cycle distributions and cell death markers for mitochondrial depolarization, caspase activation, phosphatidylserine exposition and compromise of the plasma membrane. Populations containing the candidate scatterers were selected based on the death markers and statistical correlations were performed to determine the strength of relationship between acoustic parameters and cell death indices.

Results: Based on TEM, negative TUNEL staining and cell cycle analysis, it was found that paclitaxel at a concentration of 1mM induces mitotic catastrophe as opposed to apoptosis in MDA-MB-231 cells. The spectral slope correlated strongly (r^2 = 0.89, p<0.05) with the percentage of cells arrested at the G2/M checkpoint and exhibited an opposite trend to that of systems undergoing classical apoptosis. MBF increases through time suggested an increase in the concentration of acoustic scatterers. The formation of condensed chromatin condensates of a 1 micron or greater diameter in the course of paclitaxel-induced mitotic catastrophe, as revealed by TEM, were hypothesized to act as large intracellular scatterers accounting for the increase in ultrasound MBF. MBF showed the strongest correlation of all parameters (r^2 = 0.99, p<0.01) when plotted against the percentage of the cell population expected to possess these scattering structures.

Conclusions: The spectral slope is suggested to serve as a prime biophysical marker for differentiating modes of cells death, as the trends observed for populations undergoing mitotic catastrophe were opposite to those undergoing apoptosis in previous studies. The comparison of changes in MBF to those of the cell-death index establishes the first such quantitative correlation involving RF-derived parameters. These

findings support that HFUS RF analysis may be used to quantify the degree and modality of cell death, thereby predicting the efficacy of chemotherapeutic treatment within a short post-treatment time frame.

1.5 **Cancer-response monitoring using a texton-based approach in locally-advanced breast cancer,** Mehrdad J. Gangeh[1, 2] , Hadi Tadayyon[1, 2], Lakshmanan Sannachi[1, 2], Ali Sadeghi-Naini[1, 2], Maureen Trudeau[3], Sonal Gandhi[3] and Gregory J. Czarnota,[1, 2] *[1]Department of Medical Biophysics, University of Toronto, [2]Physical Sciences, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, [3]Division of Medical and Haematologic Oncology, Department of Medicine, Sunnybrook Health Sciences Centre and [4]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, Canada, mgangeh@gmail.com*

Background and Motivation: Assessing the efficacy of cancer treatments in preclinical and clinical treatments is presently limited; results may not be available to the clinician for typically months. This can lead to ineffective cancer treatments continued needlessly as no faster feedback mechanisms have yet reached broad biomedical adoption. Quantitative ultrasound (QUS) methods provide a promising alternative framework that can noninvasively, inexpensively and quickly assess tumour response to cancer treatments using standard ultrasound equipment. Due to heterogeneous responses developed in tumors as a result of treatment, texture methods can potentially characterize these responses and assist to quantify the assessment of cancer-response monitoring. In this research, texton-based methods as the state-of-the-art technique for texture analysis was used to model locally advanced breast cancer (LABC) responses to chemotherapy.

Methods: Fifty-six patients with locally-advanced breast cancer (LABC) who received neoadjuvant chemotherapy treatments were imaged before and at 4 times during treatment, i.e., weeks 1, 4, 8 and preoperatively. Data were acquired using a Sonix RP ultrasound machine at a central frequency of ~7 MHz. Mid-band fit and 0-MHz intercept parametric maps were computed by deploying quantitative ultrasound spectroscopy techniques. The patients were grouped into good- and poorly-responding based on their ultimate clinical and pathological response to treatment. Codebooks of textons were constructed for each patient by extracting 500 random patches of 11×11 segments from parametric maps and by using *k*-means methods with the *k* value of 30. Subsequently, a histogram of textons was computed for each parametric map using the associated codebook as the model/feature set to represent the pre- and during-treatment images for each patient at a specific time frame after treatment. The distance between these features for each subject was used as a criterion of the effectiveness of the treatment, which was ultimately submitted to a naïve Bayes classifier to classify the patients to responding or non-responding in a leave-one-subject-out manner.

Results: The classification of patients with LABC to responding and non-responding using the proposed texton-based system achieved an accuracy of 83.85 ± 10.06% and 85.00 ± 6.79%, area under curve (AUC) of 80.30 and 83.14, sensitivity of 86.92% and 86.36% and specificity of 80.77% and 83.64% after 4 and 8 weeks of treatment, respectively.

Conclusion: In this study, texture methods based on a texton-based approach was proposed to quantify the assessment of LABC response to neoadjuvant chemotherapy. The proposed system achieves a promising accuracy and sensitivity 4 weeks after treatment initiation. This would permit clinicians to receive feedback and switch to alternate treatments far earlier, in a step towards the goals of *personalized medicine*.

1.6 **Study of the effect of microbubble vascular perturbation on mice-tumor radiation response,** Lin Su[1], Chen. Yang[2], Jinyuan Zhou[3], Reem. Malek[1], Phuoc Tran[1], Yin Zhang[1], Bin. Zhang[1], Esteban Velarde[1], Ken Kang-Hsin Wang[1], John W. Wong[1] and Kai Ding,[1] *[1]Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University, School of Medicine, Baltimore, MD, [2]Department of Ultrasound, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China and [3]Division of MR Research, Department of Radiology, Johns Hopkins University, School of Medicine, Baltimore, MD, kding1@jhmi.edu*

Purpose: It has been reported that the ultrasound-mediated microbubble vascular perturbation could increase tumor responses to radiation. In this study, we tested the effect of microbubble vascular disruption on mice-tumor radiation response.

Methods: Contrast-Enhanced Ultrasound (CEUS) uses microbubbles as contrast agents. The microbubbles consist of high molecular mass gas wrapped by a thin shell. The median diameter of the microbubbles is several micron, which enables the bubbles pass through systemic circulation. Ultrasound of certain frequencies can burst the microbubbles and in turn cause microdamage to the structure of cells and tissue. Tumors typically have higher density capillary distribution and higher blood supply. Therefore, the bubble burst could cause more severe damage to tumor than normal tissue. When combined with radiotherapy, the treatment effect may be further enhanced. In this study, the microbubble from Visual Sonics (Toronto, Canada) was used. A low-intensity ultrasound (3 MHz; continuous output, D150 Plus, Dynatronics Corp, Salt Lake City, UT) was used to burst the microbubbles. Vivo 2100 (Visual Sonics, Toronto, Canada) was employed as ultrasound imaging tool. In total, 15 mice with implanted flank tumors were tested in this study. All the mice were divided to three groups. The first group was treated with microbubble bursts plus radiotherapy (MB+R); the second group was treated with radiotherapy only (R) and the third group was treated with microbubble bursts only (MB). For the microbubble burst treatment, the microbubbles were injected into the tumor and then followed by 9 min ultrasound treatment. For the radiotherapy, the tumor was treated with 8 Gy in single fraction on small animal research platform (SARRP). The tumor size and blood relative volume of the tumor were determined in Vivo 2100 as indices of tumor development. The data were obtained before treatment and 36 hours after treatment.

Results and Discussion: The results of all mice with the same group were averaged to make an easy comparison. The tumor size change 36 hours after treatment for MB+R, R and MB grous are 6.2%, 12.8% and 17.7% respectively. The blood relative volume changed for three groups are -11.1%, 19.6% and 75.4%, respectively. These results shows the MB+R treatment have higher containment of tumor development than other two single-treatment methods, which indicates that the microbubble burst may enhance the tumor response to radiation on mice.

1.7 **Changes in quantitative ultrasound parameters of excised mouse kidney and liver due to decellularization,** L.A. Wirtzfeld, E.S.L. Berndl, E. Hysi and M.C. Kolios, *Department of Physics, Ryerson University, Toronto, ON Canada, lauren.wirtzfeld@ryerson.ca*

The fundamental sources of ultrasound scattering in tissue has been extensively studied, with particular emphasis on the contributions from the cell and cell nucleus. The extracellular matrix is also likely to contribute to ultrasound backscatter but is harder to isolate its contributions to tissue scatter from the cellular scattering. In order to investigate the scattering contributions from the extracellular matrix (ECM), a recently-developed technique was employed to remove cells from tissue or "decellularize" the tissue. Decellularizing involves washing the tissue in sodium dodecyl sulfate (SDS) for 24 hours, followed by washing with Triton-X for 24 hours and finally washing and storing in PBS. In this process, the ECM of the tissue is isolated from its inhabiting cells, leaving an ECM scaffold of the tissue.

In this study, excised murine livers and kidneys were imaged after being maintained in PBS. Subsequently the organs were decellularized and were reimaged. Attenuation was estimated by an insertion loss method using the VisualSonics Vevo770 and a nominal 25 MHz transducer. The spectral slope and midband fit were estimated using a VisualSonics VevoLAZR using a nominal 40 MHz transducer.

Subsequent to decellularization, the organs were significantly smaller in volume. A decrease in attenuation of 0.3 dB/cm-MHz was observed after decellularization for both organs. A decrease in spectral slope was observed after decellularization, while the midband fit increased for both organs with a larger increase observed for the liver compared to the kidney.

The ability to study the ultrasound backscatter from organ extracellular matrices offers a new opportunity to further our understanding of the potential sources of scattering within tissue and variations across organs.

1.8 **Classification of kidney and liver tissue using ultrasound backscatter data**, Fereshteh Aalamifar, Hassan Rivaz, Juan J. Cerrolaza, James Jago, Nabile Safdar, Emad M. Boctor and Marius George Linguraru, *Johns Hopkins University, Baltimore, MD 21218, fereshteh@jhu.edu*

Ultrasound (US) tissue characterization provides valuable information for the initialization of automatic segmentation algorithms and can further provide complementary information for diagnosis of pathologies. US tissue characterization is challenging due to the presence of various types of image artifacts and dependence on the sonographer's skills. The latter can be mitigated by using 3D US probes such as matrix arrays and collecting 3D US images. In addition, for the purpose of tissue characterization, one may benefit from focusing on the underlying physics of US wave-tissue interaction rather than the post-processed image adapted for better interpretation by human eye. On the other hand, most clinically-acquired US images are available in their post-processed, i.e., compressed B-mode, form.

In line with the above statements, our goal in this work was to perform tissue characterization of 3D volumetric US data, using the distribution of backscatter US data, recovered from end-user displayed B-mode images available in clinical systems. Specifically, we aimed to use this method to differentiate between kidney and liver tissue in 3D US abdominal images taken from pediatric patients with hydronephrosis. The motivation comes from the fact that hydronephrosis is a common disease in pediatric patients, affecting 2-2.5% of children, and US imaging is currently used for early diagnosis of hydronephrotic patients, but yet is limited by its subjective and qualitative assessment. Hence, many of these patients have to then undergo other imaging modalities such as CT. US tissue characterization and segmentation makes possible objective and quantitative assessment of the US images, eliminating the need for ionizing-radiation imaging.

The first step is to differentiate between the two most commonly-appearing tissue types in 3D abdominal US images, i.e., liver and kidney. To this end, we first reverse the compression and convert the US image to its envelope-detected data. We then extract the speckle-distribution parameters. We propose the computation of a large feature set based on the homodyned-K distribution of the speckle as well as the correlation coefficients between small patches in 3D images. We then utilize the random forests framework to select the most important features for classification. Experiments on *in-vivo* 3D US data from nine pediatric patients with hydronephrosis showed an average accuracy of 94% for the classification of liver and kidney tissues, showing a good potential of this work to assist in the classification and segmentation of abdominal soft tissue.

2. ELASTICITY 1

2.1 **Viscoelastic tissue-mimicking phantom validation study with shear-wave elasticity imaging and viscoelastic spectroscopy,** Carolina Amador, Randall R. Kinnick, Matthew W. Urban, Mostafa Fatemi and James F. Greenleaf, *Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN, amadorcarrascal.carolina@mayo.edu*

Objectives: Acoustic-radiation-force shear-wave elasticity imaging (SWEI) methods are being used to characterize soft-tissue pathologies by assessing the viscoelastic properties of tissue. Tissue-mimicking phantoms are frequently used in the evaluation of SWEI methods. To adequately evaluate the SWEI methods, it is extremely important to characterize the viscoelastic properties of tissue-mimicking phantoms with an independent method. In this study, we developed viscoelastic tissue-mimicking phantoms that were then tested with SWEI and viscoelastic spectroscopy methods.

Methods: Viscoelastic tissue-mimicking phantoms consisting of 8% gelatin (Sigma-Aldrich, St. Louis, MO), 1% potassium sorbate (Sigma-Aldrich), 1% cellulose (Sigma-Aldrich), 6% propaneidol and 0.5% xanthan gum were made by volume concentration. To vary the viscoelastic properties, two phantoms were made with 5% (Phantom 2) and 10% (Phantom 3) of Vanicream Lite (Pharmaceutical Specialties, Inc., Rochester, MN). The control phantom (Phantom 1) did not have Vanicream. From a given batch, each phantom mixture was poured into 3 cylinders (9.4 mm diameter and 44 mm height) for viscoelastic spectroscopy measurements and in a cube with 40 mm x 60 mm x 38 mm (width x length x height) dimensions for SWEI measurements. For the viscoelastic spectroscopy experiments, a RheoSpectris C500 (Rheolution, Inc., Montreal, QC, Canada) with the cylinder fixture was used. For each cylinder, 4 repeated measurements were made at frequencies between 10 Hz and 2000 Hz. For SWEI experiments, a Verasonics ultrasound system (Verasonics, Inc., Redmond, WA) equipped with a linear-array transducer

(L11-4v, Verasonics, Inc., Redmond, WA) was used. A focused push with F/2.0 configuration was used. Four measurements were made on each phantom. Both SWEI and viscoelastic spectroscopy experiments were completed one day and one week after phantom preparation. Phantoms were covered with paraffin film and stored at room temperature in between the study dates.

The complex modulus from viscoelastic spectroscopy experiments was compared with complex modulus estimated from fitting a Kelvin-Voigt fractional derivative (KVFD) model to shear-wave speed measurements at frequencies from 100-500 Hz. The KVFD model was also fit to the complex modulus from viscoelastic spectroscopy measurements from 100-500 Hz. The KVFD model parameters μ_1 [Pa], μ_2 [Pa·s], α and the fit's mean-squared error (MSE) from SWEI and viscoelastic spectroscopy were compared.

Results: One week post-phantom preparation, the complex modulus from both viscoelastic spectroscopy and SWEI measurements showed good agreement. Within the 100-500 Hz bandwidth used for comparison, the KVFD model parameters (μ_1 [kPa], μ_2 [Pa·s], α) for Phantom 1, were 3.95, 1.8, 0.8 for SWEI and 3.71, 2.4, 0.77 for viscoelastic spectroscopy; for Phantom 2, were 3.89, 2.4, 0.8 for SWEI and 3.79, 3.11, 0.75 for viscoelastic spectroscopy; and for Phantom 3, were 3.76, 10, 0.6 for SWEI and 3.8, 8.5, 0.63 for viscoelastic spectroscopy. The MSE for all fits was less than 0.01.

Conclusions: In this study, we have proposed a new formulation for viscoelastic tissue-mimicking phantoms and evaluated the complex modulus of these phantoms with two independent methods: viscoelastic spectroscopy and SWEI. The study found that for the proposed viscoelastic phantoms, one week of aging after phantom preparation is required for material stability. The results showed an increasing viscosity (μ_2) as the Vanicream concentration increased from 0% to 10%. This study was supported in part by NIH grants R01DK092255 and R01CA168575.

2.2 **In-plane anisotropy method for the measurement of the elastic behavior of anisotropic materials**, Sara Aristizabal[1], Ivan Z. Nenadic[1,] Carolina Amador[1], Bo Qiang[1], James F. Greenleaf[1], and Matthew W. Urban[1], *[1]Ultrasound Research Laboratory, Mayo Clinic College of Medicine, Rochester, MN, 55905, aristizabaltaborda.sara@mayo.edu*

Ultrasound radiation-force-based methods have been used for more than two decades for the evaluation of the material properties of soft tissues. These methods have proven successful for assessment of tissues and materials that are incompressible, homogenous and isotropic. Mechanical properties of tissues such as the kidney, myocardium and skeletal muscle are directionally dependent, a property commonly known as anisotropy. The evaluation of anisotropic tissues' mechanical properties by the traditional radiation force methods can lead to an incomplete and inaccurate characterization.

To investigate this phenomenon, anisotropic phantoms and *ex vivo* tissue samples are assumed to be transversely isotropic and their properties are evaluated by measuring the shear-wave velocity as a function of the angle between the tissue fibers and the direction of shear wave propagation in the range 0-360˚. The anisotropic behavior is characterized by the shear-wave velocity decreasing progressively as the transducer is rotated with respect to the anisotropic material to an angle perpendicular to the fibers while the shear-wave velocity values increase gradually as the transducer is rotated to an angle parallel to the fibers.

The characterization of their behavior by the aforementioned method has several limitations, one of which is being time consuming as it requires transducer rotation. The sample also needs to be wide enough to allow for the transducer to rotate freely without losing contact with the surface, which might present some difficulties in an *in vivo* setting.

To improve the characterization of anisotropic tissues, it might be possible to estimate the transversely isotropic shear moduli by measuring the shear waves in a single B-mode imaging plane. By measuring the shear wave velocity values at the angles centered around 0˚, it is possible to fit a transversely isotropic model using the obtained values to estimate the complete anisotropic velocity behavior. We refer to this method as In-Plane Anisotropy (IPA) technique.

