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CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Each year, about 800,000 Americans experience a new or recurrent stroke. The long-term goal of this research program is to create non-invasive methods utilizing ultrasound to identify plaques at high risk for initiating a cerebrovascular accident. The core of the current research project is a pilot clinical study to enroll 100 subjects who are scheduled for carotid endarterectomy (CEA). From each subject, the research effort obtains ultrasound data from the carotid plaque (or carotid artery for Normal subjects) prior to surgery and then creates histology slides of the removed plaque tissue which are used to train a statistical classifier for determining plaque composition. The main accomplishments from the prior year include: increasing enrollment to 53 CEA subjects, determination of attenuation compensation approach, testing of the use of the nonlinearly generated harmonic in addition to the fundamental for plaque characterization, and the production of a course machine learning algorithm based on data from the first 32 subjects enrolled in year 1 with accuracy of 77%.

15. SUBJECT TERMS
Atherosclerosis, stroke, cerebrovascular accident, carotid endarterectomy, ultrasound, spectral analysis, tissue characterization, machine learning, noninvasive, carotid plaque

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1. INTRODUCTION

Each year, about 800,000 Americans experience a new or recurrent stroke with a disproportionate number of these being veterans. Over 15,000 veterans are hospitalized each year for cerebrovascular accidents including stroke. The long-term goal of this research program is to create **non-invasive** methods utilizing ultrasound to identify plaques at high risk for initiating a cerebrovascular accident. As a critical first step toward achieving this long-term goal, the objective of this research project is to test the hypothesis that non-invasive spectral analysis of diagnostic ultrasound backscatter from carotid plaque can accurately and reproducibly determine plaque composition. The core of this research project is a pilot clinical study to enroll 100 subjects who are scheduled for carotid endarterectomy (CEA). From each subject, the research effort obtains ultrasound data from the carotid plaque prior to surgery and then creates histology slides of the removed plaque tissue which are used to train a statistical classifier for determining plaque composition. Future studies will apply this tool to the task of predicting stroke in "at risk" populations.

2. KEYWORDS:

Atherosclerosis, stroke, cerebrovascular accident, carotid endarterectomy, ultrasound, spectral analysis, tissue characterization, machine learning, noninvasive, carotid plaque

3. ACCOMPLISHMENTS:

This research project, *Vascular Plaque Determination for Stroke Risk Assessment*, contains five major tasks in order to create the non-invasive tool for determining plaque composition.

Major Task 1: *Clinical Study Preparation*
This effort covered the items necessary to begin clinical enrollment for the Ultrasonic Mapping of Carotid Plaque Composition Study (UMP Study). The statement of work proposed completion of these tasks by the second month (i.e. 14 Nov 2016) of the effort. These items with their completion dates are as follows:

- Development of the ultrasound standard operation procedures
  - ***Completed*** prior to contract effective date of 15 Sep 2016
- Creation of required clinical documentation (Clinical Trial Folder, Case Report Forms, etc.)
  - ***Completed*** prior to contract effective date of 15 Sep 2016

The first two *milestones* of the project were the human studies approvals required to begin recruitment. The original planned completion dates are in parentheses:

- Local Institutional Review Board Approval: 23 Aug 2016 (14 Nov 2016)
- HRPO Approval: 31 Aug 2016 (14 Jan 2016)

Major task 1 was completed prior to the effective date of the contract**.**

Major Task 2: *Subject Enrollment and Data Acquisition*
The enrollment target for this effort is 100 subjects following the contract effective date. The enrollment and data collection is ongoing with original targeted completion by 15 Jun 2019.

Actual enrollment in Year 1 was 32 subjects with a planned enrollment of 25, while actual enrollment in Year 2 was 21 subjects, planned enrollment for Year 2 was 42.

The subtasks and first year status are as follows:

- Consent Subjects (**goal 67%**): 53% complete
- Research Ultrasound Imaging: (**goal 67%**): 53% complete
- Collection of Plaque from Carotid Endarterectomy (**goal 67%**): 51% complete

Major Task 3: Data Processing
These tasks are ongoing for processing of the plaque tissue and ultrasound data. Tasks were begun 4 November 2016 and are expected to continue through July 2019. These tasks include the following:

- Prepare Serial Histology of Carotid Plaque 51% complete
- Ultrasound Signal Processing Development and Testing in process
- Match Slides to Ultrasound Grayscale *In Vivo* Data 32% complete

The Task 3 milestone is extraction of the ultrasound spectral parameters from all regions of interest (ROI) with matched histology. Original targeted completion is August 2019.

