AD______________

AWARD NUMBER: W81XWH-16-1-0608

TITLE: Vascular Plaque Determination for Stroke Risk Assessment

PRINCIPAL INVESTIGATOR: Vince, David Geoffrey

RECIPIENT: The Cleveland Clinic Foundation
Cleveland, OH 44195

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE Oct 2019	**2. REPORT TYPE** Annual	**3. DATES COVERED** 15 Sep 2018 - 14 Sep 2019

4. TITLE AND SUBTITLE
Vascular Plaque Determination for Stroke Risk Assessment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0608

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Vince, David Geoffrey
Fedewa, Russell J.

E-Mail: vinceg@ccf.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cleveland Clinic Foundation, The
9500 Euclid Ave
Cleveland, OH 44195-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Diabetic patients are at higher risk of atherosclerotic driven carotid stenosis and subsequent stroke. Point-of-care information regarding plaque composition is needed for all patients, especially diabetic patients. 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 81 CEA subjects, creation of software for producing composition overlay maps based on machine learning algorithms, streamlining the software for producing machine learning algorithms, demonstration of the benefit of the second harmonic, and the production of an algorithm based on data from the first 35 subjects.

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

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U	U	U	UU	25	USAMRMC **19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Diabetic patients are at higher risk of atherosclerotic driven carotid stenosis and exhibit riskier plaques for the same amount of blockage as compared to the non-diabetic population. Point-of-care information regarding plaque composition is needed for all patients, especially diabetic patients. In addition, diabetes and clinically relevant carotid stenosis occur in the veteran population at significantly higher rates as compared to the civilian population. 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 first step toward 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 excised tissue in order 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:** Provide a brief list of keywords (limit to 20 words).

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***
Major task 1 was completed prior to the effective date of the contract**. (Details in 2017 Annual Report)**

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. For Year 3, actual enrollment was 28 with a targeted enrollment of 33. (Under-enrollment of 19 subjects is discussed below.)

The subtasks and **third** year status are as follows:

- Consent Subjects (**goal 100%**): 81% complete
- Research Ultrasound Imaging: (**goal 100%**): 81% complete
- Collection of Plaque from Carotid Endarterectomy (**goal 100%**): 80% 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 2020. These tasks include the following:

- Prepare Serial Histology of Carotid Plaque — 67% complete
- Ultrasound Signal Processing Development and Testing — in process
- Match Slides to Ultrasound Grayscale *In Vivo* Data — 48% 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. Revised completion date as stated in no-cost extension is June 2020. Delay is primarily due to low enrollment.

Major Task 4: Create Statistical Classifiers
Initial statistical classifier creation achieved on August 2018 with a target of August 2018.
Milestone: Final statistical classifiers – original target Aug 2019, revised target of Aug 2020.

Major Task 5: Validation of Statistical Classifiers
Planned start Aug 2019 – Revised start as stated in No-cost extension Aug 2020.
Milestone: Validated final statistical classifiers – original target of Sep 2019 with revised target Sep 2020.following no-cost extension.

What was accomplished under these goals?

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
	Q9	**Q10**	**Q11**	**Q12**	**Q9***	**Q10***	**Q11***	**Q12***
Projected	*12*	*12*	*9*	*0*	***10***	***8***	***2***	***0***
Actual	**14**	**5**	**4**	**5**	TBD	TBD	TBD	TBD
Actual Cumulative	67	72	76	81	TBD	TBD	TBD	TBD

*****New projection for 12 month no-cost extension
TBD – To Be Determined

1. Clinical study enrollment
At the beginning of Year 3, clinical study enrollment rebounded as the newly hired surgeons became established and created a steady stream of subjects for the study. However, this rebound did not persist beyond Quarter 9 (see Table 1). This lull is ending as we prepare the current report with 10 to 15 scheduled surgeries at the Cleveland Clinic. We anticipate achieving over 90%

enrollment by the end of Quarter 13 (first quarter of the 12 month no-cost extension). We remain dedicated to approaching all patients scheduled for a carotid endarterectomy (CEA) until we reach 100 enrolled subjects. New target date to complete enrollment is April 2020 (start of Quarter 15).

2. Carotid Plaque Tissue Processing

Since enrollment was underperforming, we decided to work on analysis and processing of tissue collected during a phase of the clinical study prior to funding of this grant. This additional data will provide both training and test data for the final algorithm and will assist in development of the processing and classification code. We had collected carotid tissue and RF data in a similar fashion under an American Heart Association grant prior to the start of this research grant. Therefore, there were 20 additional data sets (histology and RF data) that required further processing in order to be used in algorithm construction. We pushed this data set through the following steps:

- serial histology
- matching histology to grayscale ultrasound
- location of homogenous zones within the matched histology (including nearest slides both proximal and distal to the match)
- Creation of regions of interest (ROI's) within the RF data based on the homogenous histology regions
- Inclusion of these ROI's with ROI's obtained from data captured under this grant. (See below for latest algorithms and corresponding confusion matrices).