To investigate this hypothesis, we evaluated the anisotropic characteristics of an *ex vivo* pork tenderloin by first using the conventional method and then comparing the results with the IPA technique. Measurements were made initially at different angles by rotating transducer with respect to the excised pork tenderloin from 0˚-360˚ in 10˚ increments. Shear waves were generated and measured by a LOGIQ

E9 (GE Healthcare) ultrasound system equipped with a linear-array transducer operating at the center frequency of 6 MHz. Subsequently, we evaluated the IPA method by measuring the shear-wave velocity in the plane where the transducer surface was aligned with the fibers orientation by first measuring the shear wave at 0˚ and then by rocking the transducer and measuring the shear waves generated around the 0˚ location from about -20° to +20°.

The shear-wave speeds for the sample of pork tenderloin obtained with the traditional method by measuring the shear wave velocity directly and with the IPA method by fitting the transverse isotropic model at 0° were 5.30 and 4.75 m/s, at 30° were 4.10 and 3.70 m/s, at 60° were 3.02 and 2.76 m/s, and at 90° were 2.71 and 2.50 m/s, respectively. There was approximately a 10, 9.7, 8.6 and 7.7% difference on the estimates of shear-wave group velocity as a function of angle using the traditional method and the IPA method. In general, the estimates of shear-wave group velocity as a function of angle using the traditional method and the In Plane Anisotropy methods were in good agreement. This work was supported in part by NIH grant DK092255 from the National Institute of Diabetes and Digestive and Kidney Diseases.

2.3 **An analytic expression for the two-dimensional Fourier-transform description of shear-wave propagation in a viscoelastic material following spatially-asymmetric Gaussian excitations,** Ned C. Rouze, Mark L. Palmeri and Kathryn R. Nightingale, *Duke University, Department of Biomedical Engineering, Durham, NC, ned.rouze@duke.edu*

Background: Viscoelastic properties of materials can be determined by observing shear-wave propagation following localized, impulsive excitations and measuring the frequency dependent phase velocity $c(f)$ and shear attenuation $\alpha(f)$. Often, a specific viscoelastic model is used to parameterize these quantities in terms of a small number of model parameters. Recently, Nenadic, et al[1, 2] have described a model-free method to obtain $c(f)$ and $\alpha(f)$ from the two dimensional Fourier transform (2D-FT) of a spatial-temporal shear wave signal. However, this approach gave large biases in the dispersion analysis of simulated data sets that modeled the excitation and shear wave propagation in experimental measurements performed in human liver by Nightingale, et al.[3] In this study, we evaluate bias introduced in the measurement of viscoelastic properties of materials using the 2D-FT analysis approach.

Methods: The equation of motion describing shear wave propagation in a viscoelastic material is derived in the Fourier domain and solved analytically for a spatially asymmetric Gaussian acoustic radiation force source. This solution is used to evaluate the 2D-FT analysis method by calculating the 2D-FT signal for a specific viscoelastic model and comparing $c(f)$ and $\alpha(f)$ to the true values determined from the parameters used in the model.

Results/Conclusions: Results indicate that the values of $c(f)$ and $\alpha(f)$ measured using the 2D-FT analysis approach gives biased results that can exceed 50% in some cases depending on the specific values of frequency, aspect ratio of the source, and the material. In particular, biases in phase velocity are observed to increase with frequency, and thereby give biased results for the dispersion slope that are sufficiently large to be an important contribution to the biases observed by Nightingale, et al.[3] These biases can be reduced by weighting the spatial-temporal shear wave signal using a factor of the form x^p where the optimal power p depends on the aspect ratio of the source to account for geometric spread of the shear wave front and the specific material properties of the imaging medium. This work has been supported by NIH grants R01EB2132 and R01CA142824.

*** [1 J. Acoust. Soc. Am. 134, 4011 (2013). [2] Proceedings of the 2014 IEEE International Ultrasonics Symposium, 987-990 (2014). [3] IEEE UFFC 62, 165-175 (2015).

2.4 **System-dependent sources of uncertainty and bias in quantitative shear-wave imaging,** Yufeng Deng, Ned C. Rouze, Mark L. Palmeri and Kathryn R. Nightingale, *Department of Biomedical Engineering, Duke University, Durham, NC, yufeng.deng@duke.edu*

Introduction: Several research groups have developed quantitative shear-wave imaging methods to measure the speed of propagating shear waves following acoustic radiation force (ARF) excitations to reconstruct tissue elasticity.[1-3] These methods estimate tissue motion from ultrasound data before and after the ARF excitation and then reconstruct shear-wave speed (SWS) from temporal-spatial tissue motion data using time-of-flight (TOF) methods.[4-6] This work investigates the sources of uncertainty and

bias arising from ultrasound system-dependent parameters, such as spatial location and timing, in quantitative shear-wave imaging.

Methods: Errors arising from both spatial and temporal uncertainties lead to errors in TOF-SWS measurements. System-derived temporal errors include master clock jitter, pulse repetition frequency (PRF) and arrival time estimation noise. Sources of spatial errors include phase aberration, beamforming misalignment and coupling medium sound speed mismatch. Beamforming errors include pitch errors in linear and phased arrays as well as radius of curvature (ROC) and sector angle errors in curvilinear arrays. Each of the error sources is investigated with the aid of Field II simulation,[7] full-wave acoustic propagation simulation[8] and experimental validation.

Results/Conclusions: Beamforming errors, coupling medium sound speed mismatch and PRF noise cause biases in SWS measurements (accuracy errors) while the other error sources cause uncertainties (variance). Arrival-time estimation noise is the dominant source of uncertainty, often leading to ~5% error; however, this error can be reduced by averaging over the number of beams used in the SWS reconstruction. Incorrect transducer parameters result in beamforming errors. A 5% pitch error would lead to biases in SWS measurements of 5% in linear arrays and ≤ 5% in phased arrays. The steering effect and the parabolic delay profile in phased array focusing bias SWS measurements in opposite directions. For curvilinear arrays, a 5% error of sector angle would lead to 5% errors in SWS measurements, while a 5% error of ROC would lead to < 5% errors depending on the imaging depth. In our experience, the calibration of transducer parameters in research systems is likely to be less rigorous than in commercial systems. Calibration of these sources of error is an important step in the development of shear wave imaging systems. Spatial errors can be characterized by calibrating the lateral beam positions, which can be accomplished by imaging point targets with known lateral translations. Supported by NIH grant R01EB002132 and RSNA/QIBA studies.

***(1) UMB 24, 1419-1435 (1998). (2) IEEE UFFC 51, 396-409 (2004). (3) JASA 115, 2781-2785 (2004). (4) UMB 34, 546-558 (2008). (5) UMB 36, 802-813 (2010). (6) IEEE UFFC 57, 2662-2670 (2010). (7) *IEEE ISBI* (2004). (8) *IEEE UFFC 56*, 474–488 (2009).

2.5 **Quantitative measurements of viscoelasticity using Viscoelastic Response (VisR) ultrasound**, Mallory R. Selzo[1] and Caterina M. Gallippi[1], [1]*Joint Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC, cmgallip@email.unc.edu*

Background: Viscoelastic Response (VisR) ultrasound is a method for assessing the viscoelastic properties of tissue. We have previously demonstrated with finite-element-method (FEM) modeling that by using two successive force impulses delivered to a single node, displacements measured in the region of excitation can be fit to the mass-spring-damper (MSD) model to accurately measure τ, the ratio of viscosity to *elasticity*. However, the ARF impulses generated using conventional transducers are volumetric and span millimeters in axial, lateral and elevation dimensions. *We hypothesize that the volumetric nature of the ARF impulses will result in an overestimation of τ, but this overestimation can be corrected using an empirically determined correction function.*

Methods: FEM models of the response of a viscoelastic isotropic media in response to successive, focused, ARF excitations were solved using LS–DYNA. Field II was used to characterize the 3D acoustic intensity field associated with a VF7-3 linear array transducer. VisR sequences were implemented using two, 70 μs ARF excitations administered to the same ROE and separated by 0.4 ms in time. Simulations were performed in 100 viscoelastic materials with Young's moduli ranging from 5-100 kPa and viscosities ranging from 0.5-9.5 Pa·s. Axial-displacement profiles were fit to the MSD model and the error-correction function was determined by fitting a 3D surface to the VisR-derived parameters relative to the true τ of the materials. Error correction was also demonstrated experimentally in a gelatin phantom using a high-speed camera to optically track the ARF-induced displacements. ViSR imaging was performed with a Siemens Antares scanner and VF7-3 linear array; two different focal configurations of the ARF excitations were employed (F/1.5 and F/3) in order to vary the volume of displaced tissue.

Results: In FEM, VisR performed using a volumetric ARF excitation overestimated τ of the material with a median error of 373.7%. After applying the correction function, the median error of τ across all materials was reduced to 5.1%. Experimentally, estimates of τ were significantly smaller (p<0.01) when using an F/1.5 focal configuration (0.568 ± 0.085 ms) than those made using an F/3.0 focal configuration

(0.816 ± 0.037 ms). After applying the correction function, τ estimates using the F/1.5 configuration (0.418 ± 0.064 ms) were consistent (p=0.59) with the F/3.0 corrected τ estimates (0.439 ± 0.046 ms).

Conclusion: These data show that when using a volumetric ARF body force to induce displacement, VisR overestimates τ in viscoelastic materials. In order for VisR estimates of τ to be quantitative, inaccuracy in the modeled forcing function can be mitigated using an error-correction function. VisR with τ correction was also demonstrated for proof of feasibility in a gelatin phantom.

Monday afternoon

3. ROBOTICS, INTERVENTION AND THERAPY GUIDANCE

3.1 **Evaluation of real-time graded control for upper-extremity prostheses using ultrasound imaging**, Nima Akhlaghi[1], Mohamed Lahlou[2], Brian Monroe[4], Huzefa Rangwala[3], Jana Kosecka[3], Joseph J. Pancrazio[2] and Siddhartha Sikdar[2]. [1]*Department of Electrical and Computer Engineering,* [2]*Bioengineering and* [3] *Computer Science, George Mason University, Fairfax, VA, 22030 and* [4] *Hanger Clinic, Burtonsville, Maryland, 20866, ssikdar@gmu.edu*

Background: With recent developments in the electromechanical design of upper extremity prosthetics, the need for more advanced control strategies has increased. Current commercially-available prostheses based on myoelectric control have limited functionality, which leads to many users abandoning use. Myoelectric control using surface electrodes has a number of limitations, such as low signal to noise ratio and lack of specificity for deep muscles. To address these limitations and enable more intuitive dexterous control, our research group is investigating a new strategy for sensing the muscle activity based on ultrasound imaging. In the past, we demonstrated that using ultrasound imaging, individual finger flexion could be decoded offline with 97% accuracy and 15 different complex grasp (e.g., power grasp and pinch) could be decoded with 91%. The objective of this study is to evaluate the feasibility of real-time graded control using a computationally-efficient method to differentiate between complex grasps based on ultrasound imaging of the activity of forearm muscle compartments.

Methods. Dynamic ultrasound images of the forearm were obtained from six healthy volunteers and two transradial amputees using a Sonix RP system with a 5-14 MHz linear probe. These images were analyzed to map muscle activity based on changes in ultrasound echogenicity of the contracting muscles during different movements, and used to control a virtual prosthetic hand. MATLAB-based custom software was developed to implement real-time decoding. The custom software received the ultrasound image stream through a research interface. Then an image processing algorithm was used to decode movements in real–time based on a predefined dictionary. After that, the correlation value between a baseline image and current image was used to generate a proportional graded signal (called completion level) indicating the level of completion for each movement. We evaluated the completion rate, or the percentage of times the motion was decoded successfully out of the attempts made, the motion initiation time and the motion completion time. To evaluate graded control, position sensors were mounted on the individual digits and the relationship between the angle of finger flexion and completion level was investigated.

Results: For healthy subjects, the real-time image-based control of a virtual hand showed an average classification accuracy of 92%. The acquisition time for all motions was 200 ms and the average processing time was 79 ms. There was an inverse linear relationship between completion level and the angle of flexion (with average r^2 = 0.94). In a preliminary feasibility study on transradial amputees, we demonstrated that the residual muscle functions can be visualized and decoded to differentiate between different complex grasps using ultrasound imaging. We were able to differentiate between four grasps (grip with index point, grasp, thumb and little finger flexion and wrist pronation) performed by the first amputee, and seven different grasps (five individual digit flexion, grasp and wrist flexion) performed by the second amputee subject with small crosstalk between the movements performed.

Discussion and Conclusion: Our current approach demonstrated the feasibility of using ultrasound imaging to reliably decode complex volitional motor and generate graded signals for real-time interactive control using able-bodied individuals and amputees. In our current implementation, the acquisition time is relatively large because a fixed number of frames are acquired before decoding every motion -- under the assumption that motion completion time of the user is the same for every motion type. We are investigating new strategies to reduce acquisition time by monitoring the percent change in the completion level to start data collection once the user initiates the motion. Changes to the dictionary would also be implemented, looking at only motion initiation activity patterns instead of full completion of motion activity patterns. Using this method, it is possible to decrease acquisition time to 60 ms, without significant change in classification accuracy. Our preliminary results demonstrate the feasibility of using ultrasound imaging for control of upper-extremity prostheses. We anticipate that this strategy will be a significant improvement over conventional myoelectric-based control of prosthetics, as well as robots and other actuated exoskeletons.

3.2 **Dual robotic-arm ultrasound tomography: system setup and error analysis,** Fereshteh Aalamifar, Haichong K. Zhang, Dengrong Jiang, Iulian Iordachita and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218, fereshteh@jhu.edu*

Most clinically-available US systems provide reflection US image and its varieties; however, reflection US imaging may miss some of the useful information that can be measured by transmission US tomographic imaging. In transmission US tomographic imaging, the transmitter and receiver transducers are located at different known positions with respect to insonified area and the received signals can be used to reconstruct tissue acoustic properties such as speed of sound and attenuation. Previous attempts to allow US tomography has shown to be effective in breast cancer detection and diagnosis. However, these systems are mostly circular and specifically designed for breast US tomography. Another alternative setup for enabling US tomography can be realized by aligning two conventional US probes; one of them acts as transmitter and the other one as receiver to enable tomographic imaging. Aligning two probes manually is a challenging task. Hence, we previously proposed robot-assisted US tomography using two conventional US probes, one operated by the sonographer as a tracked freehand and the other one moved by a robotic arm that keeps aligned with the freehand probe. Such a system could facilitate tomographic reconstruction for more general examinations.

In this work, we propose a new system setup that includes two robotic arms holding the two US probes. One of the robotic arms is operated by the sonographer to determine the desired location for the tomographic imaging; this probe can also provide the B-mode US image during the search. The other robotic arm can then move automatically to align the two probes. Compared to our previous setup, the dual arm system does not require line of sight for tracking, has the potential to be used as a fully robotic or tele-operated system or in cooperative mode and provides the operator with the option to fix the cooperative probe for tomographic imaging after the area of interest is found. In comparison, the latter, in freehand mode, allows for a spatially-stable probe, leading to more accurate registration between the robotic arms and probes and therefore a more accurate reconstructed image.

To enable automatic alignment of the two probes, accurate calibrations between the robot bases, robot tooltips and US images were performed. After the calibrations were done, we captured a single point in five different locations using the two US probes and then used the calibration matrices to reconstruct the points in one of the robot bases. The mean distance between the reconstructed points were [3.30, 0.58, 1.67] mm. This alignment error will affect the image reconstruction. In this work, we also describe a sample algorithm for reconstructing speed of sound in US tomographic images using two aligned linear probes and then provide an analysis on how the tracking error propagates through the whole system and how the in-plane translational error affects the tomographic reconstruction.

3.3 **Synthetic-Tracked Aperture Ultrasound (STrAtUS) Imaging using robotic guidance,** Haichong K. Zhang[1], Nick Bottenus[2], Alexis Cheng[1], Gregg E. Trahey[2] and Emad M. Boctor[1], *Johns Hopkins University, Baltimore, MD 21218 and Duke University, Durham, NC, 27708, hzhang61@jhu.edu*

In ultrasound imaging, it is challenging to acquire decent image quality in deep tissue. Center frequency, focusing depth and aperture size are three main factors contributing to ultrasound image

resolution. It is known that high-frequency ultrasound signal is required to attain high resolution. Attenuation, however, is proportional to ultrasound frequency and hinders high-frequency signals from insonifying deeper tissue. Thus, low-frequency signals can be used to image deeper regions. As the way to utilize a wider aperture, synthetic aperture (SA) is a technique proposed to increase image resolution by synthesizing information from multiple subapertures. But still, the resolution improvement is limited by the physical size of the ultrasound array transducer. Hence, it is hard to achieve high resolution in deep regions without extending the effective aperture size.

We propose a novel method to extend the available aperture size using tracking information from a robotic arm. We apply an SA reconstruction algorithm on these tracked apertures and hencem our imaging approach is called synthetic tracked aperture ultrasound (STrAtUS) imaging. Typically, we sweep the ultrasound transducer while tracking its orientation and location and the tracking information of the probe is used to synthesize the signals received at different positions. Since the wider aperture size can be kept in the final image, it is possible to improve the ultrasound image quality.

Considering the realistic implementation of this approach, it is important to recognize that there are two uncertain factors limiting the quality of the STrAtUS imaging: the accuracy of the tracking device and the precision of ultrasound calibration. We estimated the effect of tracking error and ultrasound calibration to final beamformed image quality through simulation. Moreover, to experimentally validate this approach, a 6 degree-of-freedom (DOF) robot arm (UR5, Universal Robot) was used as a mechanical tracker to hold an ultrasound transducer and to apply in-plane lateral translational motion. Results indicate that the technique to synthesize the tracked aperture has the potential to improve the ultrasound image quality.