Major Task 4: Create Statistical Classifiers
Initial statistical classifier creation: August 2018.
Milestone: Final statistical classifiers - target Aug 2019.

Major Task 5: Validation of Statistical Classifiers
Planned start Aug 2019
Milestone: Validated final statistical classifiers – target Sep 2019.

What was accomplished under these goals?

1. Clinical study enrollment

Clinical study enrollment proceeded as anticipated until the departure of key vascular surgeons from the study. Three surgeons left the Cleveland Clinic Department of Vascular Surgery over the course of Year 2 including our largest enrolling surgeon. This led to an immediate decrease in enrollment in Quarter 6 of this study as shown in Table 1. The Department of Vascular Surgery has hired surgeons to fill these vacancies, however there was a significant lag in enrollment during the beginning of the tenure of these new surgeons. By Quarter 8, we experienced a strong return to our prior enrollment numbers and during the first two weeks of Year 3 we have seen 4 more enrolled subjects whose numbers are not included in Table 1. Based on these numbers, we expect our enrollment completion date to have changed by 3 months from the original target date to 15 September 2019. Currently, all vascular surgeons that perform carotid endarterectomies (CEA) at the main campus of the Cleveland Clinic are involved in patient recruitment. Thus all patients scheduled for a CEA are evaluated for the clinical study, *Ultrasonic Mapping of Carotid Plaque Composition* (UMP).

Table 1: Projected versus actual enrollment for the UMP Study.

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Projected	*0*	*7*	*9*	*9*	*9*	*9*	*12*	*12*
Actual	**4**	**7**	**11**	**10**	**9**	**3**	**1**	**8**
Actual Cumulative	4	11	22	32	41	44	45	53

2. Carotid Plaque Tissue Processing

A study representative collects the tissue following excision of the plaque during the CEA procedure. The plaque is placed in saline and RF ultrasound data is collected using the same settings as used during the in vivo imaging prior to the CEA. The primary reason for the ex vivo data collection is to aid in the matching of the in vivo transverse sites with histology slides from the plaque. An important dataset in locating these same sites between the ex vivo and in vivo data are systematic RF data collections while moving the transducer from proximal end to the distal end of the transducer. These images obtained from sweeping through the plaque greatly aid the site matching since they depict how the plaque is changing in both proximal and distal directions. After the tissue is paraffin embedded, serial histology through the matched sites is performed.

As of 14 September 2018, 19 of the 21 plaque samples that were collected over Year 2 had been processed with histology slides produced and all samples from Year 1 have been processed and matched to the target grayscale image.

3. Attenuation Compensation Comparison and Investigation

An evaluation of attenuation compensation approaches to determine which approach is best for differentiating carotid plaque tissue types was performed and completed. The following approaches were evaluated:

1. Reference phantom only based compensation
2. Adventitia based approaches, where data from normal subjects is used to provide an estimate of the average attenuation experienced by the ultrasound waves.
    a. Single path: depth and frequency dependent compensation
    b. Two-path: depth and frequency dependent compensation (user input to determine the depth of patient fat/skin versus primarily muscle for the remainder of the path).
3. Centroid methods including: (K. Samimi, T. Varghese, Optimum Diffraction-Corrected Frequency-Shift Estimator of the Ultrasonic Attenuation Coefficient, IEEE Trans. UFFC, 2016, vol 63 (5), 691-702.)
    a. nominal power spectral shift estimator
    b. optimum power spectral shift estimator

A summary of the results are contained in the abstract located in the Appendix. Short summary is that the best performing attenuation compensation was the patient adaptive two-step attenuation compensation (number 2a in the above list).