The tissue processing of plaque collected in years 2 and 3 continued as well. As of 14 September 2019, slide sets were obtained for the first 67 subjects enrolled in the study (into Quarter 2 of Year 3). Matching has been completed for the first 48 enrolled subjects under this effort and ROI's have been obtained from homogenous regions for 35 of the subjects. The total number of ROI's now available for use in creation of statistical classifiers is 602. The three main categories are as follows with current number of ROI's listed (ROI's added in Year 3):

F: Fibrous/Fibro-Fatty: 88 ROI's (added 29)

H/NC: Hemorrhagic and/or Necrotic Core: 434 ROI's (added 133)

Ca: Calcium: 80 ROI's (added 15 ROI's)

3. Signal Processing Framework and Future Testing

Significant effort was dedicated to streamlining the coding framework within Matlab in order to insure reliable computations and to provide controlled flexibility regarding processing choices to prepare for the final algorithm production once we have obtained and processed all ROI sets. As part of the construction of the processing framework was the integration of tools able to draw lumen and vessel boundaries in order to segment the plaque and the creation of code capable of applying the classification algorithm to this segmented plaque in order to create a color overlay as shown in Figure 1.

The preparation efforts for the processing framework is in anticipation of testing the following processing choices once all homogenous ROI's are created:

- ROI size:
  - 0.6 mm by 0.6mm
  - 1.2 mm by 1.2 mm (15 lines by 64 points, current use)
  - 2.4 mm by 2.4 mm
- Attenuation Compensation (see abstract in appendix for more details):
  - 0.5 dB/cm-MHz (standard phantom)
  - One-Sep Attenuation (based on backscatter from healthy adventitia)
  - Two-Step Attenuation (based on backscatter from healthy adventitia)
- Auto-Regressive Order number (currently using 24 but will retest)

4. Signal Processing Investigations

Investigations into the use of harmonic data and attenuation compensation approaches continued.

Harmonics

In October of 2018, the harmonic data was presented at the 2018 IEEE International Ultrasonics Symposium and a proceedings paper was submitted (see appendix). In addition, the harmonic content was re-analyzed with additional ROI's in early 2019 and the results were accepted to both the 2019 Military Health System and Research Symposium (MHSRS) (abstract in the appendix) for a poster presentation and the 2019 IEEE International Ultrasonics Symposium (IUS) for an oral presentation (abstract in the appendix). The 2018 results demonstrated that harmonic parameters contain information that can aid in distinguishing between tissue types. The 2019 analysis used more data to back up the early 2018 analysis and created statistical classifiers along with color overlays to demonstrate some of the benefits of using the harmonic information. See section 5 below for discussion on the latest versions of the classification algorithm.

Attenuation

Early attenuation analysis was presented at the 176th Meeting of the Acoustical Society of America in November 2018. Additional analysis was performed in early 2019 with more data and no statistically significant results were found to distinguish between the different attenuation compensation approaches. The 2019 analysis was accepted for an oral presentation at the 2019 IEEE International Ultrasonics Symposium (abstract in the appendix). This 2019 analysis was performed on a very small data set and involved the use of random forest statistical classifier (in contrast to the 2018 presentation which simply looked at parameter statistics and found a significant benefit from using the 2-Step adventitial based compensation. Thus there could be significance found when analyzing the data using the latest (i.e. larger) data set.