3.4 **Tracked ultrasound-based bone registration for robotic orthopedic surgery,** Lei Chen, Seth Billings, Russell H. Taylor, Peter Kazanzides and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218 lchen121@jhu.edu*

Background: In the United States, each year about 250,000 patients need total hip replacement (THR) surgeries. These surgeries are commonly guided by computer-aided orthopedic surgery (CAOS) systems. A typical image-based CAOS system consists of a pre-operative model of the bone, intraoperative real-time tracking system, and navigation algorithms to fuse pre-operative models to surgical scene. Also, there are a number of CAOS systems include robots to perform precise machining. The registration/fusion step between CT model and the femur requires invasive insertion of one pin at the proximal-end and two or more pins at the distal-end. This invasive registration approach needs to be replaced by an accurate, robust, and most importantly non-invasive method.

Methods: We presented a pin-less registration method by using tracked ultrasound B-mode images. Our goal in this project is to automatically segment ultrasound B-mode images to localize the bone surface of distal femur. First, the bone surface is extracted from B-mode images with the difference of Gaussian blob detector. Second, the border between the fat and muscle is detected by segmenting the fat from muscle with the fast marching methods. Third, the locations of the bone surface are then recalculated based on the corrected speed of sound in fat and muscle. Finally, the rigid-body registration is performed by the standard ICP and the projected iterative most-likely oriented point algorithms.

Results: The proposed methods were evaluated with a porcine bone phantom. Two optical markers were rigidly affixed to the proximal and distal ends of a fresh pig shoulder, which established a ‘patient’ coordinate system. The ground-truth was established by transforming the manually segmented bone surface from CT coordinate to the patient coordinate. The transformation was determined by a point-to-point registration with the tracked fiducial and the segmented fiducial from CT image by using a model-based localization method. Tracked Ultrasound-based bone registration has high potential to reduce the invasiveness and improve the registration accuracy of THR procedure.

3.5 **Robot-assisted mirror ultrasound scanning for deep venous thrombosis using twin RGB-D**, Bo Meng, Lei Chen, Fereshteh Aalamifar and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218, eboctor@jhmi.edu*

Deep Venous Thrombosis (DVT) is a major cause of morbidity and mortality. Ultrasound (US) imaging is the modality of choice to evaluate the risk of DVT. Usually, this exam is applied bilaterally on both legs, in which the sonographer spends about 45-90 minutes scanning manually and sequentially both

legs. This paper proposes a novel co-robotic "mirror" US scanning system to assist sonographers getting rapid, detailed and consistent bilateral scans.

The co-robotic mirror system is composed of a two-arm robot and an US probe with IR markers for both master and slave side. On the master side, sonographers hold one robot arm and operate the probe to inspect one leg. On the slave side, the robot follows the master probe and scans the other leg utilizing available surface information, depth maps and kinematics of both arms. 3D depth maps of both legs are segmented and registered to get the "mirror" transformation matrix. The master probe's position and orientation are obtained and mapped to the slave side. This co-robotic ultrasound system has been developed, integrated with Kinect sensors, and several experiments were conducted.

The transformation matrixes between legs, Kinect and probes were obtained. The corresponding position and orientation of the slave-probe was calculated after applying registration and mirror process. The correlation coefficient of the mirrored leg to the other leg is 0.9547, with an average error of 7.66 mm. The experiments show an early feasibility of a new robot-assisted US scanning system.

3.6 **Surgical fiducial segmentation and tracking for pose estimation based on ultrasound B-Mode images,** Lei Chen, Nathanael Kuo, Fereshteh Aalamifar, David Narrow, Devin O'Brien-Coon, Emad M. Boctor and Jerry Prince, *Johns Hopkins University, Baltimore, MD, 21218, lchen121@jhu.edu*

Background: Each year over 250,000 patients in the U.S. undergo anastomosis, a blood-vessel reconnection procedure. One failed reconstruction caused by blood clots is $45,000 and the annual cost of such failures is over $150 million. Doppler ultrasound is a noninvasive diagnostic tool for the quantitative measurement of blood flow and may be used for monitoring blood clots in a surgical anastomosis. However, this technique requires an experienced sonographer to perform frequent ultrasound screenings at bedside. We therefore developed an image-guidance system based on ultrasound B-mode images to guide an unexperienced user to position the ultrasound probe to the same surgical site repeatedly in order to acquire a relevant time series of Doppler readings.

Methods: The system includes a specially-designed biodegradable fiducial as well as complementing software composed of fiducial detection, key points tracking, probe pose estimation and graphical user interface (GUI) modules. The implantable fiducial is made of PLGA. It will be degraded and absorbed within a few weeks postoperatively, depending on the monitoring requirement. The fiducial is an echogenic marker that is implanted at the surgical site and can be detected and tracked during ultrasound B-mode screening. The workflow starts from the marker detection using the difference of Gaussian blob detector. The detection are then used to extract six key points with the algorithm of robust point set registration using Gaussian-mixture models. The key points on the marker can next be used to determine the pose of the ultrasound probe with respect to the echogenic marker. Here, the Kalman filter is applied to reduce the noise from the pose measurements. The 3D representation of the probe and the fiducial is implemented using the Visualization Toolkit. In the 3D view, the relative pose (the position and orientation) between the probe and the fiducial are then displayed in the GUI for user guidance.

Results: The system provides a low cost, efficient and expert-independent solution for the blood vessel monitoring at bed side. We tested the system on a phantom and showed that it can detect and track the fiducial marker while displaying the probe pose in real-time. The validation data consists of five data sets (three translations and two rotations around the center of maker). Each one contains B-mode images and pose information for one translation or one rotation. It was collected using a linear probe holding by a UR5 robot arm, 1 mm step for translation and 1 degree step for rotation. Therefore the estimated pose between two successive frames can be evaluated with the relative pose provided by a UR5 robot.

3.7 **Out-of-plane detection and tracking with active acoustic sources,** Alexis Cheng, Haichong K. Zhang, Xiaoyu Guo, Hyun Jae Kang and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218, eboctor@jhmi.edu*

There is a need for tracking catheters in many surgical procedures, such as cardiac catheterization or central venous catheterization. The standard approach is to use fluoroscopy intermittently during the process of guiding the catheter to its destination. This has the obvious disadvantage of delivering ionizing radiation to the patient. Other real-time imaging modalities such as ultrasound may be useful in certain procedures but catheters are often visualized poorly in a standard ultrasound brightness-mode image. The

main focus of this work is in the use of an active source placed at the catheter tip to aid in catheter tracking when combined with real-time ultrasound imaging. In previous work, we demonstrated how the active ultrasound pattern injection system (AUSPIS) could aid the surgeon in localizing the catheter tip within the ultrasound mid-plane. However, the AUSPIS system was limited by the detection range of the catheter tip because it relied on the transmitting pulse from the ultrasound transducer. Thus, it could only be tracked successfully when it was within the ultrasound beam profile.

In this work, we demonstrate the use of an active source that does not rely on the transmitting pulse from the ultrasound transducer. The active source is continuously transmitting while the ultrasound transducer is set to receive-only mode. With this change, the detection range of the active source is no longer limited by the transducer's beam profile, but by its receiving angular sensitivity. We present the overall system, associated methods, and experimental results to demonstrate the feasibility of this approach.

3.8 **Direct ultrasound-to-video registration using photoacoustic markers from a single image pose**, Alexis Cheng, Xiaoyu Guo, Russell H. Taylor and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218, acheng22@jhu.edu*

Many modern surgical scenarios provide surgeons with additional information via fusion of video and other imaging modalities. This functionality requires the use of interventional guidance equipment and surgical navigation systems to register independent tools and devices. In this work, we focus explicitly on registering ultrasound with a stereo-camera system using photoacoustic markers. In previous work, we showed that photoacoustic markers can be used to register three-dimensional ultrasound with video resulting in target registration errors better than the state of the art. Photoacoustic markers are noncollinear laser points projected onto some surface. They can be simultaneously visualized by a stereo-camera system and in an ultrasound volume because of the photoacoustic effect. Being able to represent the same set of points in two independent coordinate systems allows one to find the registration between these two coordinate systems. This work replaces the three-dimensional ultrasound volume with images from a single ultrasound image pose.

An ultrasound image is less informative than an ultrasound volume but it does have certain advantages, such as lower cost and faster acquisition rate. However, it is difficult to register two-dimensional with three-dimensional spatial data. We propose the use of photoacoustic markers viewed by a convex array ultrasound transducer. The channel data of each photoacoustic marker provides a wavefront that gives some information about its elevational position, resulting in three-dimensional spatial data. This development enhances the method's practicality as convex array transducers are more common in surgical practice than three-dimensional transducers, as well as the advantage of not having to use an ultrasound volume. This work is demonstrated on a synthetic phantom. The resulting target registration error for this experiment was 2.47 mm and the standard deviation was 1.29 mm, which is comparable to currently-available systems.

3.9 **Photoacoustic image guidance for robot-assisted skull-base surgery,** Sungmin Kim, Hyun Jae Kang, Alexis Cheng, Muyinatu A. Lediju Bell, Peter Kazanzides and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218, sungminkim@jhu.edu*

We are investigating the use of photoacoustic (PA) imaging to detect critical structures, such as the carotid artery, which may be located behind the bone being drilled during robot-assisted endonasal transsphenoidal surgery. In this system, an optical fiber coupled to a laser is mounted on the drill and the 2D ultrasound (US) probe is placed on the temporal region of the skull. Both the drill and the US probe are tracked relative to the patient-reference frame. In PA imaging, a laser penetrates thin layers of bone and the PA image displays targets that are in the laser path. Thus, the laser can be used to extend the drill axis noninvasively, thereby enabling reliable detection of critical structures that may reside in the drill path. This setup creates alignment challenges because the image plane of the US probe must intersect the laser in the neighborhood of the target anatomy (as estimated from preoperative CT images). This work reports on a navigation system developed to assist with this alignment task. Results of our phantom experiments demonstrate that a critical structure can be detected with an accuracy of approximately 1 mm relative to the drill tip. upported by NSF NRI 1208540 and supported in part by NIH K99-EB018994.

Tuesday morning

4. IMAGING 1

4.1 **Breast ultrasound tomography with two parallel transducer arrays: Initial clinical results,** Lianjie Huang[1], Jun Seob Shin[1], Youzuo Lin[1], Ting Chen[1], Miranda Intrator[1], Kenneth Hanson[1], Katherine Epstein[2], Daniel Sandoval[2] and Michael Williamson[2], *[1]Los Alamos National Laboratory, Los Alamos, NM 87545 and [2]University of New Mexico Health Sciences Center, Albuquerque, NM 87131, ljh@lanl.gov*

We have designed and manufactured a synthetic-aperture ultrasound tomography system with two parallel phased-transducer arrays for breast imaging. The system consists of 768 transducer elements and takes approximately 2-3 minutes to scan one breast. The center frequency of the ultrasound transducers is 1.875 MHz. We recently started to use this breast ultrasound tomography system to acquire patient data at the University of New Mexico Hospital. We plan to scan 200 patients using this system and conduct whole breast ultrasound imaging and tomographic reconstructions to obtain sound speed and attenuation distributions within the breast. We present some initial clinical imaging and tomographic results for a number of patients with different breast abnormalities. The purpose of this study is to demonstrate to the capability of ultrasound bent-ray and waveform tomography for characterizing breast tumors. Supported by the Breast Cancer Research Program of the U.S. DoD Congressionally Directed Medical Research Programs.

4.2 **System for 2D Ultrasonic Temperature Imaging (TI) based on the change in backscattered energy in real-time with or without motion compensation**, R. Martin Arthur, and Weiyuan Zhao, *Electrical & Systems Engineering, Washington University in St. Louis, St. Louis, MO 63130, rma@ese.wustl.edu*

Background: Thermal therapies from cryosurgery to ablation would benefit from a noninvasive, safe, inexpensive and convenient thermometer to monitor heating patterns. Ultrasound is a modality that meets these requirements. Agreement among predicted, simulated and measured change in the backscattered energy (CBE) from both our *in-vitro* and *in vivo* experiments has shown that CBE can be used for temperature imaging (TI) in 3D.[(1, 2)] Building on these previous studies, we combined initial, real-time CBE TI estimates with refined CBE ultrasonic temperature images in 2D.[(3)]

Methods: To achieve real-time TI, we implemented a two-computer architecture. Our 7.5 MHz phased-array Terason 3000 ultrasonic imaging system collected and sent raw images over an internet connection to our TI computer. The TI machine was an HP Envy Phoenix 810 with a GeForce GTX 770 GPU card. It performed motion compensation (MC), estimated CBE and extracted temperature images over the image region. Six turkey specimens were imaged during heating with hot water (75^{o}C) in a 1 cm tube through the center of the specimen. Total heating time was 1200 s. The interval between image acquisitions was 30 s. Tissue temperature was monitored with thermocouples.

Results: In six experiments at three thermocouple sites over 1200 s, the accuracy of CBE TI was 0.8±0.7^{o}C. Using its CPU, the TI computer updated TIs using nonrigid MC in 7 s. MC time was reduced to 0.2 s using the GPU processor along with optimization of the MC algorithm, which means TI imaging was performed in real time with the GPU. In over 240 image pairs from six experiments, MC increased the correlation coefficient (CC) between temporally-adjacent images taken at 30 s intervals from 0.994±0.006 to 0.996±0.023. Because the CCs were high even without MC, we saw no significant increase in CBE temperature estimation errors without MC.

Conclusions: Calculation of CBE and conversion of CBE to a TI took about 0.2 s using only the CPU. Therefore, CBE-based TI without MC can be done in real time using a conventional workstation at a 1 Hz frame rate with <1^{o}C error for ultrasonic images taken at <30 s intervals. Supported by R21-CA90531, R01-CA107558 and the Wilkinson Trust at Washington University, St. Louis.

(1) Arthur et al. *IEEE Trans UFFC 57*, 1724-1733, (2010). (2) Basu, *PhD Dissertation*, Washington University (2010). (3) Zhao, *MS Thesis*, Washington University (2011).

4.3 **Using two-dimensional impedance maps to study weak scattering,** *Adam Luchies* and Michael Oelze, *Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering, The University of Illinois at Urbana-Champaign, Urbana, IL 61801, luchies1@illinois.edu*

The impedance map (ZM) is a tool for modeling acoustic properties of tissue microstructure. Three-dimensional (3D) ZMs are constructed from a series of adjacent histological tissue slides that have been stained to emphasize acoustic impedance structures. The slides are digitized using a camera, a realignment and registration process is applied to the series of images and each pixel is assigned an impedance value based on color. The result is a 3D spatial map of acoustic impedance, whose power spectrum can be related to the ultrasound backscatter coefficient (BSC), which can be further reduced to a form factor.

The goal of this study was to demonstrate the ability to estimate form factors using two-dimensional (2D) ZMs instead of 3DZMs. Compared to 3DZMs, fewer histological slides are required and a slide realignment process is not needed when using 2DZMs, resulting in reduced computational and financial cost. Two methods were explored for processing 2DZMs. The first assumes that the medium is isotropic and the second that the medium is transverse isotropic, e.g., structures aligned in one direction. In both methods, the correlation coefficient was estimated from slices before estimating a form factor. Simulated collections of objects (spheres, ellipsoids, cylinders) with known form factors in the case of weak scattering were used to study using 2DZMs to estimate form factors.

The mean absolute error (MAE) between estimated and theory for the logarithm of the form factor when using one slice having a size that included 50 object cross sections was 2.3, 1.3, 1.4 and 1.7 for monodipserse spheres, polydisperse spheres, monodisperse randomly oriented ellipsoids and monodisperse aligned ellipsoids. Increasing ZM slice thickness was found to produce form-factor estimates representing larger objects than those that were simulated. The results suggest that 2DZMs are a feasible alternative to 3DZMs when estimating form factors for collections of objects.

4.4 **Robustness of estimating hemodynamics around carotid artery plaques using computational fluid dynamics and 3D ultrasound imaging** , Khalid AlMuhanna[1], Bong Jae Chung[2], Md Murad Hossain[1], Limin Zhao[3], Juan R. Cebral[2], Brajesh K Lal[3, 4], and Siddhartha Sikdar[1, 2], *Departments of [1]Electrical and Computer Engineering and [2]Bioengineering, George Mason University, Fairfax, VA, [3]University of Maryland Medical Center, Baltimore, MD, and [4]Department of Veterans Affairs Maryland Health System, Baltimore, MD, ssikdar@gmu.edu*

Background: Debris from carotid artery plaque contributes to about 20% to 30% of ischemic strokes. The current clinical method of assessing the severity of carotid stenosis uses Doppler ultrasound. Evidence is mounting that severity of stenosis alone is not a good indicator of vulnerable plaque that has a higher likelihood of rupture. Hemodynamic effects on the plaque may provide more information on carotid plaque vulnerability. Computational fluid dynamic (CFD) simulations, such as wall shear stress, have been used to study carotid-artery atherosclerosis. CFD simulations are typically performed on volumes generated using magnetic resonant angiography (MRA) or computed tomography angiography (CTA) imaging. The objectives of this study are to perform CFD simulations on stenosed carotid artery volumes generated from manually-segmented 3D ultrasound images and study the sensitivity of hemodynamic factors due to differences in volumes segmented by multiple observers.

Methods: Ultrasound image volumes were acquired from patients with asymptomatic carotid stenosis using an Ultrasonix MDP system equipped with a 4D L14-5/38 transducer as part of the ongoing Asymptomatic Carotid Stenosis and Cognitive Function (ACCOF) study. The plaque volumes were manually segmented by three trained observers using a standardized protocol developed by our research group. The Stradwin software(1) was used for visualizing and segmenting the volumes. CFD simulations were performed using a fluid solver based on a finite element method (FEM) developed by Cebral et al.(2) In previous work, our group performed inter- and intra-observer variability/reliability analyses of the carotid artery volume segmentations.(3) In this study, these volumes were used to study the sensitivity of CFD parameters.