4. Harmonic Parameter Analysis

An initial analysis of the nonlinearly generated second harmonic was performed and an abstract was submitted and accepted to the 2018 IEEE International Ultrasonics Symposium, 22-25 October 2018. The abstract is located in the appendix and demonstrates that the nonlinearly generated second harmonic portion of the backscatter (formed from nonlinear propagation to the target) provides information that is distinct from the information contained in the fundamental portion of the backscatter. In Figure 1, boxplots of the intercept spectral parameter demonstrate the difference between the fundamental spectral parameter and the nonlinearly generated second harmonic spectral parameter. This investigation demonstrates that the spectral parameters for the nonlinearly generated backscatter do provide some different information from that contained within the fundamental spectrum. Side note, the spectral parameters are all computed relative to adventitia since it was used as a baseline for attenuation compensation. In addition, the source of the differences could be multi-faceted since the apparent additional information from the Harmonic can simply be explained via the differences in bandwidth rather than a nonlinear scattering effect. See the appendix for further details.

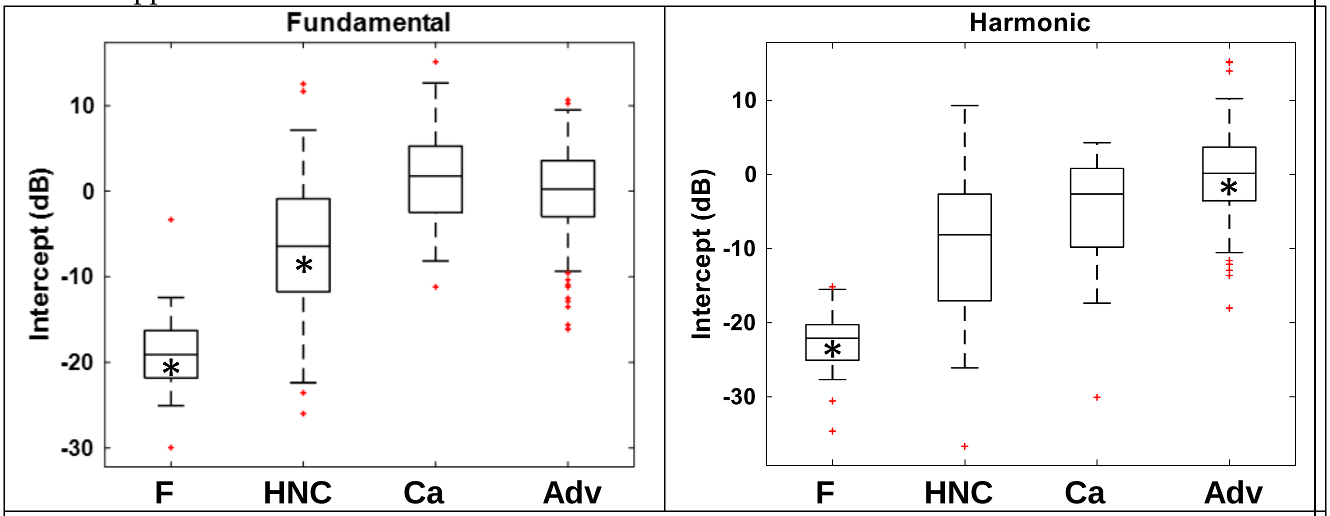


Figure 1 Boxplots of the Intercept spectral parameter for the fundamental and second harmonic portions of backscatter. F- Fibrous/Fibro-Fatty, HNC – Hemorrhagic/Necrotic Core, Ca – Calcium, Adv – Adventitia. The asterisks denote tissue types where the mean values are significantly different than the means of the other tissue types.

5. *Produce First Statistical Classifier*

Histology slide matches were obtained from 32 subjects. Initially, there were 117 potential sites from which 62 matches between histology and grayscale ultrasound image sites were obtained. Homogenous regions were found in 57 of these 62 matches. Each region of interest (ROI) was drawn on the recreated grayscale image using the ROI selection tool within Matlab. This tool permits the investigator to draw a precise region and obtain the location of the ROI within the raw RF ultrasound data. This information then gets analyzed to obtain the average power spectrum and a single-step, frequency and depth dependent attenuation compensation was applied (2.a from above) to obtain the estimate of the backscatter transfer (eBTF) function and cepstral parameters. The number of ROI's is as follows:

1. Calcium (Ca): **65:** 140 used for training
2. Hemorrhagic and/or Necrotic Core (HNC): **301:** 206 used for training
3. Fibrous and/or Fibro-Fatty (F): **59:** 35 used for training
4. Adventitia (Adv) from normal volunteers: **209:** 140 used for training

A random forest containing 100 trees was created within Matlab and the relative importance was extracted and is shown in Figure 2 with the out of bag (OOB) error shown in Figure 3.