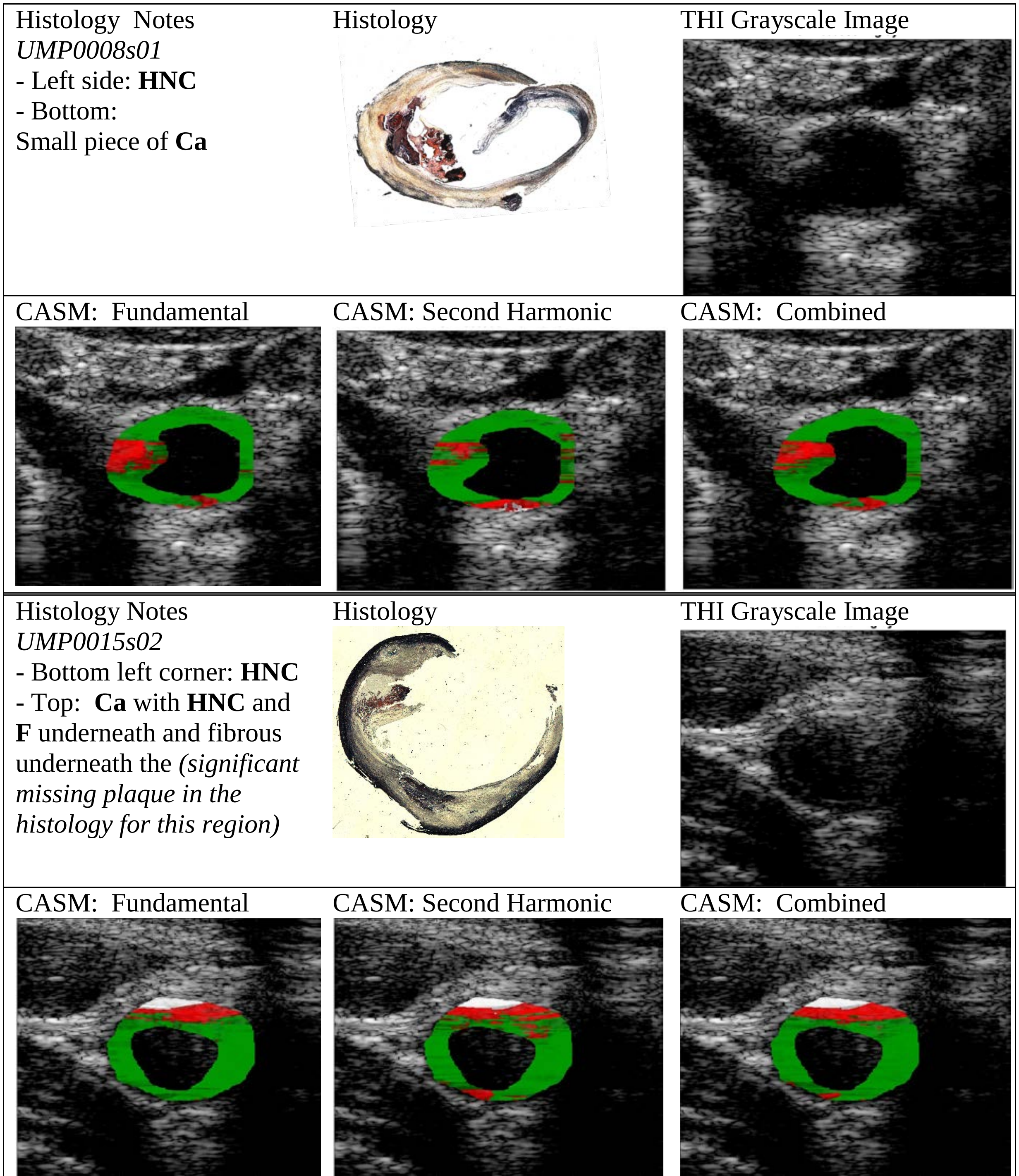

Figure 1: Two examples of a cover overlay depicting plaque composition. In both cases the Harmonic portion adds information that is not present in the Fundamental data. F-Green, Ca – White, HNC – Red. Transparency level represents confidence of classifier.

5. Early Application of Machine Learning Classification

In Year 3, we created a set of classifiers to compare Fundamental and Harmonic frequency bands. This data formed the basis of the Harmonic abstracts sent to the 2019 MHSRS and the 2019 IEEE IUS conferences. In Figure 5, we compare for two instances where the harmonic data appears to improve the sensitivity to calcium on the far all of the vessel in the upper set of images in Figure 5 (UMP0008s01) and locates the necrotic core on the far wall of the vessel in the lower set of images (UMP0015s02). This is the first use of the nonlinearly generated second harmonic portion of the backscattered signal for tissue characterization based on spectral analysis in the world. Key aspects that need improvement are that edges around calcium regions are often misclassified as hemorrhagic and/or necrotic core regions. We will be working to minimize this artifact through additional parameters and automated location of regions with low signal to noise. The goal is enable the avoidance of classifying regions where there is insufficient signal.

In order to obtain an understanding of the strengths and weakness of the current limited machine learning classification. We used the data set of ROI's obtained by the end of Year 3 to produce a Random Forest classifier. A total of 80 ROI's of each type were randomly chosen without replacement (chose to have balanced training and test sets): 57 of each type were used for training a random forest and 27 of each type were reserved for a test set. The random forest using parameters from both the Fundamental and Harmonic bandwidths was created and the test set was run with the results shown in the confusion matrix of Table 2. The predictive accuracy is 64%, which is low but not surprising for the following reasons:

- We are using a limited number of parameters for classification: integrated backscatter, mid-band fit, slope, intercept, maximum and minimum values and their respective frequencies. We will be adding classifiers throughout the next year that are more sensitive to local stricter rather than intensity of the backscattered ultrasound.
- We have not performed a thorough analysis of which parameters to keep as input parameters and using too many parameters can overfit the training data.
- The final comparison and most important is with histology and we are building a comparison set for use by pathology experts to truly see how well our machine learning algorithm performs.

Table 2 Confusion matrix using the test data for the random forest with 27 ROI's of each type used for testing. Predictive accuracy is 64%.

		Histology		
		F	**H/NC**	**Ca**
Predicted from Random Forest Classifier	**F**	23	10	2
	H/NC	3	11	7
	Ca	1	6	18

Stated Goals Not Met

The enrollment target was not met by the end of Year 3 and thus we applied for and obtained a no-cost extension. We have spent this year on testing the code framework for processing the ROI's, for creation of machine learning based classifiers, border drawing for plaque segmentation, and creation of color overlay for comparing to histology.

The under-enrollment led to missing the dependent milestones and as stated in the no-cost extension we have modified the timeline for completion of key items as follows:

1. Complete Enrollment into UMP Study: Month 7 of Year 4.
2. Tissue and Data Processing: Month 9 of Year 4.
3. Creation and testing of statistical classifiers: Month 11 of Year 4.
4. Final validation based on reserved data and prepare publications: Month 12 of Year 4.

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

We submitted abstracts to two professional conferences and the abstracts were accepted. These included:

- The 2019 Military Health System Research Symposium in August 2019: Russell Fedewa attended and presented a poster at this conference.
- The 2019 IEEE International Ultrasonics Symposium in October 2019: Russell Fedewa and Sheronica James both submitted abstracts which were accepted for oral presentations.

Hired a summer intern, Anna Phillips, and trained her in the use of signal processing and basic science skills.

How were the results disseminated to communities of interest?

Abstract was submitted and a poster presented at the following conference (abstract is located in the appendix):

2019 Military Health System Research Symposium, Orlando, Florida, 19-22 August 2019. "Non-invasive Measurement Of Carotid Plaque Composition To Improve Risk Assessment Of Stroke", AUTHORS: R. Fedewa, S.L. James, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, Ohio.

Abstracts were submitted and accepted to the following conference for oral presentations (abstracts located in the Appendices): 2019 IEEE International Ultrasonics Symposium, Glasgow, UK, 6-9 October 2019.

1. "Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque", AUTHORS: R. Fedewa, S.L. James, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, Ohio.

2. "Attenuation Compensation Comparison for Human Carotid Plaque Characterization Using Spectral Analysis of Backscattered Ultrasound", AUTHORS: S.L. James, R. Fedewa, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, Ohio.