Results: Nine subjects with asymptomatic carotid stenosis (age: 66 ± 7 years) were included in this study. The mean total luminal volumes measured were 509.3 ± 151.1 mm^3, with an intra-class correlation coefficient (ICC) of 0.72 between observers. The CFD measurements' ICC values were: mean wall shear

stress (WSS) 0.58; percent of areas with low WSS 0.73; shear concentration index (SCI) 0.80; mean oscillatory shear index (OSI) 0.21; and maximum velocity 0.60.

Discussion: Our results indicate that 3D ultrasound imaging has the potential to be used for CFD simulations in addition to CTA and MRA images. Some CFD measurements, such as percent area with low WSS, and SCI, were robust to small changes in luminal geometry introduced by variability in segmenting 3D ultrasound images. Several other indices had lower ICC, indicating more sensitivity to geometric differences. In addition, there are a number of challenges. Although the volume measurements have a relatively good correlation, the calculated peak velocities from CFD did not correlate with the measured velocities from Doppler ultrasound in this analysis (average correlation coefficient of -0.16). One possible cause of this is the relatively short length of the segmented carotid (around 3 cm total) for CFD simulations in the present study. CFD results from ultrasound would need to be validated against results from alternative imaging methods such as MRA and CTA, which are planned for future work.

(1) Treece et al. *IEEE TMI* (2000). (2) Cebral et al. *Academic Radiology* (2002). (3) AlMuhanna et al. J Vasc Surg, (2014).

4.5 ***Ex vivo*** **measurements of ultrasonic wavefront distortion using large synthetic apertures across ribs and abdomen**, Marko Jakovljevic, Nick Bottenus and Gregg Trahey, *Department of Biomedical Engineering, Duke University, Durham NC 27708, marko.jakovljevic@duke.edu*

We have acquired large synthetic 2-D aperture data sets of point targets through canine ribs and abdomens *ex vivo*, and used them to estimate the shape and magnitude of the respective tissue-induced aberrations. For the transcostal acquisition, we observe the estimated aberrator profile to determine whether wavefront coherence is preserved across the ribs; this issue is of particular importance in transthoracic ultrasound, given the trend of increasing aperture size and element-counts in modern matrix arrays. In addition, for both transcostal and abdominal acquisitions we measure the point-spread-functions (PSF) to assess the loss of focus quality due to aberrators.

Large coherent 2-D aperture data sets were created by collecting full synthetic (transmit and receive) data from a clinical matrix array at five overlapping lateral array positions. The tissue samples were placed directly underneath the array. The arrival-time profiles were estimated from the channel data using a least-squares method. For the transcostal acquisition, channels that were blocked by the ribs were detected based on their low-amplitude values and were excluded from the arrival-time estimates. Arrival-times estimates were also obtained for wavefronts propagating through a water-path only. These control estimates were subtracted from the tissue estimates to obtain true aberrator profiles. Images of point targets were beamformed to assess the effect of phase aberration on focus quality.

Estimated aberration profiles indicate that chest wall causes more severe degradation of ultrasonic wavefronts than the abdomen. The root-mean-square (rms) values of arrival-time profiles were computed to be 58 ns for the transcostal acquisition, 24.8 ns for the abdominal acquisition and 10.8 ns for the control acquisition at the transmit frequency of 2.5 MHz. Similar trends are observed in the resulting PSF deformations. Point target images acquired through the ribs experienced 18.6 % increase in full-width at half maximum (FWHM) of the mainlobe and 13.8 dB rise in the side-lobes relative to the control acquisition. Imaging through the abdomen increased FWHM by 16.7 % while sidelobe levels increased by 1.9 dB. We also discuss potential ways to beamform signals from large apertures depending on severity of the aberrator. Supported by NIH grant R01-EB017711. The authors wish to thank the Ultrasound Division at Siemens Medical Solutions USA, Inc. for their in-kind and technical support as well as to Shalki Kumar and Lily Kuo for their efforts on harvesting the tissue samples and preparing the point-target phantom.

4.6 **Model-based clutter suppression for separating the impact of reverberation and phase aberration in abdominal imaging,** Kazuyuki Dei and Brett Byram, *Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, kazuyuki.dei@vanderbilt.edu*

In vivo ultrasound imaging is used more often than any other medical imaging modality besides X-ray, as it is completely noninvasive, relatively inexpensive and substantially advances diagnosis. Most ultrasound image-quality research has focused on image degradation due to diffraction limitations and phase aberration due to tissue inhomogeneity. Recent studies reveal that multipath scattering is also a

primary cause of clutter. In order to compare the relative contributions of multipath scattering and phase aberration, we introduced a model-based signal decomposition algorithm that can suppress off-axis and reverberation clutter. After suppressing these sources of clutter, we can reconstruct channel data, which can be used to estimate properties of phase-aberration.

By applying this scheme to clinical data sets, we generated B-mode images and measured the contrast before and after decluttering the channel data. The contrast of the decluttered images showed 6.3±0.6 dB improvement. Next, we applied near-field phase-aberration correction schemes to the data and found that contrast decreased 1.3±2.4 dB after applying aberration correction. When applying the aberration correction to decluttered data, there was a negligible change in contrast – 0.2±1.3 dB for the aberration corrected and decluttered data compared to decluttered-only data. After our image analysis, we characterized aberration characteristics on the original and decluttered channel data. Aberration profiles were characterized using the autocorrelation length full-width at half-maximum (FWHM), the aberration profile's root-mean-square (RMS), and the profile's isoplanatic patch size. The FWHM before and after decluttering are 1.63±0.76 mm and 3.13±0.34 mm, respectively (higher is better). The average RMS values are 34.2±10.0 ns and 16.2±4.5 ns, respectively (lower is better). Results show that our decluttering process decreased measured levels of aberration. Further, isoplanatic patch sizes using a 0.5 one-sided normalized cross-correlation width were 1.35±0.39 mm and 1.04±0.25 mm, with and without decluttering, respectively. Isoplanatic patch sizes were 0.60±0.12 mm and 0.39±0.06 mm, respectively, for a 0.7 correlation limit. Overall, after decluttering aberration profiles became less degrading and more stable suggesting that phase-aberration was not a significant source of degradation in our data. These results are consistent with simulation studies by others.

5. ARFI 1

5.1 ***In-silico*** **demonstration of ARFI Surveillance Of Subcutaneous Hemorrhage (Assh) imaging,** Tomasz J. Czernuszewicz, Robert M. Hinson, Jr. and Caterina M. Gallippi, *Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC, cmgallip@email.unc.edu*

Background: ARFI-surveillance of subcutaneous hemorrhage (ASSH) is a novel imaging technique developed for assessing hemostasis onset pre-clinically in dog models of inherited bleeding disorders and clinically in patients undergoing percutaneous coronary intervention. In ASSH, acoustic radiation force (ARF) is used to mechanically excite tissue surrounding a vascular injury, and regions of hemorrhage are identified by elevated variance in the measured displacement profiles. However, it is well known that increased variance, or "jitter", can be caused by a number of factors nonspecific for the presence of hemorrhage, such as low ultrasonic signal-to-noise ratio (SNR) or small tracking kernel size. In this work, a model of a growing subcutaneous hemorrhage under ARF excitation is developed using finite-element method simulation and Field II, and the model is utilized to quantify the performance of the ASSH technique for hemorrhage identification under various system conditions.

Methods: Soft tissue dynamics in response to an impulse of ARF were simulated using LS-DYNA and ultrasonically tracked using Field II. To mimic extravasated blood, additional scatterers were added to the Field phantom (at 10%, 30%, and 50% concentration) with amplitudes 10 times smaller than background scatterers and assigned either ±1 μm or ±5 μm 3D-motion following peak displacement. White Gaussian noise was added (to simulate system SNRs of 5-50 dB), and data sets were motion tracked with normalized cross correlation using six kernel sizes (1.5λ, 2.5λ, 3.5λ, 5λ, 7λ and 10λ). Finally, the variance of acceleration (VoA), i.e., variance of the second derivative of displacement profiles, was computed from the last 2 ms of the ensembles. Field simulations were repeated with 30 independent speckle realizations to determine mean VoA values for a given condition and compared with two sample t-tests to show statistical significance ($\alpha = 0.05$).

Results: Increasing the SNR of the system from 5 to 50 dB resulted in almost 5 orders of magnitude decrease in measured VoA values when no extravasated blood scatterers were present. When extravasated blood scatterers were added, the mean VoA increased with both concentration of scatterers and scatterer

motion, but was only measureable at higher system SNR levels. Using a 1.5λ kernel, simulations with 5, 10 and 15 dB SNR did not result in any statistical differences in VoA measured from blood compared to background. As the SNR was increased higher than 15 dB, VoA values from blood started to be statistically differentiable; at 20 dB SNR, VoA from blood scatterers with ±5 μm motion and 50% concentration was statistically different (p = 0.011) than background VoA. At 45 dB, all test cases resulted in statistically-significantly different VoA values ($p < 0.001$), even when the blood concentration and motion was reduced to their lowest values (10% and ±1 μm, respectively). As kernel size was increased, a smaller amount of decorrelation from blood scatterers was necessary for statistically-different VoA. For example, blood scatterers with ±5 μm motion and 10% concentration at 25 dB resulted in significantly-different VoA when using a 7λ kernel (p = 0.009) but not with 1.5λ (p = 0.6735).

Conclusions: The results of this study suggest that, given adequate system SNR (≥40 dB) and optimal displacement tracking kernel selection, ASSH may be implemented to distinguish pixels corresponding to hemorrhage from those of soft tissue by analysis of VoA in response to ARF excitation. Future work will explore performance in a 2D imaging scenario.

5.2 **Experimental validation of Time to Hemostasis (TTH) and Bleeding Rate (BR) metrics derived from ARFI Surveillance of Subcutaneous Hemorrhage (ASSH) in tofu tissue-mimicking model,** *Rebecca E. Geist, Chase DuBois and Caterina M. Gallippi, Biomedical Engineering Department, The University of North Carolina at Chapel Hill, Chapel Hill, NC, regeist@ncsu.edu*

Background: Current bleeding-detection mechanisms after peripheral vascular injury primarily rely on detection of superficial hematoma or hemorrhage at the skin surface, which may not be present during subcutaneous bleeding. Developing alternative subcutaneous bleeding-detection methods could improve patient-bleeding outcomes. An Acoustic Radiation Force Impulse (ARFI)-derived method, called ARFI Surveillance of Subcutaneous Hemorrhage (ASSH), has been shown previously to discriminate bleeding phenotype *in vivo*[1-3] but lacks experimental validation of Time to Hemostasis (TTH) and Bleeding Rate (BR) metrics. This work explores validation of ASSH-detected TTH and BR metrics through the infusion of a blood substitute into a tissue mimic for a known time at a known rate. We hypothesize that ASSH BR and TTH measurements will reflect known differences in experimental infusion rate and infusion time.

Methods: Evaporated milk (My Essentials) was utilized as a blood substitute while firm tofu (Vitasoy) was used as a tissue mimic. Using ultrasound guidance (Siemens VF7-3), an 18F catheter was introduced less than 5 mm in elevation from the imaging plane. The catheter tip was centered laterally in the ultrasound image and positioned at the approximate axial focus of 2 cm in depth. Through this catheter, a peristaltic pump directed dyed evaporated milk into tofu. The evaporated milk was infused into the tofu as prescribed by cohort protocol. Three protocols were developed with two infusion rates and two infusion times (Protocol 1: 5 mL/min, 5 min, n=6; Protocol 2: 2.5 mL/min, 5 min, n=6; Protocol 3: 5 mL/min, 7 min, n=6). ARFI images (4.21 MHz 300-cycle push, 6.15 MHz 2-cycle track) were taken once per minute for 15 minutes total. These ARFI images were separately processed using the ASSH method to estimate area of hemorrhage versus time. From these data, the positive rate of change in hemorrhagic area prior was collected as ASSH BR, and the time at which the BR was sustained at ≤ 0 mm^2/min was collected as ASSH TTH.

Results: For protocol 1, the mean ASSH-detected BR was 3.09 mm^2/min (σ= 0.95mm^2/min); ASSH TTH was 5 min for 4 trials, 4 min for one trial and 6 min for 1 trial. For protocol 2, mean ASSH BR was 1.61 mm^2/min (σ= 0.32 mm^2/min), and ASSH TTH was 5 min for 4 trials, 4 min for 1 trial and 6 min for 1 trial. For protocol 3, mean ASSH BR was 2.88 mm^2/min (σ = 0.9 0mm^2/min) and all 6 trials of Protocol 3 had an ASSH-detected TTH of 7 min.

Conclusions: When the calibrated flow rate was reduced by 50% in Protocol 2, the mean ASSH-detected BR was reduced by 44-48%, suggesting that ASSH is sensitive to changes in volumetric bleeding rate. Further, ASSH TTH matched the known infusion time for 14/18 total trials and the remaining 4 trials had ASSH-detected TTH within 1 min of the known infusion time. These data suggest that ASSH-detected TTH discriminated infusion cessation. Overall, this work supports that ASSH is a viable tool for noninvasively monitoring subcutaneous bleeding dynamics.

(1) Behler et al. *Ultrasonic Imaging 31*, 159-171 (2009). (2) Scola et al. *Ultrasound Med Biol 37*, 2126-2132 (2011). (3) Geist et al. *IEEE IUS*, 2296-2299 (2014).

5.3 **FEM analysis of the effect of focal configuration on acoustic radiation force (ARF) induced and ultrasonically tracked displacements in a transversely isotropic elastic material**, Md Murad Hossain[1], Tomasz J. Czernuszewicz[1] and Caterina M. Gallippi[1], [1]*Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill,NC,cmgallip@email.unc.edu.*

Background: A transversely isotropic (TI) material is defined by an axis of symmetry perpendicular to a plane of isotropy. Previous work(1) showed elliptical shear wave group velocity propagation in the plane perpendicular to the plane of isotropy in a modeled TI material, and this phenomenon was demonstrated experimentally in muscle by Wang et al.(2) Rather than directionally-dependent shear wave propagation, the objective of this work is to demonstrate the directional dependence of ARF-induced peak displacement (PD) in TI materials. We further evaluate the impact of ARF focal configuration and demonstrate the potentially confounding effects of acoustic displacement underestimation. We hypothesize that ARF-induced PDs will be higher when the larger dimension of a spatially-asymmetric ARF excitation is along versus perpendicular to the axis of symmetry in TI materials. We further hypothesize that acoustic displacement underestimation does not inhibit the detection of directional differences in PD in TI materials with elastic properties similar to those of muscle.

Methods: A TI elastic material was modeled using LS-DYNA3D with the following elastic constants:

$$E_T = 32.76\,Kpa, E_L = 91.0\,Kpa, \mu_L = 25.0\,Kpa, \mu_T = 9.0\,Kpa, \upsilon_{LT} = 0.499\,, and\ \upsilon_{TT} = 0.82,$$

where E = Young's modulus, μ =shear modulus, υ = Poisson's ratio, L = direction along the axis of symmetry, and T = direction perpendicular to the axis of symmetry. The material was excited with ARF excitations, modeled using Field II as the 3D acoustic intensity fields associated with a Siemens VF7-3 linear array transducer centered at 4.21 MHz with F/1.5, F/3, F/4, and F/5 focal configurations. The resulting displacements directed the motion of scatterers in Field II simulations of acoustic displacement tracking using the VF7-3 at 6.15 MHz with F/1.5 focal configuration and ensemble length of 6.5 ms. Axial (1D) cross-correlation between sequentially-acquired tracking lines was used to estimate the ARF-induced displacement.

Results: The anisotropic properties of the TI materials were validated by measuring shear wave velocity (SWV) along and perpendicular to the axis of symmetry. The error in measured SWV relative to theory was < 2%. Using an asymmetric ARF focal configuration of F/1.5, PD increased as the elevational (larger) dimension of the ARF excitation was oriented from across (PD = 10.02 μm) to along the axis of symmetry (PD = 14.30μm). However, as focal configuration increased from F/1.5 to F/5, the difference in PD achieved with the elevational dimension of the ARF excitation oriented across versus along the axis of symmetry progressively decreased:

$$(\Delta PD_{F/1.5} = 29.94\%, \Delta PD_{F/3} = 13.32\%, \Delta PD_{F/4} = 4.49\%,\ and\ \Delta PD_{F/5} = 1.91\%.$$

When PDs were acoustically tracked, there were statistically-significant directional differences for F/1.5, F/3 and F/4 ARF focal configurations (p<0.001, paired t-test), but there was no statistically significant directional difference in PD for F/5.0 (p=0.36).

Conclusions: This FEM analysis illustrates that ARF-induced PDs are directionally-dependent in TI elastic materials but that the directional difference in PD is diminished as the ARF excitation approaches spatial symmetry. Further, acoustic displacement underestimation does not corrupt the detection of directionally-dependent PD differences in TI materials with elastic properties similar to those of muscle. These data support that material anisotropy may be selectively exploited or obviated in ARF-based imaging by employing asymmetric or symmetric ARF excitations, respectively.

(1) Rouze et al. *J Biomech 46,* 2761-2768 (2013). (2) Wang et al. *IEEE Trans Med Imag 32,* 1671-1684 (2013).