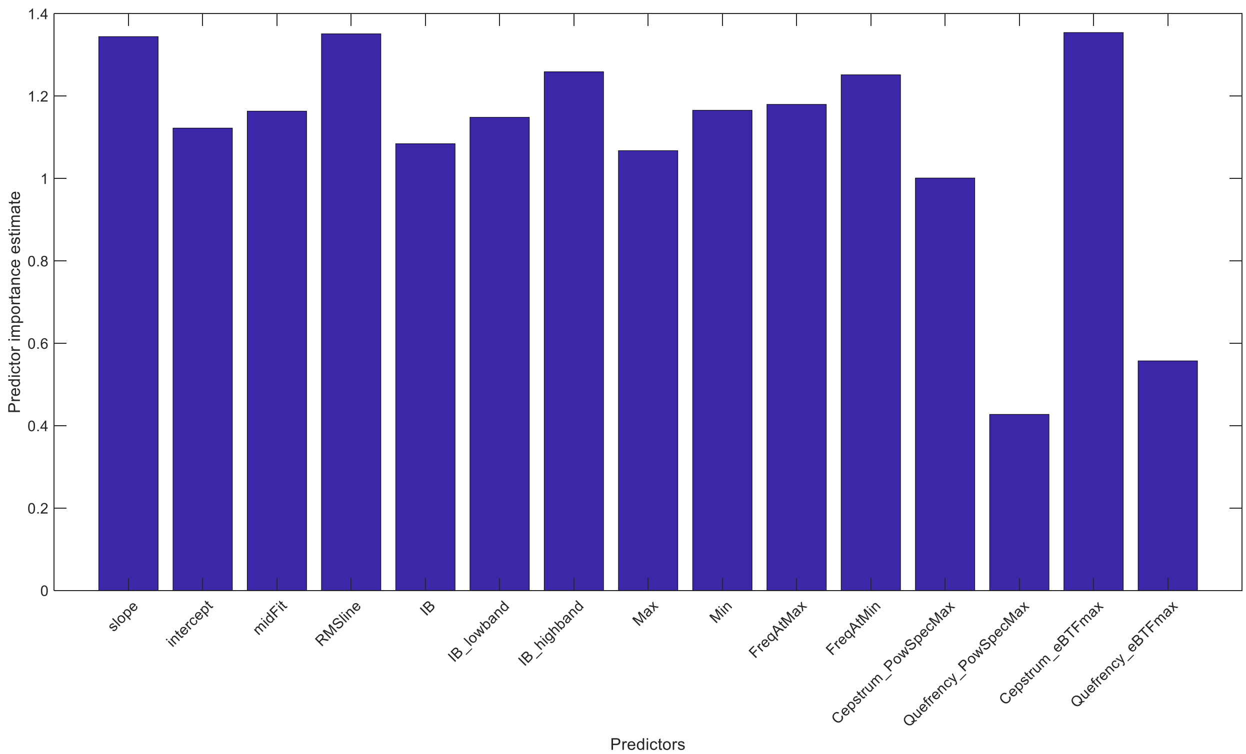


Figure 2 Relative importance for the 15 parameters used in the Random Forest. Higher bars indicate that the parameter provided greater discriminatory ability for the data set.

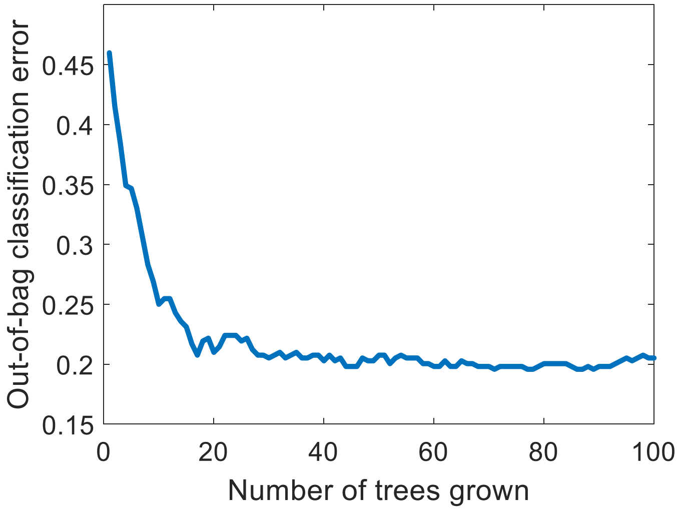


Figure 3 Out of bag (OOB) error as a function of the number of trees in the forest.