Abstract was submitted and Sheronica James presented the research (abstract was submitted in the 2018 Annual Report Appendix):

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.

Proceedings paper was submitted for the following conference (paper located in the Appendix, abstract was submitted in the 2018 Annual Report):

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?

Complete Study Enrollment

The team will continue to approach all patients scheduled for a carotid endarterectomy for enrollment. New target for completion is April 2020 with 19 subjects needed to complete enrollment as of 14 September 2019.

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 additional spectral parameters that are more sensitive to scatterer size and arrangement as compared to pure intensity.
3. Investigate ratios of parameters obtained from fundamental and second harmonic bands
4. Implementation of support vector machines (SVM), including cross validation within the training set to determine the best kernel to implement. *(Delayed due to low enrollment.)*
5. Determination of the correlation between the different parameters and weed out parameters that effectively duplicate others and remove parameters that are of low importance for classification. *(Delayed due to low enrollment.)*
6. Re-test ROI size, auto-regressive order, and attenuation compensation based on color overlay output.

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 (Delayed due to low enrollment.)

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?

A few items of impact:

1. The backscatter of the nonlinearly generated second harmonic appears more sensitive to smaller inclusions along the far side of the plaque, while the fundamental bandwidth appears to have better penetration through highly attenuating plaque.
2. This work is the first use of the nonlinearly generated second harmonic for tissue characterization based on spectral analysis of ultrasonic backscattered signals.
3. Further analysis of the attenuation compensation demonstrates that, statistically, the benefit from the adventitial based attenuation compensation approaches are small at best and color-overlay comparison to histology will be needed to determine to what extent they are beneficial.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

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 rebounded in Quarter 1 of Year 3 (Quarter 9) for our best enrollment numbers of the study but then declined for the remainder of Year 3. We have noted another rebounding in Quarter 1 of Year 4 (Quarter 13) but at this point anticipate another declined come Quarter 2 of Year 4 (Quarter 14). Even with a decline in enrollment following Quarter 13, we anticipate having less than 10 to enroll in 2020 (based on recent enrollment and scheduled surgeries at the time of preparation for this report).

All other delayed actions were a response to the low enrollment.

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

Dr. Heather Gornik left the Cleveland Clinic in the f+all of 2018 and was not replaced and thus her salary expenditure ceased at this time (duties were assumed by other personnel already involved in the study which is discussed in Section 7).

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 - 2019 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:

Journal publications.

Nothing to Report

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

Nothing to Report

Other publications, conference papers and presentations.

Presentation (poster) abstract is attached as an Appendix
2019 Military Health System Research Symposium, Orlando, Florida, 19-22 August 2019. “Non-invasive Measurement Of Carotid Plaque Composition To Improve Risk Assessment Of Stroke”, AUTHORS: R. Fedewa, S.L. James, D. Vince, Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio; S. Lyden, Vascular Surgery, Cleveland Clinic, Cleveland, Ohio.

Presentation (oral): abstract was attached to the 2018 Annual Report as an Appendix
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.

*Presentation (poster) with Proceedings Paper: paper located in the Appendix
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.

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

Nothing to Report

- **Technologies or techniques**

1. Development and demonstration of using the nonlinearly generated second harmonic for tissue characterization. (see Appendix: 2019 IEEE IUS Proceedings Paper, *Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque*)
2. Testing of technique for attenuation compensation of backscatter from carotid arteries and tissues. (see Appendix: 2019 IEEE IUS Proceedings Paper, *Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque*)

Both of these technologies are to be presented at the IEEE International Ultrasonics Symposium in Glasgow, Scotland on 7 Oct 2019 (proceedings papers were submitted in September 2019).

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

Nothing to Report

- **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. As a result of the no-cost extension of 1 year, the deadline for uploading this data is also extended by one year by 1 Aug 2021.

In vivo data from 81 human carotid plaques and the surrounding tissue have been collected and archived internally in the Lerner Research Institute at the Cleveland Clinic. In addition, RF data has been collected from 6 normal human subjects. This data comprises the following:

- Reference Phantom Data for Normalizing the collected RF data
- 279 Static Sites:
  - *in vivo* RF ultrasound backscattered signal from human carotid plaque with an approximate separation of 1 cm to insure independence.
  - 10 frames of data per file
    - Each Frame: 456 lines by 2076 points at 40 MHz.
    - Collected using pulse inversion (thus both fundamental and harmonic data are included)
- 81 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 No Change
Name:	Russell J. Fedewa No Change
Name:	Sheronica James No Change
Name:	Anna Phillips
Project Role:	Summer Intern
Research Identifier (e.g. ORCID ID):	NA
Nearest Person Month Worked:	2
Contribution to Project:	Signal processing support and extension of spectral analysis approach.
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?

Heather Gornik left the Cleveland Clinic in November 2018. The study has carried on in her absence without a replacement. Her key task of training and monitoring the sonographers has fallen to the lead sonographer for the study Alia Grattan and has been minimal since we have not had any new sonographers join the study and do not anticipate the need for additional sonographers to complete the study. To replace her clinical expertise we are relying on the other key personnel, specifically Sean Lyden who is the chair of the Department of Vascular surgery at the Cleveland Clinic.