5.4 **Characterization of bright scatterer-induced bias in ARFI displacement estimation**, Willie Long, Peter Hollender and Gregg E. Trahey, *Department of Biomedical Engineering, Duke University, Durham, NC, willie.long@duke.edu*

Speckle-induced bias is described as a correlated noise source in ultrasound displacement estimation that results from preferential tracking of bright or constructively interfering speckle within an ultrasound tracking beam. Due to dominating echoes from bright scatterers, displacement estimation can produce a skewed representation of scatterer deformation within a tracked volume. Given the submillimeter displacements observed in acoustic radiation force impulse (ARFI) and other elasticity imaging techniques, this phenomenon can have appreciable impact on the accuracy and robustness of ultrasound tissue characterization.

In this study, we explore the influence of speckle-induced bias on displacement estimation, specifically in the context of ARFI imaging. Preliminary investigation examines the scenario of a single bright scatterer located at the focal depth along the imaging plane. Via finite element and Field II simulation, scattering amplitude and location of the biasing scatterer are systematically varied. In each condition, ARFI deformation is tracked using F/1, F/2 and F/3 tracking configurations in order to evaluate bias in the estimated displacement. Each scatterer is simulated within homogenous speckle and a total of 40 unique speckle realizations are evaluated to ensure statistical convergence of the displacement measurements.

Results suggest a strong connection between displacement of a bright scatterer in the imaging medium and the overall tracked ARFI displacement. Increased correlation with the biasing scatterer's displacement (i.e. speckle-induced bias) and decreased estimation jitter are observed for scatterers of increasing reflectivity and proximity to the imaging axis. Broader tracking beams are furthermore shown to be associated with greater risk and severity of underestimation resulting from speckle-induced bias. For a scatterer of brightness on the order of 16 dB, maximum percent underestimation relative to a no biasing scatterer condition increases from 13.4% to 48.9% as track beam F-number is increased from F/1 to F/3.

On the basis of these findings, we will examine the influence of this phenomenon on ARFI displacement estimation, identify the tracking scenarios most vulnerable to this source of correlated noise, as well as discuss the broader clinical implications of scatterer bias in other echo correlation-based ultrasound technologies.

5.5 **Shear viscosity imaging with acoustic radiation force,** Yiqun Yang[1], Matthew W. Urban[2] and Robert J. McGough[1], [1]*Department of Electrical and Computer Engineering, Michigan State University, East Lansing, MI and* [2]*Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN , mcgough@egr.msu.edu*

We have recently created images of the shear viscosity with an applied acoustic radiation force. Shear viscosity images were obtained for both simulated and measured shear wave data, where the shear waves were generated by an applied acoustic radiation force produced by a focused ultrasound beam. Shear viscosity images were obtained after the effects of diffraction, dispersion, and attenuation were all taken into account. Simulated shear displacements and velocities were obtained from a three-dimensional (3D) Green's function and 3D finite difference calculations of shear waves in a viscoelastic medium after a 3D intensity field was simulated in FOCUS (http://www.egr.msu.edu/~fultras-web) for a linear array transducer. Simulations were performed in homogeneous computer phantoms, and then an algorithm for constructing an image of the shear viscosity was applied to the simulated shear wave velocities. In the images obtained from the simulated shear-wave data, accurate values for the shear viscosity were recovered. Shear wave velocities were also measured with a Verasonics system in cooked and uncooked chicken breast and then images of the shear viscosity were also constructed from the measured values. In the results obtained from measured values, the images show significant contrast between the shear viscosities in cooked and uncooked chicken breast. These images indicate that the shear viscosity is effective for distinguishing between different soft tissue states.

5.6 **Acoustic Radiation Force based quantitative stiffness estimation within the region of excitation using a Bayesian estimator,** Kristy M. Walsh[1], Douglas M. Dumont[1,] Mark L. Palmeri[2] and Brett C. Byram[1], [1]*Department of Biomedical Engineering, Vanderbilt University, Nashville, TN and* [2]*Department of Biomedical Engineering, Duke University, Durham, NC, Kristy.m.walsh@vanderbilt.edu.*

In traditional shear wave elasticity imaging (SWEI), the velocity of shear waves are measured away from the acoustic radiation force (ARF) axis to obtain quantitative estimates of tissue.(1) In this study, the

tissue displacement is measured along the ARF axis, and we use a look-up table to obtain quantitative estimates of tissue stiffness. Specifically, by assuming the medium is linear, elastic and isotropic, we can relate the shear wave speed to a proportional time-to-peak displacement. Stiffness is then estimated by extracting the time-to-peak displacement as a function of depth from look-up tables.[(2)] In either method, measuring displacements on the order of microns requires high precision. This study applies a Bayesian estimator to reduce the displacement estimation variance to enable the quantitative on-axis approach.

This study uses 3D finite element analysis to model the soft tissue response to the acoustic radiation force impulse excitation. Field II routines simulate the radio-frequency data of the tissue response. The Bayesian estimator is applied to this RF response data to improve tissue-displacement estimates. This study modeled the focal configuration of an ATL L12-5 50 mm linear array transducer with a transmitted center frequency of 7.8 MHz and a focal depth of 2 cm. The look-up tables were generated using the average displacement data from 20 speckle realizations of each tissue stiffness (3, 6 and 9 kPa) and an attenuation of 0.7 dB/cm/MHz. The average data for the look-up table was also interpolated to a PRF of 50 kHz and smoothed using LOWESS regression. Then, 20 experimental measurements were simulated at 6 kPa and measured from look-up tables generated by either Bayesian or normalized cross-correlation (NCC) estimates. Simulation results showed that Bayesian displacement estimates resulted in better quantitative stiffness estimates with lower MSE compared to NCC based estimates. At each depth, the estimates were compared with the true mean of 6 kPa to get the mean squared error (MSE) of the 20 realizations. Within the range of 0.62 and 0.77 cm depths, the MSE for the Bayesian estimator has a mean of 1.1643 kPa^2 (ranging from 0.6294 kPa^2 to 1.5979 kPa^2) and NCC has a mean of 7.8354 kPa^2 (ranging from 2.4649 kPa^2 to 63.1941 kPa^2) which shows the Bayesian estimator improves estimates where there is low SNR in the near-field. Both methods perform best between depths of 0.89 and 1.66 cm and the Bayesian estimator also has a lower MSE. Here, the mean of the Bayesian estimator is 0.2590 kPa^2 (ranging from 0.0875 kPa^2 to 0.6099 kPa^2) and NCC has a mean of 1.2744 kPa^2 (ranging from 0.3992 kPa^2 to 3.8678 kPa^2). Both the NCC and Bayesian estimators have a slight increase in MSE as the depth approaches and exceeds the focal depth of 2 cm, but Bayesian estimates also outperform NCC. Overall, the Bayesian estimator lowers the MSE of the stiffness estimates.

(1) Sarvazyan et al. *Ultrasound Med Biol 24*, 1419-1435 (1998). (2) Palmeri et al. *IEEE IUS* 2009-2012 (2008).

5.7 **Low-push radiation force elasticity imaging with a Bayesian estimator**, Douglas M. Dumont and Brett C. Byram. *Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, douglas.m.dumont@vanderbilt.edu*

Recently, it has been demonstrated that limitations in radiation-force elasticity imaging due to low displacement magnitudes and low displacement SNR can be overcome with greater deposition of ARF energy into the insonified tissue1. In this work, we also seek to examine the relationship between ARF displacement magnitude and displacement SNR, but rather than trying to improve SNR by increasing the applied ARF, we instead approach the problem from the concept of ALARA (As Low as Reasonably Achievable). We hypothesize that reductions in displacement SNR – due to reductions in displacement magnitude – can be largely offset by improved displacement estimation using Bayesian techniques.[(2)]

ARF-induced displacements were simulated in a homogeneous 6.5 kPa phantom. The applied ARF was adjusted to vary the ARF-induced peak displacement in the simulation from 0.27 to 14.1 μm (peak FEM displacements). Twenty-five independent phantom realizations were created for each tested displacement-magnitude level. The radiofrequency data were then corrupted with 30 dB of additive, white Gaussian noise. Displacement estimates were then computed within the region-of-excitation using normalized cross-correlation (NCC) and a previously-described Bayesian estimator.[(2)] Displacement SNR was computed for each realization as the ratio of the mean displacement to the standard-deviation of the displacement data (computed over the 25 realizations).

The mean axial displacement SNR at the approximate time of the peak ARF-induced displacement (as determined from the tracked data) was found to be 7.98 ± 0.58 dB (range 7.18 to 8.78 dB, $n = 7$ displacement magnitudes, 0.27 to 14.1 μm, 25 realizations each) higher for the Bayesian estimator than normalized cross-correlation over the range of investigated, ARF-induced displacement magnitudes. The results also show that compelling images can be made with the Bayesian estimator with only a few

microns of displacement and that peak displacement magnitudes can be reduced by almost an order of magnitude (2.0 µm vs. 14.1 µm datasets, peak FEM displacement, Bayesian vs. NCC, respectively) with only a marginal loss in displacement SNR (15.16 dB vs. 16.92 dB, Bayesian at 2.0 µm vs. NCC at 14.1 µm, respectively).

(1) Deng et al. *2014 IEEE Ultrasonics Symp*, pp.719-722. (2) Dumont et al. *2014 IEEE Ultrasonics Symp*.

Tuesday afternoon

6. PHOTACOUSTICS 1

6.1 **Functional and quantitative photoacoustic imaging for monitoring and predicting cancer treatment response,** Eno Hysi[1], Lauren A. Wirtzfeld[1], Jonathan P. May[2], Shyh-Dar Li[2] and Michael Kolios[1], *[1]Department of Physics, Ryerson University, Toronto, ON Canada and [2]Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC Canada, eno.hysi@ryerson.ca*

Emerging evidence suggests that evaluating cancer treatment efficiency early in the treatment process has a significant impact in treatment outcome. Evaluating the changes in tumor vasculature during treatment is of paramount importance as the degree of malignancy and treatment outcome are related to the organization and functionality of tumor blood vessels. In this area, photoacoustic (PA) imaging has a significant advantage over its ultrasonic (US) imaging counterpart: it is capable of providing estimates of vessel oxygenation by utilizing the oxygen-dependent optical absorption changes of blood. In addition, quantitative information contained in its radiofrequency data could provide clues about the changes in the structure and hierarchy of vasculature.

In this study, 31 female Balb/c mice were inoculated with EMT-6 murine mammary carcinoma cells in the left footpad. The tumors were grown for one week prior to being treated by a thermosensitive liposome (Heat-activated cytoToxic – HaT-DOX) loaded with doxorubicin (DOX) (n = 12), systemic DOX (n = 7) or saline (n = 12). In order to treat, the tumor-bearing footpad of all mice was immersed in a water bath at 43°C for 1 hour. Treatment progress was monitored by imaging the tumors using the FUJIFILM VisualSonics VevoLAZR – integrated US/PA system. Tumors were imaged in 3D using the 40 MHz linear-array probe and illuminating the tumor at 750 and 850 nm pre-treatment, 30 minutes, 2 hours, 5 hours, 24 hours and 7 days post-treatment. At every time point, oxygenation maps were reconstructed for multiple tumor slices and a histogram of tumor oxygenation was computed. PA frequency-spectra at both wavelengths were normalized by a depth-matched PA reference phantom consisting of 10 black carbon beads (diameter 2-12 µm) per probe resolution volume. Parametric maps of the PA spectral slope (SS) were computed at all time-points. The SS was used in conjunction with the changes in tumor volume and oxygenation at early time points to predict treatment response.

On average, tumors that were treated with HaT-DOX and free DOX exhibited a 15-20% decrease in oxygenation as early as 30 minutes after treatment. This decrease persisted for the first 24 hours before a significant 50% increase in oxygenation at 7 days over pre-treatment. Saline-control tumors did not exhibit a significant change in their oxygenation until 7 days post-treatment. In the HaT-DOX group, 1 of the 12 animals did not respond to treatment and its size grew to 300% of its pre-treatment volume, as assessed by caliper measurements. For this animal, the oxygenation at 30 minutes did not drop from pre-treatment like it did for responders. The responder's PA SS at 750 nm decreased by 46% at 30 minutes while the non-responder PA SS did not change until 7days. These results suggest that the combination of structural information from PA spectral parameters with the functional information of oxygenation could serve as early predictors of cancer treatment response.

6.2 **Correlates of cell death and tumor morphology in breast tumors using quantitative ultrasound and diffuse optical spectroscopy imaging,** William T. Tran[1, 2], Charmaine Childs[2], Heidi

Probst[2], Gregory J. Czarnota[1 ,2, 3], *[1]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto Canada, [2]Centre for Health and Social Care Research, Sheffield Hallam University, Sheffield, UK, [3]Physical Sciences, Sunnybrook Research Institute, Toronto, ON, Canada and [4]Department of Medical Biophysics, University of Toronto, Toronto, Canada, Gregory.Czarnota@sunnybrook.ca*

Background and Objectives: Locally-advanced breast cancer (LABC) accounts for approximately 10-15% of all breast cancer diagnoses and is often treated with neoadjuvant chemotherapy. Since 60-70% of patients may fail therapy, treatment-monitoring approaches would benefit patients to develop adaptive therapies and thus improve survival. Previously, quantitative ultrasound has been shown to be sensitive to the cellular changes during treatment and have demonstrated promising results to predict response. Multiparametric approaches have been useful in detecting apoptotic cell death in tumors however; the coincident changes in tumor-morphological features such as blood vasculature, oxygenation and tissue components have yet to be understood. Diffuse optical spectroscopy (DOS) has been shown to reflect these morphological features and here, 22 women were imaged and studied using quantitative ultrasound and diffuse optical spectroscopy during neoadjuvant chemotherapy treatment.

Methods: Breast imaging was performed on 22 patients using quantitative ultrasound and diffuse optical spectroscopy in parallel during neoadjuvant chemotherapy for LABC. Analysis of the tumor regions of interest (ROIs) were selected and quantitative measurements were acquired for both imaging parameters. For QUS, calibration using a reference phantom was performed in order to obtain the mid-band fit, 0-MHz intercept and the spectral slope. For DOS, ROIs were selected and analyzed using custom Matlab software to obtain hematological and tissue features within the tumor using photon scattering and absorption models.

Results: QUS and DOS parameters demonstrated co-incident changes during treatment. Clinical responders showed a significant increase ($p<0.05$) in MBF (10.02 dBr ± 1.48 [SE]) and 0-MHz intercept (12.26 dBr ± 2.02) QUS parameters, but not spectral slope. These patients also demonstrated a reduction in the total hemoglobin concentration (HbT = 15.13 ±1.97), and other parameters such as water, oxygen saturation and oxygen desaturation after 4 and 8 weeks of treatment. For clinically-nonresponding patients, QUS and DOS parameters showed insignificant changes ($p>\mathbf{0.05}$) compared to before treatment. The sensitivity and specificity testing for QUS demonstrated that the MBF and 0-MHz intercept were good predictors for response at week 4 (sensitivity 92.9%, specificity 100%, $p<0.05$), and this corresponded to blood-related parameters for DOS such as hemoglobin (sensitivity 85.7%, specificity 87.5%, $p<0.05$).

Conclusion: QUS and DOS demonstrated coincident and complementary surrogate markers for treatment response and may facilitate response-guided therapies for breast cancer.

6.3 **Evaluation of Gleason scores by photoacoustic spectral analysis,** Guan Xu, Scott A. Tomlins, Javed Siddiqui, Mandy A. Davis, Lakshmi P. Kunju, John T. Wei and Xueding Wang. *University of Michigan Medical School, Ann Arbor, Michigan 48109, xdwang@umich.edu*

Ultrasound (US) guided biopsy is the standard procedure for evaluating the presence of prostate cancer. US biopsies are not targeted and randomly sample broad areas of the prostate, which can either miss cancer or undersample aggressive cancer. Aggressive cancers are defined by high Gleason scores. The Gleason scoring system is an architectural assessment of tumor grade that is highly prognostic and performed by pathologists on biopsy tissue. The Gleason patterns, *per se* the clustering patterns of the cancer cells, can be visualized by cancer targeting optical contrast enhancing nanoparticles (NPs). In this work, the possibility of identifying cancerous areas by using photoacoustic (PA) techniques for improved US biopsy is investigated. By illuminating the prostate tissue with a side-firing fiber integrated to the biopsy needle and receiving the PA signals with a hydrophone, the Gleason patterns visualized by the NPs could potentially be evaluated *in vivo* without the need to harvest tissue. The newly-developed method of PA spectral analysis (PASA) characterizes the power spectrum of PA signals with a linear model and quantifies the microstructures in tissue with slope, intercept and midband-fit of the linear model. As a preliminary study, we scanned sliced human prostate tissues with Hematoxylin and Eosin staining. Normal prostate tissues and prostate cancer tissues with Gleason scores of 6, 7 and 9 were studied. Statistical analysis has shown that the PASA parameters have strong correlations to the Gleason scores.

6.4 **Comparison of light delivery methods for photoacoustic imaging of prostate brachytherapy seeds**, Muyinatu A. Lediju Bell,[1,2] Xiaoyu Guo,[3] Nathanael P. Kuo,[3] Danny Y. Song[4] and Emad M. Boctor,[1-3] *Departments of [1]Computer Science, [2]Radiology, [3]Electrical Engineering and [4]Radiation Oncology, Johns Hopkins University, Baltimore, MD, mledijubell@jhu.edu*

Prostate brachytherapy, a treatment option for prostate cancer, requires implantation of radioactive seeds according to a defined treatment plan. Intraoperative updates to the treatment plan are limited by suboptimal ultrasound image quality. This work compares the advantages and disadvantages of three light delivery methods (spherical transperineal, end-fire transurethral and side-fire transurethral) for a complementary photoacoustic imaging approach to seed detection.