The OOB error indicates that a forest of 40 trees is adequate and that the predictive error is roughly 20%. This forest was created using only 2/3 of the ROI data with the histology being the 'gold-standard' for the creation of this statistical classifier. The remaining 1/3 of the data was run through the classifier and the confusion matrix in Table 2 was created. Overall predictive accuracy is 77% which is comparable to the OOB error shown in Figure 3. This result is encouraging but far from ready for clinical implementation. The following items still remain to be implemented: 1. Noise detection to eliminate ROI's where there is insufficient signal to process. 2. Addition of 2D spectral parameters. 3. Addition of spectral parameters from the nonlinearly generated second harmonic. 4. Implementation of the two-step attenuation compensation.

The relative importance data demonstrates that there are three top performing spectral parameters: slope, root-mean-square (RMS) deviation from the line fit, and the maximum value from the cepstrum computed using the *eBTF*. Only one of these values is intensity based and thus closely tied to the intensity of the grayscale image, the other two (RMS and slope) are independent of the intensity and are closely associated with scatterer size and orientation. Thus the spectral information is providing actionable data that is not available via standard grayscale imaging.

Table 2 Confusion matrix using the test data for the random forest.

	Random Forest Classifier			
	Adv	**H/NC**	**F**	**C**
HISTOLOGY				
Adv	59	10	0	0
H/NC	5	88	0	2
F	0	14	10	0
Ca	5	11	0	6

Stated Goals Not Met

Due to the loss of large enrolling surgeons the enrollment of patients was greatly diminished. This has recovered as demonstrated by Q8 (see Table 10) numbers and the first two weeks of Year 3. We estimate that this disruption in patient enrollment has delayed the enrollment completion date by one quarter with a new date of 15 September 2019.

What opportunities for training and professional development has the project provided?

1. We submitted abstracts to two professional conferences. These conferences are meeting in the first quarter of Year 3 and had not occurred at the time of this report.

2. Russell Fedewa and Geoffrey Vince both spent time training Sheronica James in ultrasound image matching to histology. This requires learning how to read and histology slide for atherosclerotic plaque that has been stained with H&E and Movat Pentachrome.

How were the results disseminated to communities of interest?

Abstracts were submitted and accepted to the following conferences (abstracts located in the Appendices):

1. 176th Meeting of the Acoustical Society of America, Victoria, BC, Canada, 5-9 November 2018. "Attenuation Compensation of Ultrasound Backscatter from Human Carotid Plaque" AUTHORS: S.L. James, R. Fedewa, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; H. Gornik, Cardiovascular Medicine, Cleveland Clinic, Cleveland, Ohio; S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, Ohio.

2. 2018 IEEE International Ultrasonics Symposium, Kobe, Japan, 22-25 October 2018. "Nonlinearly Generated Second Harmonic Ultrasonic Backscatter for Determining Composition of Human Carotid Plaque", AUTHORS: S.L. James, R. Fedewa, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; H. Gornik, Cardiovascular Medicine, Cleveland Clinic, Cleveland, Ohio; S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, Ohio

What do you plan to do during the next reporting period to accomplish the goals?

Continue Study Enrollment

This research effort depends on continuing enrollment of subjects into the study. We expect to meet to reach the target enrollment for the study by the end of Year 3.

Refine Statistical Classifier and Perform Final Validation

The statistical classifier work requires the following additional steps:

1. Noise detection to eliminate ROI's where there is insufficient signal to process.
2. Implement 2D spectral parameters
3. Addition of spectral parameters from the nonlinearly generated second harmonic. This effectively doubles the number of parameters since each parameter from the fundamental spectrum can be obtained from the harmonic spectrum.
4. Implementation of the two-step attenuation compensation. This step requires user defined boundary information for locating the interface of the fat/skin and muscle.
5. Implementation of support vector machines (SVM), including cross validation within the training set to determine the best kernel to implement.
6. Determination of the correlation between the different parameters

Final step, once the best random forest and SVM classifiers are obtained is to compare the two of them using the reserved test set (1/3 of the data).