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

8. SPECIAL REPORTING REQUIREMENTS

NA

9. APPENDICES:

- **Abstract**: 2019 Military Health System Research Symposium, “Non-invasive Measurement Of Carotid Plaque Composition To Improve Risk Assessment Of Stroke”
- **Abstract**: 2019 IEEE International Ultrasonics Symposium, “Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque”
- **Abstract**: 2019 IEEE International Ultrasonics Symposium, “Attenuation Compensation Comparison for Human Carotid Plaque Characterization Using Spectral Analysis of Backscattered Ultrasound”
- **Proceedings Paper:** 2018 IEEE International Ultrasonics Symposium: “Nonlinearly Generated Second Harmonic Ultrasonic Backscatter for Determining Composition of Human Carotid Plaque”

Abstract for 2019 Military Health System Research Symposium: August 19-22
Non-Invasive Measurement of Carotid Plaque Composition to Improve Risk Assessment of Stroke

Russell Fedewa, PhD[1], Sheronica James, PhD[1], Sean Lyden, MD[1], D. Geoffrey Vince, PhD[1]
[1] Cleveland Clinic Foundation, Cleveland, OH

Breakout Session: Predictive Analytics within the Military Health System
Learning Objective 1: Describe ultrasound tissue characterization and how it can be used to find unstable carotid artery plaque before a stroke occurs.
Learning Objective 2: Discuss the approaches of improving predictive analytics for ischemic stroke and how ultrasound provides a cost-effective tool for this effort.
Learning Objective 3: Examine the efficacy of ultrasound tissue characterization for non-invasively measuring plaque composition.

Abstract: (8000 character limit)

Carotid artery plaque composition is a known predictor of future cardiovascular accidents (ie. stroke), but knowledge of plaque composition is generally unavailable to clinicians at the point of care. Currently, risk assessment of future stroke from atherosclerotic carotid stenosis for an asymptomatic population is based largely on the degree of stenosis but numerous studies have shown that ischemic strokes often arise from only carotid arteries with only a moderate degree of stenosis. A non-invasive and relatively inexpensive clinical tool is needed to improve risk assessment of carotid arteries by providing a measure of plaque composition. Current approaches attempting to provide this information include magnetic resonance imaging (MRI), computed tomographic angiography (CTA), and ultrasound. Key components of carotid plaque that are associated with an increased risk of future stroke are the presence of ulcerations and hemorrhage within the plaque or the presence of necrotic core tissue comprising a significant portion of the plaque tissue. If these elements are present being able to measure the thickness of the fibrous cap will aid the risk assessment. State-of-the-art MRI devices allow the characterization of fibrous cap and intraplaque lipid pools and also offer the ability to visualize intraplaque hemorrhage; however, this technique is expensive and requires intense operator input to obtain accurate information. CTA shines are determining calcium within the plaque but struggles to distinguish between soft tissue types and involves the use of ionizing radiation. Both CTA and MRI are significantly more expensive than ultrasound. Ultrasound efforts to date have utilized four main approaches: grayscale imaging, acoustic radiation force impulse (ARFI), photo-acoustic, and spectral analysis. Grayscale imaging is insufficient to distinguish between hemorrhage and calcium. ARFI approach performs well when the ultrasound beam is relatively perpendicular to the artery wall (top and bottom of the vessel for a standard duplex ultrasound image), but currently struggles when deriving composition from the sides. Photo-acoustic has a limited depth penetration that currently requires an approach from inside the vessel. Spectral analysis of ultrasonic backscatter encompasses all the tissue information contained in grayscale image and can be implemented in a non-invasive approach that is able to estimate the plaque composition from all parts of the plaque. All of these ultrasound approaches are significantly cheaper than MRI or CTA and the spectral analysis approach can be implemented within current ultrasound imaging systems and probes with changes to the software to permit further analysis of the radiofrequency (RF) raw backscattered signals (same ones used to create the grayscale image).

The long-term goal of this research effort is to develop non-invasive methods utilizing ultrasound to identify carotid plaques at high risk of stroke. To accomplish this goal we have

created a statistical classifier using machine learning. Data were collected from patients who were scheduled for a carotid endarterectomy in response to significant carotid stenosis with or without symptoms. We obtained measurements of the RF ultrasound backscattered signals from the carotid plaque prior to surgery using a Siemens S3000 with 9L4 probe. This is a standard clinical imaging system with the addition of a piece of software that saves the post-beamformed RF data. Following surgery, we obtained the excised tissue. Serial histology was performed on the plaque tissue and key frames of ultrasound data are matched to specific histology slides. Then regions of homogenous tissue were located in the histology and comparable regions of interest (ROIs) were located within the matching grayscale image and the corresponding RF data is obtained. A spectral estimate of the RF data was obtained and the affects from attenuation, diffraction, and the ultrasound system were removed to isolate the effects from scattering which is dependent on scatterer size, distribution, composition, and density. Parameters from each of these ROIs are extracted from this modified power spectrum to use as inputs for machine learning. Since each ROI represents data from a homogenous plaque type, this set of data (spectral parameters and histology reading) form the training and testing data sets for creating the CASM based on a random forest statistical classifier.