A brachytherapy procedure was performed on an intact canine prostate. A 1-mm optical fiber coupled to a 10 Hz, 1064 nm, Nd:YAG laser was utilized to deliver the light after modification of the fiber tip. A SonixTouch ultrasound scanner (Ultrasonix, Richmond, BC, Canada), transrectal ultrasound probe with curvilinear (BPC8-4) and linear (BPL9-5) arrays, and SonixDAQ were utilized for synchronized laser light emission and photoacoustic signal acquisition. Postoperative CT images confirmed seed locations. Seeds within 5 mm of the transperineal light source and 6, 9 and 12 mm of the transurethral light sources were visualized. The side-firing transurethral method offered selective visualization of seeds and identification of erroneous seeds that appeared in ultrasound images, while the spherical transperineal and end-firing transurethral methods enabled visualization of all seeds simultaneously. Although each method suffered from unwanted acoustic artifacts (i.e., clutter), the side-firing transurethral light delivery offered the most promise for artifact reduction. Supported in part by NIH K99-EB018994 and a UNCF-Merck Postdoctoral Research Fellowship, both awarded to M. A. Lediju Bell.

6.5 **Environmentally-sensitive contrast agents for photoacoustic ultrasonic imaging of tumors,** Trevor Mitcham[1], Samit Guha[2], Katherine Dextraze[1], Albert Lee[1,3], Konstantin Sokolov[1,3], Brad Smith[2] and Richard Bouchard[1], *[1]University of Texas MD Anderson Cancer Center, Department of Imaging Physics, Houston, TX, [2]University of Notre Dame, Department of Department of Chemistry and Biochemistry, South Bend, IN and [3]University of Texas at Austin, Department of Biomedical Engineering, Austin, TX, TMMitcham@mdanderson.org*

Background and Objective: The tumor microenvironment is known to show inflammation and dysregulation in pH. The tumor recruits macrophages by manipulating the body's natural inflammatory response, and a shift in intra- and extra-cellular pH is caused by the tumor's dysregulation of membrane transporters. Two liposome-encapsulated agents were investigated in this study that may be sensitive to these changes in the microenvironment: liposomal ICG (lipoICG) and liposomal croconaine (lipoCroc). LipoICG can be used as a sensor of inflammation, as macrophages that accumulate in response to the cancer cell signaling will endocytose the lipoICG. Upon endocytosis, liposomes are disrupted, releasing the ICG and causing a spectral shift in the NIR range, which is detectable by spectroscopic photoacoustic (PA) imaging. LipoCroc is sensitive to pH, where a distinct spectral shift will be observed in the NIR range when the lipoCroc is exposed to low or high pH environments. In this study, we investigated the feasibility of using spectroscopic PA imaging to distinguish the spectral shifts of lipoICG in a phantom with tissue-mimicking scattering properties and lipoCroc in a phantom and murine model.

Methods: All imaging was performed on the Vevo 2100 LAZR (VisualSonics) using a wavelength range of 680 nm-970 nm. The lipoICG phantom consisted of six targets: ICG monomer with and without triton, lipoICG with and without triton, cells alone and lipoICG engulfed by cells. The lipoICG phantom was imaged in a milk solution designed to mimic tissue scattering properties. The lipoCroc phantom contained five targets: croconaine acid in acidic solution, croconaine acid in basic solution, lipoCroc in pH 5 solution, lipoCroc in pH 7.5 solution and ICG as a reference. To demonstrate the potential for *in-vivo* PA imaging using croconaine, lipoCroc was injected into the peritoneal fluid of a living mouse, known to have a pH between 6.1-6.3. The lipoCroc was imaged in the murine model using spectroscopic PA imaging with co-registered B-mode ultrasound imaging.

Results: For the liposomal targets, unique PA spectra were observed in both *in-vitro* and *in-vivo* models, demonstrating spectral shifts in response to environmental conditions. *In-vivo* image analysis shows a peritoneal pH of 6.0-6.5 using ratiometric PA scans, correctly identifying the weakly-acidic pH of the peritoneal fluid. Spectroscopic PA imaging with these environmentally-sensitive agents may

facilitate *in-vivo*, noninvasive assessment of emerging hallmarks of cancer, such as inflammation and pH dysregulation.

7. ELASTICITY 2

7.1 **Finite-element simulation of VisR imaging in a custom transversely-isotropic viscoelastic material,** Christopher J. Moore[1] and Caterina M. Gallippi[1, 2], *[1]Department of Electrical and Computer Engineering, North Carolina State University, Raleigh, NC and [2]Department of Biomedical Engineering University of North Carolina, Chapel Hill, NC, USA, cmgallip@email.unc.edu*

Background: Viscoelastic Response (VisR) ultrasound, an acoustic radiation force (ARF)-based imaging method, has been demonstrated in FEM simulations for characterizing the viscoelastic properties of isotropic materials. However, unlike isotropic materials, transversely isotropic (TI) materials exhibit directionally-dependent mechanical properties. This directional dependence must be carefully considered when interpreting VisR imaging results and/or developing VisR beam sequences for analysis of TI tissues. The objectives of this work are 1) to develop, validate and characterize a custom TI viscoelastic material model and 2) to implement the model for evaluating the effects of transverse isotropy on VisR imaging. *We hypothesize that the VisR-derived relaxation time constant for constant stress (τ) parameter will exhibit directional dependence when an asymmetric ARF excitation is used for VisR imaging, but not when a symmetric ARF excitation is used*.

Methods: A structured finite element mesh was constructed such that two viscoelastic, isotropic materials populate the mesh. The materials were arranged with one acting as a background medium and the other acting as a parallel matrix of fibers infused in the background medium. This method was chosen so that properties of each material could be changed independently and therefore the degree of anisotropy adjusted. For the model used in this work, compressive moduli of 1 kPa and 50 kPa were chosen for the background and fiber matrix, respectively. Using the finite element (FE) solver package LS-DYNA, the custom viscoelastic TI material was subjected to VisR ARF excitations using three different ARF focal configurations (F/1.5, F/3.0, and F/5), in two orthogonal imaging planes, with the fibers of the material either parallel or perpendicular to the face of a linear array transducer. The ARF excitation for this VisR sequence consisted of two consecutive 70 μs pulses separated by 0.6 ms. The displacements at the focal point of the ARF excitation were computed in the FE solver, and the results were fit to a mass-compensated Voigt model. The solution to this model yielded a time constant, τ, which is the ratio of viscosity to elasticity of the material.

Results: The TI behavior of the custom viscoelastic material is confirmed by the ellipsoid shape of the propagating shear wave in the X-Y plane. VisR-derived τ values measured with the long axis of the transducer oriented parallel (0°) versus perpendicular (90°) to the fiber direction of the material differ by 1.05 ms (0°: 7.83 ms, 90°: 8.88 ms) for the asymmetric F/1.5 ARF focal configuration and by 0.08 ms (0°: 6.90 ms, 90°: 6.98 ms) for the symmetric F/5.0 focal configuration.

Conclusions: A material that exhibits the behaviors of a viscoelastic solid as well as transverse isotropy has been modeled in this work. The creation of this type of model facilitates the analysis of imaging methods that aim to investigate viscoelasticy as well as transverse isotropy. The results of the work also show that VisR imaging has the ability to selectively exploit or obviate the effects of anisotropy in a transversely isotropic viscoelastic material by adjusting the focal configuration of the ARF excitation.

7.2 **Feature extraction for breast-lesion classification in ultrasound elastography**, Congxian Jia[1], S. Kaisar Alam [2, 3] and Brian Garra,[1,4,5] *[1]The Food and Drug Administration, Silver Spring, MD, [2]Rutgers University, Piscataway, NJ, [3]Improlabs Pte Ltd, Singapore, [4]The Washington DC VA Medical Center, Washington, DC, and [5]The George Washington University, Washington, DC, congxian.jia@fda.hhs.gov*

Objective: External compression-based ultrasound strain elastography can detect alterations of mechanical properties in breast tissue and has been getting acceptance as an adjunct method for breast-cancer diagnosis. The strain ratio has been proposed as one quantitative measure to assess the relative stiffness of breast lesion with respect to the surrounding normal tissue. However, the strain ratio depends

on factors such as the selection of the regions of interest (ROI) in both lesion and normal tissue and lesion size, as shown in our previous study using finite element simulation and phantom experiments.[(1)] So far, no classification study has objectively assessed the effects of these factors on the efficacy of differentiating malignant and benign lesions using strain ratio. In this study, we evaluate whether incorporating information on the relative locations between ROIs and lesion size along with strain ratio improves the performance of breast lesion classification.

Methods: 41 breast lesions (10 malignant and 31 benign lesions) were used in a pilot study. The study was approved by the University of Vermont Institutional Review Board. In this study, two additional features were defined to characterize the above factors to classify breast lesions along with strain ratio. One feature is the angle of ROI in the background with respect to the acoustic beam passing through the center of the ROI in the lesion. The other feature is the distance between the centers of the two ROIs normalized by the effective radius of lesion. Two classifiers (linear discriminant analysis (LDA) and support vector machine (SVM) performing a nonlinear classification) were applied to combinations of one (LDA only), two or three features using leave-one-out cross-validation methodology. The performance of these classifiers was evaluated by calculating areas under the receiver operating characteristic curve (AUC) for each combination of features.

Results: Using LDA, the AUC using strain ratio only achieved the highest AUC of 0.919. Using nonlinear SVM, the AUC using two features (strain ratio and normalized distance between two ROIs) achieved the highest AUC of 0.948 among the different combinations of two to three of the factors described above. The number of lesions reported here is not adequate to draw statistically-significant conclusions. We are acquiring additional data in an ongoing *in vivo* study to confirm the preliminary findings reported here. We are also working to model more complex breast tissues to strengthen the theoretical foundation supporting these clinical results.

(1) Jia et al. *IEEE Trans Ultrason Ferroelect Freq Contr 61*, 611-619 (2014).

7.3 **Quality criteria for automatic strain selection in ultrasound elastography,** Congxian Jia [1], S. Kaisar Alam [2, 3] and Brian Garra, [1,4,5] *[1]The Food and Drug Administration, Silver Spring, MD, [2]Rutgers University, Piscataway, NJ, [3]Improlabs Pte Ltd, Singapore, [4]The Washington DC VA Medical Center, Washington, DC, and [5]The George Washington University, Washington, DC, congxian.jia@fda.hhs.gov*

Objective: Ultrasound strain elastography has been used clinically for breast cancer diagnosis by demonstrating the increased stiffness of cancers relative to normal breast tissue. In a clinical environment, several cine clips (10-100 frames) of strain elastograms over multiple compression-relaxation cycles are obtained by the clinician. The quality of these strain images depends, not only on the clinician's skill of performing the compression (and/or relaxation) but also on the processing of the raw data. Displaying poor quality strain images interleaved with high quality strain images severely interferes with the proper interpretation of strain images. To avoid displaying poor quality images in cine clips, some manufactures have attempted to select only "good" quality strain images for display. Unfortunately, no reliable automatic strain image selection scheme is currently available for clinical strain elastography. This leaves the selection of high quality strain elastograms to the interpreting clinicians, who usually perform this task in a subjective and variable manner. Therefore, the purpose of this study is to develop criteria to automatically, objectively and reliably select strain maps for ultrasound strain elastography.

Methods: We assessed five criteria, quantifying strain quality reduction, due to factors such as the decorrelation, uneven compression, poor strain signal to noise ratio (SNR) as well as other unexpected effects. Finite element method (FEM) simulations were performed to establish optimal (empirical) values for these quality criteria. A 2D plane stress case was simulated emulating a lesion embedded in normal tissue, mimicking the conditions described above, e.g., uneven compression. The various selection criteria were then applied to this data set to find an appropriate selection threshold for each criterion. Data from 135 patients previously acquired with a clinical ultrasound machine modified to recover radio frequency ultrasound data (HDI 1000, Philips – ATL, Bothell, WA) were used. The study was approved by the University of Vermont Institutional Review Board. The data acquired from each patient consists of 8 to 9 cine loops (These included 4 to 5 freehand data acquisitions and 4 to 5 machine controlled data acquisitions). Each cine loops contains 30 frames complex IQ data. We have varied the step size (no frame skipped: frames 1 and 2 and so forth, 1 frame skipped: frame 1 and 3 and so forth, etc.), producing

more than 100 strain maps. These strain images were used to verify the individual criteria and the stepwise selection scheme using all criteria. We visually inspected selected images to verify proper selection.

Results: We have evaluated five quality criteria using data from FEM simulation. These criteria were used to exclude the strain images with poor quality due to decorrelation, excessive lateral motion, uneven compression and poor strain SNR. The remaining strain images were then cross correlated with all other selected images and ranked based on the average cross correlation value. From each cine loop, the selected images with (a maximum of) five highest average cross-correlation values were selected as the final image set for clinical viewing. In our *in vivo* study, the above scheme of using the combination of these quality criteria successfully selected a desired number of high quality strain maps (such as 5). We have observed that proper compression can produce an acceptable number of stain maps while poor compression produces as few as zero selected strain images (maps). Examples of selected images *vs.* those not selected will be shown. Future work will include an observer study to verify the diagnostic performance of observers viewing only selected images compared to observers viewing the entire image set. Also a study comparing observers' subjective impression of the image quality of selected images vs. those not selected will be performed.

7.4 **Measurements of shear-wave velocity and attenuation in the porcine and human pancreas**, Ivan Z. Nenadic[1], Carolina Amador Carrascal[1], James F. Greenleaf[1] and Matthew W. Urban[1], [1]*Ultrasound Research Laboratory, Mayo Clinic College of Medicine, Rochester, MN, 55905, nenadic.ivan@mayo.edu.*

Pancreatic cancer is the fourth most common cause of cancer-related deaths in the United States with the one- and five-year survival rates of 25% and 6%, respectively. In 80% of the cases, patients report pain in the advanced metastatic stage of the disease with no well-established biomarkers for the disease. We have been investigating the use of shear-wave elastography methods to quantify mechanical properties of the pancreas. We studied three excised pig pancreases before and after formalin treatment to assess the ability to detect changes in shear wave velocity and attenuation of the pancreatic tissue. We performed *in vivo* transabdominal measurements of shear wave velocity and attenuation of the pancreas in a pig. Finally, we performed *in vivo* measurements of pancreatic shear wave velocity and attenuation in healthy volunteers.

Ultrasound radiation force was used to excite shear waves in the pancreas and pulse-echo ultrasound was used to track the motion of the tissue. Autocorrelation was used to calculate tissue displacement as a function of time and propagation distance, $u(x,t)$. A two-dimensional fast Fourier transform (2D FFT) of $u(x,t)$ yielded the k-space whose coordinates are the wave number (k) and frequency (f). Shear wave velocity (c) and attenuation (α) at multiple frequencies were calculated from the k-space. A fully-programmable ultrasound system (Verasonics, Inc. Redmond, WA, USA) operating a phased-array probe (P4-2 Philips Healthcare, Andover, MA) was used to excite 900 μs impulse in the pancreases and track the motion by sector imaging at 3.906 kHz. First, we studied shear-wave propagation at several different locations in 3 excised porcine pancreases and repeated each measurement 5 times. In addition, we submerged an excised pig pancreas in a formalin bath and measured the shear wave velocity and attenuation every 20 minutes for a total of 100 minutes. *In vivo* pig pancreas measurements were performed both transabdominally and with an open abdomen allowing a direct acoustic access to the organ. In the formalin and the *in vivo* studies, shear-wave velocity and attenuation were measured at 100 Hz. The study protocol for the healthy volunteer studies was approved by our Institutional Review Board. Shear-wave measurements were made in the tail, body and head of the pancreas.

The group velocities in the three excised pancreases ranged from around 0.65 m/s to 1.1 m/s with the largest standard deviation around 9% of the mean. The formalin studies showed that the velocity increased from 0.5 m/s at t = 0 minutes to 1.1 m/s at t = 100 minutes while the attenuation decreased from around 400 Np/m to around 200 Np/m. The formalin studies suggest that shear wave vibrometry can track changes in pancreatic shear wave velocity and attenuation. *In vivo* porcine transabdominal values of shear wave velocity and attenuation at 100 Hz were c = 1.07 m/s and α = 173 Np/m. The *in vivo* porcine open-abdomen values were c = 1.14 m/s and α = 170 Np/m. The values of shear-wave velocity in healthy volunteers were in agreement with the previously reported MRE studies with the values ranging from 1.02 to 2.19 m/s. These results demonstrate the feasibility of using shear-wave elastography to assess

mechanical properties of the pancreas.

7.5 **Shear-wave dispersion in a rhesus macaque model of the uterine cervix,** Ivan M. Rosado-Mendez[1], Lindsey Carlson[1], Bin Huang[1], Quinton W. Guerrero[1], Mark Palmeri[2], Helen Feltovich[1,3] and Timothy Hall[1], *[2]Biomedical Engineering, Duke University, Durham, NC and [3]Maternal Fetal Medicine, Intermountain Healthcare, Provo, UT, rosadomendez@wisc.edu*

Objectives: Tissue viscoelasticity assessed through shear-wave dispersion has been used to diagnose pathologies in tissues such as liver and breast. Our group is extending this analysis to distinguish between sufficient cervices (which gradually soften and hydrate during pregnancy) and rapidly-softening cervices that can lead to preterm birth. Significant viscoelastic changes of ripened and unripened cervices have been demonstrated in *ex-vivo* animal models that may not fully represent the *in-vivo* human case. This study presents a comparison between ripened and unripened states of *ex-vivo* samples of a nonhuman primate (NHP) model, the rhesus macaque (RM), by means of shear-wave dispersion.