Perform Inter-Pathologist Review

Inter-pathologist review will take place to compare the performance of the classifier to three pathologists. A key step in this effort is the creation of a 2D color overlay to classify and visualize the plaque composition.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Two items that were learned or better understood: 1. The backscatter of the nonlinearly generated second harmonic is useful for determining tissue composition. 2. The two-step attenuation compensation approach that breaks the propagation path into two sections (skin/fat and muscle) provides measureable improvement in distinguishing between plaque tissue types.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Invention disclosure was submitted with that combines the use 1D and 2D spectral parameters and cepstral parameters for the determination of plaque composition including the use of nonlinearly generated second harmonic data along with novel attenuation compensation.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Enrollment decline during Q5 and Q6 of this 3 year effort has delayed the targeted enrollment completion by a quarter. We currently enrolled 53 subjects at the end of Year 2 which missed the target enrollment of 67. This was a direct result of the loss of the top surgeon enrollers and the lag of carotid endarterectomy surgeries that occurred as the hired replacements became established. Recovery of our rate of enrollment is demonstrated in Table 1 as Q8 enrollment is beginning to approach our target quarterly enrollment goals. Also, in the first 2 weeks of Year 3, we enrolled 4 additional subjects. Thus we anticipate that enrollment should be close the target of 12 per quarter for the remainder of year 3.

We will continue to evaluate and approach all patients scheduled for carotid endarterectomy at the Cleveland Clinic main campus.

Changes that had a significant impact on expenditures

The reduced enrollment had a significant impact on the histological processing costs and sonographer charges since there were not as many cases to process.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No significant changes in the use or care of human subjects.

Annual IRB Review and Approval - 2018 August
IRB Study Number 12-797
"Ultrasonic Mapping of Carotid Plaque Composition (UMP)"
PI: David Geoffrey Vince, PhD
Cleveland Clinic Foundation, Cleveland, OH
HRPO Log Number A-19566

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Invention disclosure was filed with the Innovations office at Cleveland Clinic in April 2018. Outline below
Title: Noninvasive Method for Determination of Carotid Plaque Composition

See appendix DD Form 882

- **Other Products**

As provided in the Data and Resource Sharing Plan, the collected ultrasound RF backscatter data along with Matlab processing code will be uploaded to the Zenodo digital research sharing site by 1 Aug 2020.

Currently, in vivo data from 53 human carotid plaques and the surrounding tissue has been collected and archived internally in the Lerner Research Institute at the Cleveland Clinic. This data comprises the following:

- Reference Phantom Data for Normalizing the collected RF data
- 198 Static Sites:
  - *in vivo* RF ultrasound backscattered signal from human carotid plaque with a 1 cm separation to insure independence.
  - 10 frames of data per file
    - Each Frame: 456 lines by 2076 points at 40 MHz.
    - Collected using pulse inversion (thus harmonic data included)
- 53 Sets of Transverse and Longitudinal Scans (Same settings as the Static Sites):
  - Transverse Scans Through the Plaque: Sonographer slowly moving the transducer from proximal to the distal end of the plaque while collecting RF data.
  - Longitudinal Scans Through the Plaque: Sonographer slowly moving the transducer from medial to lateral while collecting RF data from the plaque.
- Data from 6 Normal Subjects (Similar to RF ultrasound data from CEA subjects)
  - 60 static sites
  - 6 sets of transverse and longitudinal scans