Two distinct frequency bands were investigated: fundamental band (2.5 MHz to 6.9 MHz) and nonlinearly generated second harmonic band (5.1 MHz to 10.1 MHz). The following parameters were extracted from each frequency band: integrated backscatter (average intensity), and the linear fit parameters from fitting a line across the usable bandwidth (intercept, slope, and mid-band fit). Three random forests were created: 1. Fundamental data only, 2. Harmonic data only, and 3. Both Fundamental and Harmonic. The predictive accuracy of the three were 90%, 92% and 93% respectively when classifying the tissue into three types. The three tissue types are: Calcium (Ca); Fibrous/Fibro-Fatty (F); Hemorrhagic and/or Necrotic Core (HNC). The random forests were built using 48 ROIs for each tissue type (144 ROIs total) and these were obtained from 45 human subjects. The random forest was implanted as a regression analysis in order to provide a measure of the confidence for the given classification when creating an image overlay. During the construction of each classifier the random forest computes an out-of-bag error: the fundamental based forest had an OOB of 0.31, the harmonic based forest had an OOB of 0.28, and the combined forest had an OOB of 0.26. We had expected the combined to be better but were a bit surprised by the performance of the nonlinearly generated harmonic based classification algorithm. Potential benefits of the harmonic backscatter: reduced clutter within the vessel and plaque, higher frequency band, wider bandwidth. However, the signal to noise ratio is greatly reduced for the harmonic data and we expect harmonic data to have a greater variance in classification since the amount of second harmonic signal that is present at the time of scattering is heavily affected by the attenuation, structure and composition of the layers of tissue between the skin and carotid artery. Graphic overlays were created to visualize the plaque composition. Since HNC is associated with increased risk of plaque, the sensitivity and specificity for the algorithm using both the fundamental and harmonic input parameters is computed for HNC. The specificity for HNC is 97% and the sensitivity is 85%.

In conclusion, using the nonlinearly generated second harmonic backscattered signal provides a noticeable improvement with the statistical classifier. The combined statistical classifier can accurately reproduce the carotid plaque composition. The final algorithm can be implemented on a standard ultrasound system and displayed to sonographers as a color overlay on the grayscale image. Next step is a clinical study to connect the plaque composition features to future stroke or transient-ischemic attack.

Abstract: **2019 IEEE International Ultrasonics Symposium**

Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque

Russell Fedewa[1], Sheronica James[1], Sean Lyden[1], D. Geoffrey Vince[1]
[1]Cleveland Clinic, Cleveland, United States,

Background, Motivation and Objective

Current state-of-the art clinical care for carotid stenosis relies on measuring the severity of the arterial narrowing; physicians use this measurement to determine clinical course of action to reduce the risk of a cerebrovascular accident (CVA). Unfortunately, this measure of stenosis does not provide sufficient information to adequately determine the risk for all patients, especially for asymptomatic patients. The objective of this research is to develop a non-invasive approach for determining the composition of carotid plaque in order to improve risk stratification of patients with carotid stenosis.

Statement of Contribution/Methods

Human carotid plaque is collected following carotid endarterectomy from 45 subjects. The plaque is processed using serial histology and matched to grayscale images created from RF data collected in vivo prior to surgery. Next regions of homogenous tissue (F-fibrous, HNC – hemorrhagic and/or necrotic core, Ca – calcium) are determined based on the histology and corresponding regions of interest (ROI's) are located within the grayscale image and the corresponding RF is extracted. This RF data is processed by computing an average power spectrum (15 lines by 64 points) over a 1.2 mm by 1.2 mm region and normalizing using a reference phantom with a 0.5 dB /cm-MHz attenuating phantom. An additional attenuation compensation is applied based on backscatter from healthy volunteer adventitia as a function of depth and frequency. A pulse inversion implementation on a Siemens S3000 ultrasound system with a 9L4 transducer was used to collect all data. The bandwidths for the fundamental and harmonic are 2.5 to 6.9 MHz and 4.9 to 10.1 MHz respectively. Random forests were created using four parameters: integrated backscattered and linear fit parameters obtained within the respective bandwidths. A total of 48 ROI's of each type were used to train each forest of size 50.

Results/Discussion

Figure 1 demonstrates the images for obtained using the harmonic and fundamental bands. The out of bag error is 0.38, 0.35, and 0.33 and predictive accuracy is 87%, 85%, and 91% for forests based on fundamental, harmonic, and combined input parameters. Backscatter from the harmonic data improves plaque characterization beyond the fundamental.

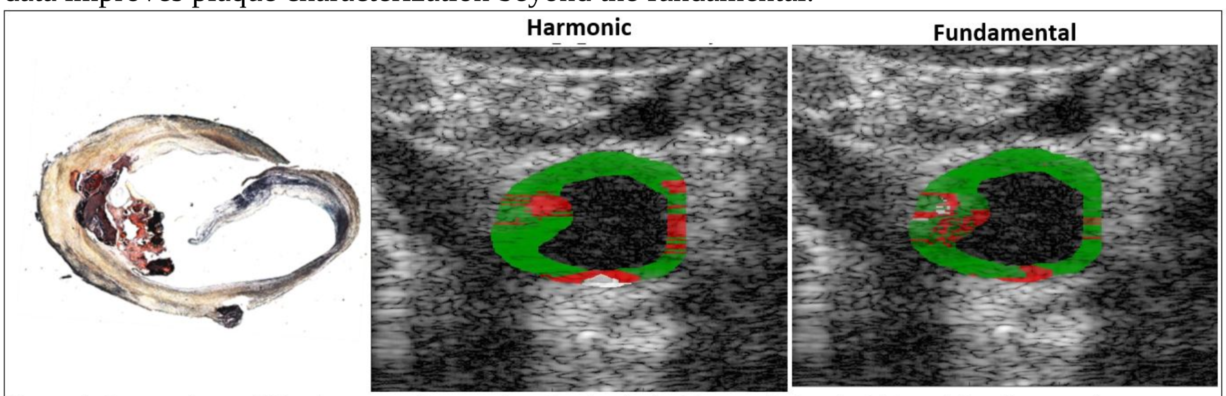

Figure 1: Comparison of histology to color overlays for both the Harmonic bandwidth and Fundamental bandwidth. There is significant hemorrhage on the right side of the plaque and a piece of calcium on the bottom. Color code: Green – F, White – Ca, Red – HNC.