Methods: This study includes 28 NHP necropsy cervix samples, 12 of which were exogenously ripened with misoprostol. Samples were pinned to a piece of sound-absorbing rubber and placed in a saline solution. Shear waves were ARFI-generated and tracked at various axial locations (along the length of the cervix) in the anterior and posterior sides of each sample with a Siemens Acuson S2000 and 9L4 transducer with the scanning aperture parallel to the cervical canal. Displacements were calculated using the Loupas' routine. The axial location with the best displacement estimates within Mid to Proximal end was selected for comparison between ripened and unripened states of the anterior and posterior sides. The phase velocity dependence on frequency was estimated by computing the Fourier transform of the displacement time-derivative. We report values of the intercept, slope and value at 0.2kHz of a linear fit applied to the phase velocity versus frequency.

Results: Evaluation of the dispersive properties of the samples in this study was challenging due to their large stiffness. Independent estimates of the dispersive properties of the anterior and posterior parts of the cervix were consistent. Differences in the slope of the phase velocity versus frequency between the unripened and the ripened states were statistically significant ($p<0.05$, 12.4 m s^{-1} kHz^{-1} and 19.0 m s^{-1} kHz^{-1}, respectively). These values and those of the phase velocity intercept (unripened: 2.3m s^{-1}, ripened: 1.7m s^{-1}) and at 0.2kHz (unripened: 4.3m s^{-1}, ripened: 4.7m s^{-1}) suggest that the dispersion curve of the ripened case starts at a lower phase velocity than the unripened case but increases at a steeper rate with frequency, intercepting the curve of the ripened curve at about 0.2kHz. Further analysis is focused at understanding the large biologic variance of the estimates and comparing to the *ex-vivo* human case. Supported by NIH grants R01HD072077, R21HD061896 and R21HD063031 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and T32CA009206. We are also grateful to Siemens Healthcare Ultrasound division for an equipment loan and technical support.

7.6 **Comparison of shear-wave speed estimates among commercial ultrasound systems with liver-mimicking phantoms,** Timothy J Hall[1], Mark Palmeri[2], Paul Carson[3], Andy Milkowski[4], Shigao Chen[5], Ted Lynch[6], and other authors, *[1]Medical Physics, University of Wisconsin - Madison, [3]University of Michigan, Ann Arbor, MI, [4]Siemens Healthcare, Inc. Ultrasound Division, [5]Mayo Clinic - Rochester, NY and [6]Computerized Imaging Reference Systems, Inc. (CIRS), tjhall@wisc.edu*

The intent of this project was to perform a systematic comparison of ultrasound imaging-system-based shear-wave speed estimation in phantoms that have viscoelastic behavior similar to that observed in normal and fibrotic livers. The goal is to understand the differences in shear wave speed estimates obtained by various systems, determine sources of bias and variance in the estimates and minimize bias and variance among systems.

Computerized Imaging Reference Systems, Inc. (CIRS) provided three ('lossy') viscous phantoms that well-approximate shear-wave properties of normal and fibrotic liver. Those phantoms were used in the interlaboratory comparison of commercial shear-wave estimation systems at six sites and included commercially-available imaging systems from Philips, Siemens, Toshiba, General Electric, and Supersonic Imagine as well as a custom system. Each site followed an established protocol for data acquisition and followed the specific manufacturer's procedures for shear-wave speed (SWS) measurements with their system. The ten available SWS observations for each combination of system,

transducer, phantom and SWS estimation depth were subjected to a measurement system analysis (MSA) / Gauge R&R with Analysis of Variance (ANOVA).

As expected, the various systems provide 'reasonably consistent results' at 3.0 cm and 4.5 cm depth but estimate variance and bias among systems increases significantly at 7 cm depth with the greatest differences were found in the more viscous phantom (C). (Absolute SWS metrics have been withheld due to the ongoing, blinded nature of the study.)

Results to date demonstrate that there is a small, but clinically-significant, difference in SWS estimates among systems for clinically-relevant scanning conditions. These results, particularly when combined with digital phantom studies, will provide further insight into methods to minimize bias and variance among ultrasound SWS estimates. Partially supported by the US FDA, CDRH. The QIBA effort is funded in part by the RSNA and a contract with the NIBIB (HHSN268201300071C). Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

Wednesday morning

8. IMAGING 2

8.1 **Model-based channel data decluttering: parameter selection**, Brett Byram and Kazuyuki Dei, *Dept. of Biomedical Engineering, Vanderbilt University, Nashville, TN, brett.c.byram@vanderbilt.edu*

Ultrasound exams can produce beautiful images of anatomic structure, hemodynamics and a number of other tissue parameters and it is also low-cost and real-time. These qualities make ultrasound an excellent choice for many applications. Despite its potential, clinical exams commonly fail. To address the problem of failed and low-quality exams, we developed a model-based approach that suppresses reverberation and off-axis clutter. Our approach relies on a combined L1/L2 regularization scheme called elastic-net. An important aspect of any regularization scheme is parameter selection to control the amount of regularization applied to the model-fit.

To solve this, we developed a unique and efficient Field II adaptation for simulating multipath and off-axis clutter. This framework allows us to exactly know all aspects of our signal so that we can precisely control the signal-to-clutter level and relative levels of multipath versus off-axis clutter. The goal of the tuning is to find parameters that minimize error between the true wavefront of interest and the reconstructed wavefront, while also minimizing energy from clutter sources. For elastic net regularization, we need to identify the relative weighting of L1 and L2 and the degrees of freedom (*df*) in the model-fit. The relative L1/ L2 weighting is denoted by α, where 0 and 1 are L2 and L1, respectively.

Using the reconstructed wavefront error and a contrast metric we identified several candidate sets of regularization parameters. The sets were α=0.5 and *df* =70, α=0.9 and *df* =70, and α=1 and *df* =86. We applied these parameters to *in-vivo* data and found contrast improvements in anechoic lesions of 22.8, 22.3, 7.2 dB, respectively. We evaluated the algorithm on simulated anechoic cysts corrupted by multipath and off-axis clutter, and signal-to-clutter ratios (SCR) of -10, 0, 10 and 20 dB. We evaluated the α=0.9 and α=1, and we saw mean improvements of 12.3, 15.7, 8.7 and 3.8 dB for α=0.9 and 3.1, 4.5, 3.2 and 1.9 dB for α=1, listed in increasing SCR. The results show that most improvement occurs when clutter power is high. Results also indicate that elastic-net regularization (i.e. $\alpha<1$) produces more improvements than L1.

8.2 **Velocity estimation in pulsed Doppler with coherent beamforming**, Jeremy J. Dahl,[1] You Li[2], Dongwoon Hyun,[2] *[1]Department of Radiology, Stanford University, Stanford, CA and [2]Department of Biomedical Engineering, Duke University, Durham, NC, jeremy.dahl@stanford.edu.*

Traditional delay-and-sum beamforming has been a staple amongst ultrasound imaging modalities due to its simplicity and speed in execution. This beamformer, along with many adaptive beamforming

methods, yields ideal results when the target is coherent, such as that of a point reflector. For example, estimating the movement of a coherent target from a delay-and-sum beamformed signal yields accurate measurements of velocity or displacement with little bias or variance. However, when the target is a field of randomly-distributed subresolution scatterers, the scatters give rise to a partially coherent wave that manifests itself as speckle in traditional B-mode images. This partial coherence generates variance and bias in the estimates of velocity and displacement. The presence of acoustic and thermal noise can exacerbate this problem.

We introduce a theory describing the delay-and-sum beamforming process as a function of the coherence between the element signals of the transducer array and show how partial coherence introduces variance and bias into the estimates of velocity and displacement estimation. We demonstrate this theory in the context of velocity estimation in pulsed Doppler. Through the elimination of incoherent signals in Kasai's velocity estimator, we reduce our bias in a low signal-to-noise ratio environment from 8.3% to 0.6% (9.17 mm/s to 9.94 mm/s for a flow of 10 mm/s in a 2 mm vessel and 10 dB noise relative to blood). In addition, the standard deviation of our velocity estimates drop from 3.38 mm/s to 0.96 mm/s. While improving the bias and variance, however, this method sacrifices spatial information, thus decreasing resolution. Supported by the NIH grants R01-EB013361 and R01-EB015506 from the National Institute of Biomedical Imaging and Bioengineering. In-kind and technical support was provided by the Ultrasound Division at Siemens Medical Solutions USA, Inc.

8.3 **Analysis of the sources of variance in ultrasonic estimators of tissue displacement using pulsed-Doppler methods**, Joseph Hecker[1] and Siddhartha Sikdar,[2] *[1]Department of Electrical and Computer Engineering and [2]Department of Bioengineering, George Mason University, Fairfax, VA, jhecker@gmu.edu.*

Conventional narrowband Doppler velocity estimation using the autocorrelation method is well known to have large variance introduced by variations of the instantaneous frequency of the propagating broadband ultrasound pulse. The two-dimensional (2D) autocorrelation method improves the variance by including a correction for the instantaneous frequency and is commonly used in practice. This paper explores the sources of variance in the estimator with the aim of developing a lower variance estimate.. We show that the correction factor in the 2D estimator does not fully account for regions where speckle produces significant destructive interference and consequently large excursions from the nominal axial frequency. These regions are also shown to exhibit larger variance in the Doppler estimates. We also demonstrate that the estimation of instantaneous RF frequency can be challenging without sacrificing spatial resolution. Two alternative methods of filtering the RF data to reduce Doppler variance are introduced. First, a matched filter is used to preprocess the RF data before estimation using the 2D autocorrelation. Second, a frequency compounding filter bank method is employed to estimate Doppler phase shifts at different frequencies. The results of these three methods are compared with that of the 2D autocorrelation estimator using a Field II tissue motion model as well as *in-vivo* data.

8.4 **Ultrasound-based muscle kinematics tracking using dense feature point fields**, Paul Otto,[1] Frances Gavelli[2] and Siddhartha Sikdar,[1,3] *[1]Department of Electrical and Computer Engineering, George Mason University, Fairfax, VA,22030 and [2]Functional and Applied Biomechanics Section, Rehabilitation Medicine Department, National Institutes of Health, Bethesda, MD, 20892, S.Sikdar ssikdar@gmu.edu*

Background and Objective: Ultrasound imaging (US) has become an important tool for dynamic functional assessment of muscles and tendons. Quantifying musculotendon kinematics provides crucial outcome measures for diagnosing movement disorders and planning rehabilitation while offering key insights into the mechanisms of injury. Unfortunately, only a limited set of musculotendon kinematics can be quantified using US because currently the entire muscle fascicle must be visualized to define the muscle kinematics. Therefore, the objective of this work is to develop a flexible muscle kinematics tracking technique that can be used in an office-based setting by using B-mode imagery from commercially-available equipment.

Methods: The tracking algorithm uses a framework of dense feature points computed from the B-mode image. The feature point distribution correlates to regions with high feature quality measure, such as

having a strongly-peaked localized autocorrelation function, which would indicate an edge. Once a high quality feature region is identified, the feature points are tracked in that region over multiple B-mode frames until the feature points' quality criteria falls below a set threshold. The feature points' locations are then "stitched together" to form a tracklet. The tracklet length is limited because of speckle decorrelation. Therefore, many short tracklets are needed to create the final image trajectory. This is accomplished by merging the results of multiple feature point detections and tracking algorithms. This automatic method enables a quantitative characterization of motion across different regions of the muscle.

We investigated the accuracy of determining the rectus femoris muscle velocities using US against cine phase contrast (CPC) magnetic resonance (MR) imaging. 9 healthy volunteers were enrolled for this study. Each subject was first placed in a supine position in the MRI scanner with their knee slightly bent and supported by a cushion. The subject performed cyclic knee flexion and extension to the rhythm of a metronome while CPC anatomic images and velocity data were collected. Then the subject remained on the scanning plinth as it was moved out of the MR scanner to an exam room. The rectus femoris was then imaged using an Ultrasonix SonixTouch US system and a 5-14 MHz linear array transducer while the subject repeated the same cyclic motion. The B-mode image sequences were analyzed offline using MATLAB to generate velocity estimates. The muscle region was manually marked on a single B-mode frame, and then the velocity waveform was automatically derived based on feature tracking in subsequent frames.

The velocity waveforms derived from US and MRI were compared over a full cycle of extension and flexion.

Results and Discussion: The ranges of measured velocities were 57.3± 21.2 mm/sec for US and 54.6 ± 22.7 mm/s for MRI. The mean absolute error (MAE) between the US and MRI velocities over a full cycle of extension and flexion for all subjects was 9.2 ± 3.6 mm/s, while the average peak velocity error was 11.0 ± 7.7 mm/s. A strong correlation of 0.84 was obtained between the peak flexion velocities for the first 7 subjects; however, peak extension velocities showed weaker correlation (0.47). This was likely due to muscle contractions causing out-of-plane motion along with shape changes that differ from the originally marked frame. The velocity estimates for the last two subjects showed significantly higher variability compared to the remaining 7 and further investigation is needed to determine the source of these errors.

These results indicate that muscle velocities estimated using US compare well with those determined using CPC MRI. The limitation of this method is that muscle contractions cause out of plane motion along with region shape changes, and requires additional processing steps to reduce the velocity error. Currently, the framework is being extended to use active contour tracking at the muscle borders to limit features to the muscle of interest and further improve the velocity measurements. Supported in part by Grant Number 0953652 from the National Science Foundation and by the Intramural Research Program of the National Institutes of Health Clinical Center, Bethesda, MD.

9. TISSUE PARAMETERS 2

9.1 **Estimating structure function from histological tissue sections,** Aiguo Han[1] and William D. O'Brien, Jr[1], *[1]Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 405 N. Mathews, Urbana, IL 61801, han51@illinois.edu*

Objective: The spatial correlation among scatterers plays an important role in acoustic scattering of dense medium. The structure function (three-dimensional Fourier transform of the scatterer positions) has been used to describe the scattering caused by the scatterer position correlation. Previous studies estimated the structure function of dense medium by analyzing the ultrasound backscatter data, and developed analytical structure function models. To test if the structure function estimated acoustically (i.e., estimated from ultrasound backscatter data) was indeed a result of scatterer position arrangement, this study develops a method to directly estimate the structure function from two-dimensional (2D) histological images. The comparison between the structure functions estimated acoustically and histologically could improve the understanding of scattering in dense biological tissues.

Methodology: Dense cell pellet biophantoms were constructed and scanned using high-frequency single-element transducers (9 – 105 MHz). H&E stained sample sections were viewed under a light microscope (Olympus BX–51, Optical Analysis Corporation, Nashua, NH, USA). A 40X image was taken using the digital camera that was in sync with the microscope. The nuclear center was determined manually for each cell on the image, and a fast Fourier transform was performed on the nuclear center positions. The squared modulus of the 2D Fourier transform was normalized to unity for large wave numbers. The structure function was obtained by radial averaging the normalized squared modulus of the 2D Fourier transform. The structure functions obtained from multiple images were averaged. The resulting structure function was compared with the acoustically determined structure function. Three cell lines, Chinese Hamster Ovary (CHO), MAT (ATCC #CRL-1666) and 4T1 (ATCC #CRL-2539), were used to demonstrate comparability.

Results: The structure function was successfully obtained from histology. The structure function curves estimated histologically and acoustically showed similar shapes for every cell line. Both structure function curves were consistent with the previously developed structure function model. Additionally, each cell line has a unique structure function curve that was significantly different from others.

Conclusion: The method for estimating structure function from histology is feasible. The acoustically-estimated structure function is indeed a result of scatterer position arrangement. The structure function model can yield useful information related to tissue structure.

9.2 **Attenuation variability in the Rhesus Macaque cervix,** Quinton W. Guerrero[1], Lindsey C. Carlson[1], Ivan Rosado-Mendez[1], Bin Huang[1], Helen Feltovich[1,2] and Timothy J. Hall[1], *[1]Medical Physics Department, University of Wisconsin, Madison, WI 53706 and [2]Maternal Fetal Medicine Department, Intermountain Healthcare, Provo, Utah, qguerrero@wisc.edu*

Objectives: Acoustic attenuation has been proposed to quantify cervical change, specifically for predicting the risk of preterm birth. Collecting quantitative ultrasound data in human patients is expensive, time consuming, and most studies are only cross sectional. Therefore use of an appropriate animal model is justified. The non-human primate (NHP) model has been shown to be an excellent model for pregnancy and we are investigating its use in cervical remodeling. Attenuation, measured in human *ex vivo* specimens, was shown to be dependent on the angle of interrogation, spatial location, parity, and cervical remodeling. In this work, we attempted to replicate the human *ex vivo* study in the rhesus macaque model. We found the anatomy of the NHP cervix more complicated than that of humans. We also found mixed, but encouraging, results compared with findings in human cervix.

Methods: Necropsy specimens (N=32) were collected, and pinned to sound absorbing rubber. The samples were positioned so that the inner cervical canal was parallel to the aperture of the transducer. Samples were scanned with a Siemens Acuson S2000 and an 18L6 linear array transducer. Radiofrequency echo signal data were collected with the acoustic beams steered from -40 degrees to +40 degrees in steps of 4 degrees. Reference phantom data was collected for the same angles and attenuation was calculated using the Reference Phantom Method. Attenuation estimate bias was minimized with respect to spectral estimation regions and parameter estimation regions using the multitaper method for spectral estimation. The internal os and external os of the cervix were then used to group the attenuation measurements among cervix specimens based on fractional distance from the external os.

Results: Attenuation showed statistically-significant differences (p<0.001) among spatial locations along the cervix. The overall trend of attenuation matched that seen in the human cervix (Attenuation highest in Mid region, tapering off on either side). The mean standard deviation of attention estimates decreased from the distal to proximal ends (0.344 dB cm^{-1} Mhz^{-1} vs. 0.209 dB cm^{-1} Mhz^{-1} in distal vs proximal, respectively), consistent with findings in the human cervix. Dependence of attenuation on angle of interrogation showed a similar relationship, with the anisotropy increasing in the same direction as found in humans, after correcting for change in cervix orientation. Results suggest that, even with significant differences in cervical anatomy, there seems to be shared structural trends in attenuation estimates in both the human and NHP cervix indicating that the rhesus macaque may be an appropriate model for QUS detection of cervical remodeling. Supported by NIH grants R01HD072077, R21HD061896 and R21HD063031 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and T32CA009206. We are also grateful to Siemens Healthcare Ultrasound

division for an equipment loan and technical support.