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	D. Geoffrey Vince
Project Role:	Principal Investigator
Research Identifier (e.g. ORCID ID):	0000-0001-6155-7118
Nearest Person Month Worked:	3
Contribution to Project:	Project management including all submissions, manuscripts, and communications. Oversight and coordination between departments involved in the research effort: Biomedical Engineering, Vascular Surgery, and Vascular Medicine. Project management and communications. Management of histology processing and interpretation. Review, guidance, and development of signal and data processing approaches.
Funding Support:	NIH/NHLBI The Cleveland Clinic Innovation Accelerator
Name:	Russell J. Fedewa
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID):	0000-0002-0690-9472
Nearest Person Month Worked:	12
Contribution to Project:	Directing the development of all signal processing approaches. Study coordinator duties. Data collection and processing. Development of clinical study protocol, procedures, and forms with assistance from co-investigators and principal investigator.
Funding Support:	NA
Name:	Sheronica James
Project Role:	Research Engineer
Research Identifier (e.g. ORCID ID):	0000-0002-5647-1106
Nearest Person Month Worked:	12
Contribution to Project:	Signal processing development to account for effects of attenuation and diffraction. Data collection and processing. Support the implementation and execution of subject recruitment and enrollment, specifically, the consent process.
Funding Support:	NA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization Name:	Siemens Medical Solutions USA, Inc.		
Location of Organization:	51 Valley Stream Parkway, Malvern PA 19355, USA		
Partner's Contribution to the Project:	In-kind support		
		Equipment Loan:	S3000 HELX Ultrasound System
			9L4 Transducer
			Axius Direct Research Interface
			Matlab Software to Access RF Data Files

8. SPECIAL REPORTING REQUIREMENTS

NA

9. APPENDIX:

- 176^{th} Meeting of the Acoustical Society of America, Victoria, BC, Canada, 5-9 November 2018.
  "Attenuation Compensation of Ultrasound Backscatter from Human Carotid Plaque"
- 2018 IEEE International Ultrasonics Symposium, Kobe, Japan, 22-25 October 2018.
  "Nonlinearly Generated Second Harmonic Ultrasonic Backscatter for Determining Composition of Human Carotid Plaque
- DD Form 882: Noninvasive Ultrasonic Determination of Vascular Plaque Composition

ABSTRACT:

Attenuation Compensation of Ultrasound Backscatter from Human Carotid Plaque

Accepted for presentation at 176th Meeting of the Acoustical Society of America, Victoria, BC, Canada, 5-9 November 2018.

S.L. James, R. Fedewa, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, OH
H. Gornik, Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH
S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, OH

Carotid atherosclerotic plaque composition may be a valuable predictor of stroke risk. Here, a patient adaptive attenuation compensation technique is introduced. A Siemens S3000, 9L4 probe and Axius Direct software were used to acquire radiofrequency (RF) data from 19 subjects prior to carotid endarterectomy. Histology slices of the excised plaque were prepared and matched to ultrasound frames. Regions of interest (ROI) were selected from homogenous area within the slide stack and matched to corresponding ROI's in the RF data. ROI's were categorized as fibrous (n=32), hemorrhagic and/or necrotic core (n=74), or calcium (n=32). Additionally, 209 adventitia ROI's were obtained from six normal subjects. ROI power spectra were computed and normalized to a uniform phantom. Five attenuation compensation methods were applied to the spectra: (1) reference phantom with 0.5 dB/cm-MHz attenuation; (2) nominal power spectral shift estimator; (3) optimum power spectral shift estimator; (4) normalized backscatter from adventitia; and (5) patient adaptive two-step attenuation compensation. A linear fit of the resulting estimated backscatter transfer functions (eBTF) was performed over the fundamental bandwidth of 3 – 7 MHz. Only the patient adaptive two-step attenuation compensation distinguished among the means of all four tissue types for the linear mid-band fit parameter.

ABSTRACT:

Nonlinearly Generated Second Harmonic Ultrasonic Backscatter for Determining Composition of Human Carotid Plaque

Russell Fedewa, Sheronica James, Heather Gornik, Sean Lyden, D. Geoffrey Vince
Biomedical Engineering, Cleveland Clinic, Cleveland, OH,
Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH
Vascular Medicine, Cleveland Clinic, Cleveland, OH

Carotid atherosclerotic plaque is an important cause of stroke. Current imaging approaches measure the extent of stenosis, while plaque composition is unavailable at the point of care outside of calcium burden provided by CT. The goal of this effort is to provide a robust non-invasive measure of plaque composition. The specific focus of this abstract is to determine if the nonlinearly generated second harmonic portion of the signal can aid the fundamental portion in determining plaque composition.