Abstract: **2019 IEEE International Ultrasonics Symposium**

Attenuation compensation comparison for human carotid plaque characterization using spectral analysis of backscattered ultrasound

Sheronica James1, Russell Fedewa1, Sean Lyden1, D. Geoffrey Vince1,
1Cleveland Clinic, Cleveland, United States

Background, Motivation and Objective

Carotid atherosclerotic plaque composition has been suggested as a valuable predictor of stroke risk. Ultrasound spectral analysis has been successfully implemented clinically for determining plaque composition in coronary arteries via intravascular ultrasound. Shifting to noninvasive implementation of such techniques for carotid plaque requires attenuation compensation for the effects of overlying tissue. This study examines the effects of four attenuation compensation techniques on the accuracy of a carotid plaque classification system using spectral analysis and random forest classification.

Statement of Contribution/Methods

Radiofrequency (RF) data was acquired from 42 subjects prior to carotid endarterectomy (CEA) using a Siemens S3000 with Axius Direct software and a 9L4 probe. Histology slices of the excised plaque were prepared following surgery and matched to ultrasound frames. Regions of interest (ROI) were selected from homogenous regions within the slide stack and matched to corresponding ROI's in the RF data. ROI's were categorized as F - fibrous (n = 41), H/NC - hemorrhagic and/or necrotic core (n = 60), or Ca - calcium (n = 54). Additionally, 219 ROI's were obtained from the adventitia (Adv) of six normal subjects. Power spectra for the ROI's were computed and normalized to a uniform phantom. Four attenuation compensation methods were applied to the spectra: (1) 0.5 dB/cm-MHz phantom; (2) optimum power spectral shift estimator (OPSSE); (3) 1-step and (4) 2-step normalized backscatter from adventitia. A linear fit of the resulting estimated backscatter transfer functions (*eBTF*) was performed over the fundamental bandwidth of 2.5 – 6.9 MHz. Eight spectral parameters were used to build the random forest classification models.

Results/Discussion

Table 1 shows the Kappa statistic and accuracy, sensitivity and specificity of each attenuation compensation method in predicating H/NC, which is associated with increased stroke risk. The 2-step adventitia-based compensation produced the best statistical classifier, with 80% accuracy, 85% sensitivity, 77% specificity, and a Kappa statistic of 0.70, indicating moderate to high level of agreement with histology. Thus, the 2-step adventitia-based attenuation compensation shows considerable potential for accurately determining carotid plaque composition using a standard ultrasound system.

Attenuation Compensation Method	**Kappa statistic**	**H/NC predictive accuracy**	**H/NC Sensitivity**		**H/NC Specificity**	
			%	**CI**	**%**	**CI**
0.5 dB/cm-MHz phantom	0.49	67%	55%	33-77	74%	59-90
OPSSE	0.52	69%	65%	44-86	71%	55-87
1-step adventitia-based	0.55	71%	60%	39-81	77%	63-92
2-step adventitia-based	0.70	80%	85%	69-100	77%	63-92

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

Russell Fedewa
Biomedical Engineering
Cleveland Clinic
Cleveland, USA
fedewar@ccf.org

Sheronica James
Biomedical Engineering
Cleveland Clinic
Cleveland, USA
jamess4@ccf.org

Heather Gornik
Cardiovascular Medicine
Cleveland Clinic
Cleveland, USA
gornikh@ccf.org

Sean Lyden
Vascular Surgery
Cleveland Clinic
Cleveland, USA
lydens@ccf.org

D. Geoffrey Vince
Biomedical Engineering
Cleveland Clinic
Cleveland, USA
vinceg@ccf.org

***Abstract*—Carotid atherosclerotic plaque is a leading 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 data obtained from the fundamental portion of the backscatter to determine plaque composition. A pilot clinical study, obtained pulse inversion backscatter from 14 subjects prior to carotid endarterectomy. The corresponding plaque was obtained following surgery and histologically processed. The pulse inversion pairs were processed to obtain the harmonic and fundamental traces with 20dB bandwidths. Power spectrum for each ROI was obtained and normalized by data obtained from a uniform 0.5 dB/cm-MHz phantom and additional attenuation compensation. The results indicate that the harmonic parameters provide additional discriminatory information for plaque composition that is not contained within the fundamental data.**

Keywords—ultrasound, carotid artery, atherosclerosis, tissue characterization, second harmonic

I. Introduction

Stroke is a major cause of morbidity and mortality among patients with cardiovascular disease and a major cause of long-term disability in the United States [1, 2]. A leading cause of ischemic stroke is the growth of atherosclerotic plaque within the carotid artery [3, 4]. Current imaging approaches including duplex ultrasound, computed tomography (CT), and magnetic resonance angiography (MRA) provide reliable measurements of the degree of stenosis which is a key input measurement for the risk assessment of patients. However, a number of studies have demonstrated that a large number of cerebrovascular accidents are caused by rupture-prone atheromas that cause only a moderate degree of luminal stenosis within the carotid artery [5, 6]. Thus knowledge of plaque composition is needed in order to improve patient risk assessment and subsequent care.