9.3 **250-MHz scanning acoustic microscopy of human lymph nodes for the derivation of ultrasound scattering models**, Daniel Rohrbach[1], Emi Saegusa-Beecroft[2], Eugene Yanagihara[3], Junji Machi[2], Ernest J. Feleppa[1] and Jonathan Mamou[1], *[1]Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY, [2]University of Hawaii and Kuakini Medical Center, Department of General Surgery, Honolulu, HI, and [3]University of Hawaii and Kuakini Medical Center, Department of Pathology, Honolulu, drohrbach@RiversideResearch.org*

Quantitative acoustic microscopy (QAM) permits the investigation of soft tissue at fine resolution. In this study, QAM was performed on deparaffinized, 12-µm-thick lymph-node samples obtained from cancer patients using an F-1.16, 250-MHz transducer having a 160-MHz bandwidth. Samples were scanned in 2D and radio-frequency signals were processed to estimate acoustic attenuation (A), speed of sound (SOS), and acoustic impedance (Z). In total, 32 QAM datasets were acquired from 14 lymph nodes from 10 patients. Following QAM scanning, adjacent 3-µm thin sections were H&E stained, imaged by light microscopy, and co-registered to QAM images. QAM images had a spatial resolution of 7 µm, which allowed identification of regions consisting of lymphocytes, fat cells and fibrous tissue by comparison with matching H&E-stained thin sections. Average properties for lymphocyte-dominated tissue were 1552.6 ± 30 m/s for SOS, 9.53 ± 3.6 dB/MHz/cm for A, and 1.58 ± 0.08 Mrayl for Z. Values for Z obtained from fresh samples agreed well with those obtained from 12-µm sections from the same node. The 2D QAM images will be used to develop improved ultrasound-scattering models specific to lymph nodes. These models will be employed in an ongoing study using quantitative ultrasound methods to detect cancerous regions within freshly-excised lymph nodes. Our hypothesis is that lymph-node-specific scattering models based on QAM results can improve sensitivity and specificity of cancer detection when compared to the Gaussian-form-factor scattering model currently used in this ongoing study.

9.4 **Effects of signal saturation on scatterer-diameter and acoustic-concentration estimates based on high-frequency-ultrasound signals acquired from isolated lymph nodes,** Kazuki Tamura[1], Jonathan Mamou[2], Alain Coron[3], Kenji Yoshida[4], Tadashi Yamaguchi[4] and Ernest J. Feleppa[2], *[1]Graduate school of Engineering, Chiba University, Japan, [2]Lizzi center for Biomedical Engineering, Riverside Research, 156 William St. 9th floor, New York, NY 10038, [3]Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, F-75006, Paris, France and [4]Center for Frontier Medical Engineering, Chiba University, Japan, acda0254@chiba-u.jp*

Introduction: Choosing an appropriate dynamic range for acquiring radio-frequency (RF) data from a high-frequency ultrasound (HFU) system is challenging because signals can vary greatly in amplitude due to focusing and attenuation effects. In addition, quantitative ultrasound (QUS) methods are highly sensitive to the effects of signal saturation. In this study, the effects of saturation on QUS estimates of effective scatterer diameter (ESD) and effective acoustic concentration (EAC) were quantified using data acquired from human lymph nodes with a single-element transducer operating at a center frequency of 26 MHz.

Methods: Raw unsaturated RF signal (x) were artificially saturated (x_c) and saturation effects were quantified using Saturate-SNR

(SSNR = $20\log(|x|/|x - x_c|)$).

ESD_c and EAC_c were estimated from x_c for a wide range of SSNR values. EAC_c correction was performed by taking advantage of a linear relationship between EAC_c and $|x_c|$, which was defined as the sum of the absolute RF signal amplitude. The slope of this linear relation was estimated by artificially saturating x_c even more. The corrected EAC value was obtained at the intersection point between the line and the value of $|x^*|$, a "desaturated" RF signal obtained using smoothing splines

Results: Using an illustrative dataset of 16 lymph nodes, ESD changed on average by less than 3.8 % when SSNR varied from infinity to 7.6 dB. However, EAC_c decreased significantly with decreasing SSNR. The maximum average change was -3.19 dB/mm^3 when SSNR was 7.6 dB. After correction,

maximum error was 0.896 dB/mm^3 when SSNR was 7.6 dB and the average error was <0.3 dB/mm^3. The average differences between non-metastatic and metastatic lymph nodes in *ESD* and *EAC* were 10.8 μm and 3.87 dB/mm^3, respectively. Therefore, the error in corrected EAC was much smaller than the difference between the values for the non-metastatic and metastatic lymph node.

Conclusions: The change in ESD estimate value in the presence of saturation was small and no method was considered necessary to correct the ESD estimate in this initial study. However, the *EAC* estimate value changed significantly in the presence of saturation and therefore potentially could cause misclassification of tissue type. Nevertheless, our proposed correction method significantly reduced the effects of saturation on the EAC estimate and therefore could be used to simplify the data-acquisition process.

9.5 **Comparison of the ultrasonic properties of fast and slow waves in equine bone determined using conventional and Bayesian analysis techniques,** Amber M. Groopman[1], Jonathan Katz[1], Mark Holland[2], Fuminori Fujita[3], Katsunori Mizuno[4], Mami Matsukawa[3] and James G. Miller, *[1]Washington University in St. Louis, St. Louis, MO, [2]Indiana University-Purdue University School of Medicine, Indianapolis, IN, [3]Doshisha University, Japan and [4]The University of Tokyo, Japan, amnphysics@gmail.com*

Objective: The goal of the present study was to compare directly the ultrasonic properties of the fast and slow waves estimated by Bayesian probability theory to the ultrasonic properties determined using conventional analysis methods, in order to further validate the use of the Bayesian analysis technique for separating overlapping fast and slow waves in trabecular bone.

Methods: Through-transmission measurements, with a center frequency of 1 MHz, were acquired on an equine cancellous bone specimen that was systematically shortened from 11.8 mm down to 0.5 mm in increments of 0.5 mm (N = 24). Bayesian analysis was applied to data from all 24 sample thicknesses. In contrast, conventional analysis methods were restricted to sample thicknesses that were sufficiently long to permit separation of the fast and slow waves using time gates (N = 13).

Results: The Bayesian analysis approach successfully separated the fast and slow waves for sample thicknesses ranging from 11.8 mm down to 3.5 (N=18), whereas conventional analysis methods were restricted to data from sample thicknesses ranging from 11.8 mm to 6.0 mm (N=13). Over the same range of sample thicknesses, good agreements between conventional and Bayesian analysis methods were observed for the phase velocity and signal loss of the fast and slow waves.

Discussion: The results of this study indicate that the Bayesian probability theory approach yields estimates of the fast and slow wave properties comparable to those determined using conventional analysis techniques. Furthermore, Bayesian methods provide reasonable estimates of the fast and slow wave properties even when the two waves overlap in the time domain, thus not permitting the use of conventional methods. Supported, in part, by NIH R01 AR057433, NIH R01 HL040302, and by the Regional Innovation Strategy Support Program of the Ministry of Education, Culture, Sports, and Technology, Japan and a Grant-in Aid for Scientific Research (B) from the Japan Society for Promotion of Science.

9.6 **Relationships among ultrasonic and mechanical properties of cancellous bone,** Keith A. Wear[1], Saghi Sadoughi[2], Srinidhi Nagaraja[1], Maureen L. Dreher[1] and Tony M. Keaveny[2], *[1]Food and Drug Administration, Silver Spring, MD and [2]University of California, Berkeley, CA, Keith.Wear@fda.hhs.gov*

Most clinical-bone sonometers measure broadband ultrasound attenuation (BUA) and speed of sound (SOS) in calcaneus. In addition, backscatter coefficient (BC) has been shown to have clinical utility. The objective of this work was to assess the extent to which ultrasonic measurements convey mechanical properties of cancellous bone. Twenty-five defatted human calcaneus samples were investigated *in vitro.* Normalized BUA (nBUA), SOS, and BC were measured using 500 kHz focused ultrasound transducers. Finite Element Analysis, based on micro-computed tomography images (Scanco microCT 100), was used to estimate stiffness and apparent modulus of the samples. Correlation coefficients from linear regressions were as follows: nBUA vs. stiffness—0.80 (p<0.0001), nBUA vs. apparent modulus—0.81 (p<0.0001), SOS vs. stiffness—0.80 (p< 0.0001), SOS vs. apparent modulus—0.84 (p<0.0001), BC vs. stiffness—0.75 (p<0.0001), and BC vs. apparent modulus—0.69 (p<0.001). In conclusion, ultrasonic

measurements are very sensitive to mechanical properties of cancellous bone. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

10. PHOTACOUSTICS 2

10.1 **Ultrasonic-based tissue oxygen saturation and perfusion assessment in a rat orthotopic liver tumor model**, Katherine Dextraze[1], Nina Munoz-Gonzalez[3], Andrew Heinmiller[2], Jim Bankson[1], Charles Kingsley[1], Keith Michel[1], Rony Avritscher[3], Richard Bouchard[1], *[1]Department of Imaging Physics, University of Texas MD Anderson Cancer Center, [2]FUJIFILM VisualSonics and [3]Department of Interventional Radiology, University of Texas MD Anderson Cancer, kldextraze@mdanderson.org*

Introduction: Oxygen saturation variations have been linked to tumor aggressiveness and poorer prognosis when compared to normally-oxygenated tumors. A spatial map of oxygen saturation within the tumor may improve guidance of tumor biopsies by sampling severely hypoxic regions, which can impact tailored treatment plans for each patient. The goal of this study was to coregister multiwavelength photoacoustic-ultrasonic (PAUS) imaging and contrast-enhanced US perfusion imaging to assess local changes in oxygen saturation and perfusion within an orthotopic rat tumor *in vivo*.

Methods: Three rats inoculated with an orthotopic model of human hepatocellular carcinoma (HCC) were assessed. To induce changes in oxygen saturation within the tissue, inhalation gas was switched from medical air to pure oxygen at 10 minute intervals.

Both ultrasound and PA imaging were performed using the Vevo LAZR system using a tunable laser source (VisualSonics) and a custom-made, low-frequency array (15 MHz). Under medical air, we acquired 3D B-mode, 3D Doppler, 3D PA with 6 wavelengths (680, 710, 750, 850, 920, & 950 nm), 2D PA on a central slice of the tumor using all wavelengths (680-970nm). Under pure oxygen, the PA acquisitions were repeated, followed by contrast-enhanced ultrasound using a 150-microliter bolus injection of MicroMarker microbubbles (VisualSonics).

Analysis was performed using the VevoLab software, VevoQC software, and customized MATLAB code. For each study, two regions of interest were assessed within the tumor: (1) a central region, (2) a peripheral region of the tumor. The change in oxygen saturation was calculated via spectral unmixing, where the signal correlated to either the oxy- or deoxy-Hb spectra. For US perfusion assessment, an additional ROI in normal liver was included and dynamics for perfusion wash-in were assessed.

Results: We observed a trend of decreasing perfusion from the peripheral to central regions of the tumor, which correlated with lower oxygen saturation in the central region than the peripheral region. This study demonstrates that PAUS imaging is capable of characterizing oxygen saturation and tissue perfusion in a orthotopic rat model of HCC. Such capabilities have potential for eventual clinical translation for diagnostic and treatment monitoring purposes.

10.2 **Evaluation of enhanced flow of human inflammatory arthritis by photoacoustic imaging**, Xueding Wang[1], Janggun Jo[1], Guan Xu[1], April Marquardt[2], Sheeja Francis[2] and Girish Gandikota[1], *[1]Department of Radiology, University of Michigan Medical School, Ann Arbor, MI 48109 and [2]Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, xdwang@umich.edu*

With the capability of assessing high resolution optical information in soft tissues at imaging depth up to several centimeters, innovative biomedical photoacoustic imaging (PAI) offers benefits to diagnosis and treatment monitoring of inflammatory arthritis, particularly in combination with more established ultrasonography (US). In this work, a PAI and US dual-modality system facilitating both imaging functions in a real-time fashion was developed and initially tested for its clinical performance on patients with active inflammatory arthritis. Photoacoustic (PA) images of metacarpophalangeal (MCP) joints were acquired at 580-nm wavelength that provides a desired balance between optical absorption of blood and attenuation in background tissue. The results from six patients and six normal volunteers used as a control demonstrated the satisfactory sensitivity of PAI in assessing the physiological changes in the

joints, specifically enhanced blood flow as a result of active synovitis. This preliminary study suggests that PAI, by revealing vascular features suggestive of joint inflammation, could be a valuable supplement to musculoskeletal US for rheumatology clinic.

10.3 **Multispectral photoacoustic decomposition with localized regularization for detecting targeted contrast agent,** Behnoosh Tavakoli, Ying Chen, Xiaoyu Guo, Hyun Jae Kang, Martin Pomper and Emad M. Boctor, *Johns Hopkins University, Baltimore, MD 21218, btavako1@jhu.edu.*

Multispectral photoacoustic imaging along with targeted contrast agents has shown promising results for cancer detection. In this method, the acoustic signal is measured at multiple illumination wavelengths and it is possible to detect exogenous absorber concentration by resolving its spectral signature over other endogenous absorbers. In this study, we have developed a photoacoustic (PA) spectral unmixing algorithm based on nonlinear least square optimization and localized regularization. Three near-infrared targeted contrast agents, synthesized for imaging prostate cancer, were evaluated with our multispectral PA imaging system while dyes were injected in a thin wall plastic tube embedded in a water tank. PA spectrums of each dye at 0.5 µM to 20 µM concentrations were measured in the wavelength range of 700 nm-850 nm. Subsequently, mixtures of those dyes were imaged with the same setup. Although there was a small separation between the dye's spectra, the concentration of each dye was estimated with about 20% error on average from almost all mixtures.

10.4 **Measurement of the corneal epithelium by optical coherence tomography and high frequency ultrasound,** Ronald H. Silverman[1, 2], Raksha Urs[1], Jonathan Mamou[2], Daniel Rohrbach[2], Timothy Archer[3], Marine Gobbe[3] and Dan Z. Reinstein[4], *Department of [1]Ophthalmology, Columbia University Medical Center, New York, NY, [2]F.L. Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY and [3]London Vision Clinic, London, UK, rs3072@cumc.columbia.edu*

Background: Keratoconus (KC) is progressive, corneal dystrophy generally first manifesting in young adults. In KC, the cornea thins and deforms to assume a conical shape. While advanced KC is easy to recognize using conventional tools such as slitlamp biomicroscopy and corneal surface topography, early KC may be difficult to diagnose. Using high frequency ultrasound (US), we have shown that the corneal epithelium, an approximately 50 µm thick layer of cells overlying the 500 µm thick stroma, remodels in response to underlying stromal irregularities. In KC, this produces a pattern of epithelial thinning in the region of the cone which may be evident before surface topographic change is evident. Recently, optical coherence tomography (OCT) systems have been developed that allow epithelial thickness (ET) measurement and mapping. Our aim was to compare ET measurements and maps produced by OCT and ultrasound.

Methods: We undertook two independent studies comparing OCT (RTVue, OptoVue, Inc) and US (Artemis, Arcscan, Inc.) In the first study, 20 eyes from 10 normal subjects were scanned by RTVue, Artemis and then repeat RTVue. In the second study, 211 virgin corneas and 191 post-laser refractive surgery (LRS) corneas were scanned. We used the generally accepted corneal speed of sound of 1640 m/s for all layers. To explore the effect of speed of sound on ET measurements, we used a 250 MHz scanning acoustic microscope (SAM) to image velocity in unfixed pig cornea.

Results: In the first study, no significant change in OCT ET maps was noted pre- versus post-immersion US. Central ET values were 53.8 ± 3.4 µm by RTVue and 55.1 ± 2.8 µm by Artemis. In the second study, central ET was 53.3±3.2 µm by RTVue and 54.0±3.1 µm by Artemis in untreated corneas. In post-LRS eyes, mean ET was 57.8±6.2 µm by RTVue and 60.3±6.6 µm by Artemis. The difference between OCT and US ET values was largest in regions where the epithelium was thick, e.g., inferiorly in normal eyes and over the ablation zone in post-LRS eyes. Regression analysis between OCT and US ET values indicated a speed of 1573 m/s for the epithelium would correct the difference. Velocity images generated from 250 MHz acoustic microscopy on an unfixed, cryosection of pig cornea indicated speed of sound values in the epithelium and stroma of 1570 and 1620 m/s, respectively, after correcting for temperature.

Conclusions: The thinner ET values obtained by OCT were surprising since OCT ET values incorporate the tear film, which is typically 3-6 µm thick. We found that a velocity of about 1570 m/s would largely correct the observed discrepancy. SAM results agreed with this finding. While this new

epithelial speed of sound will allow better correspondence between optical and US ET measurements, the presence of the tear film tends to smooth out features in OCT ET maps that are observed with higher contrast by US. Thus, while the methods are strongly correlated, ET maps even after correction are not equivalent. Supported in part by NIH grants EY019055, P30 EY019007 and an unrestricted grant to the Department of Ophthalmology of Columbia University from Research to Prevent Blindness. We thank OptoVue, Inc. for loan of instruments for the purpose of this study.