Statement of Contribution/Methods:

A pilot clinical study, enrolled 14 subjects and obtained pulse inversion RF backscatter prior to carotid endarterectomy using a Siemens S3000 with Axius

Direct software and a 9L4 probe. Transverse serial histology slices of the surgically removed plaque were prepared and matched to ultrasound frames.

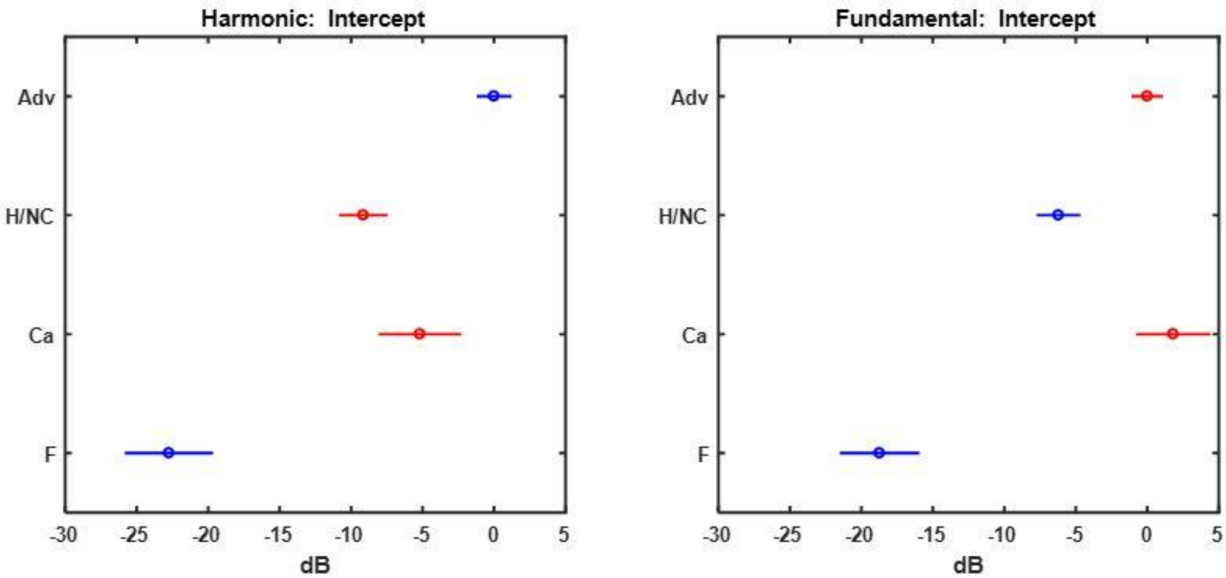
Homogenous regions of interest (ROI) were located within each stack of slides and placed into one of three categories (# of ROIs): Ca – calcium (20), F – fibrous (18), and H/NC – hemorrhagic and/or necrotic core (53). Ultrasound data was collected from adventitial (Adv) regions of six normal subjects to obtain 202

ROI's. Each ROI contained 15 pulse inversion pairs by 64 points (40 MHz) and corresponded to a 1.2mm square. The pulse inversion pairs were processed to obtain the harmonic and fundamental traces with 20dB bandwidths of 2.5 - 6.9

MHz and 4.9 - 10.1 MHz. Power spectrum for each ROI was obtained using a

Yule-Walker approach of order 20. The power spectra were normalized by data obtained from a uniform 0.5 dB/cm-MHz phantom. The estimate of the backscatter transfer function was adjusted by a depth and frequency dependent attenuation estimate derived from adventitial backscatter of normal subjects.

The figure displays Bonferroni adjusted 95% confidence intervals of the means of each tissue type for the fundamental (right) and harmonic (left) intercept linear fit parameter. Non-overlapping confidence intervals indicates that the parameters are significantly different. A significant difference for both bandwidths for the mean of F was found. The fundamental has the mean of H/NC being significantly different from other types, in contrast the harmonic has the mean of Adv being significantly different from other types. In conclusion, the harmonic parameters provide additional discriminatory information for plaque composition.



Harmonic: Intercept
Adv
H/NC
Ca
F
-30
-25
-20
-15
-10
-5
0
5
dB
Fundamental: Intercept
Adv
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Ca
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