Multiple modalities are able to provide limited composition information. Non-contrast CT and CT angiography (CTA) are capable of quantifying calcium burden, plaque ulceration, and presence of lipid in the plaque, however CT approaches are limited for further composition segmentation and they use ionizing radiation [7]. Magnetic resonance angiography (MRA) [8] and intravascular ultrasound (IVUS) employing Virtual Histology (VH-IVUS) technology [9-11] can be used for assessment of plaque composition. A number of acoustic based approaches are currently being investigated and include acoustic radiation force impulse (ARFI), acoustic attenuation, and spectral analysis approaches. These approaches by themselves or potentially combined via machine learning could provide a point of care tool for monitoring carotid stenosis in a non-invasive and cost-effective manner.

In this report, we expand on standard fundamental spectral analysis to examine backscatter from the nonlinearly generated second harmonic. Applying spectral analysis approaches to the backscatter of the nonlinearly generated second harmonic has been applied previously to monitor liver ablations which benefits from the nonlinear generation arising from scattering off bubbles created during the ablation procedure [12]. This is in contrast to the current effect where tissue ablation is not involved. 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.

This work was supported by American Heart Association SDG Award 15SDG25700258 and the Office of the Assistant Secretary of Defense and Health Affairs, through the Peer Reviewed Medical Research Program under Award No. W81XWH-16-1-0608. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense, U.S. Army, or American Heart Association.

Ultrasound imaging system loaned by Siemens Medical Solutions USA, Inc.

II. Methods

A. Human Data Acquisition and

Recruitment involved two distinct groups of subjects: normal group and a group of patients scheduled for a carotid endarterectomy (CEA group). Both groups were limited to people over the age of 40 since stenosis in younger patients is often due to other processes besides atherosclerosis. The CEA group was limited to patients having surgery for atherosclerotic stenosis. The plaque location is required to be within the range from the distal end of the common carotid artery through the internal carotid artery including the common carotid artery bulb. In addition, patients with prior ipsilateral carotid surgery or stent placement are excluded. The normal group is comprised of subjects who have not had and there is no plan to have a carotid artery intervention.

A total of 14 subjects were enrolled into the CEA group after being scheduled for surgery. This group includes both asymptomatic (10) and symptomatic (4) subjects of which three had trans-ischemic attacks and two experienced aphasia. The enrolled CEA group includes 10 male and 4 female subjects. The volunteers for the normal group include three female subjects and three male subjects. The normal group is used for determining an attenuation compensation algorithm.

Each of the CEA group received a research ultrasound exam not more than 30 days prior to surgery. This research ultrasound exam is performed using a 9L4 probe with a Siemens S3000 Ultrasound System with Axius Direct software (Siemens Medical Solutions USA, Inc., Malvern, PA). The Axius Direct software package enables the acquisition of received RF signals post beamformation and prior to further image processing. The time gain compensation (TGC) and overall gain are applied prior to the point at which the RF is saved and thus the gain and TGC settings are controlled during data acquisition. Additional settings are maintained constant as well to insure consistent data collection and include the following: pulse inversion, nominal 9.00 MHz transmit frequency, 4cm depth, 2cm focus, 456 lines per frame, 2076 RF data points per line, TGC pushed all right, and gain set to -10dB.

The RF data collection procedure begins with the sonographer determining the extent of the plaque and placing the transducer at the proximal end of the plaque in a transverse orientation with respect to the carotid artery. Pulse inversion ultrasonic backscatter is collected from this site while holding the probe still (grayscale image shown in Upper Left of Fig. 1). The probe is moved approximately 1 cm in the distal direction and RF data is acquired again. This is repeated until the distal end of the plaque is reached. Then the transducer is slowly swept from proximal to distal ends of the plaque in order to capture the plaque in between these sites and provide better understanding of how the plaque is changing as a function of distance. The 1cm step between sites ensures independent plaque regions for analysis and aids in histology processing.

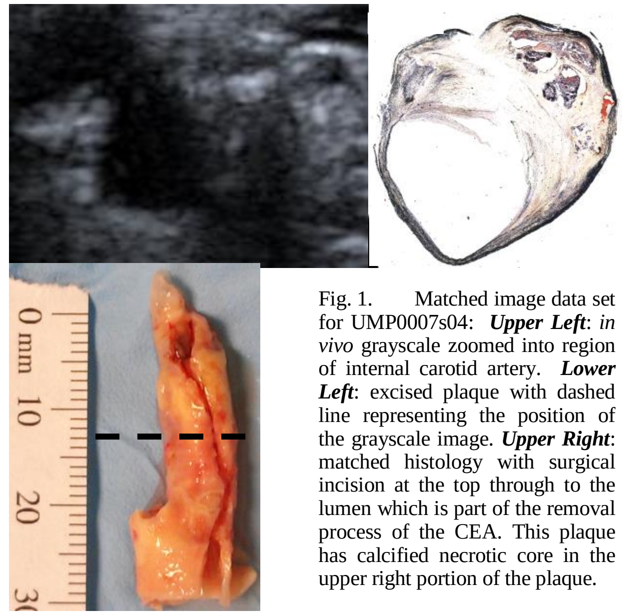

Fig. 1. Matched image data set for UMP0007s04: ***Upper Left***: *in vivo* grayscale zoomed into region of internal carotid artery. ***Lower Left***: excised plaque with dashed line representing the position of the grayscale image. ***Upper Right***: matched histology with surgical incision at the top through to the lumen which is part of the removal process of the CEA. This plaque has calcified necrotic core in the upper right portion of the plaque.

In addition to the ultrasound RF data, the plaque was obtained following excision during the CEA with a sample shown in the Lower Left image of Fig. 1. Within 12 hours from the excision, the plaque was imaged in saline and RF ultrasound data was collected at the *in vivo* site. Not all *in vivo* sites are found since a portion of the excised plaque is often sent to clinical pathology, the site did not have plaque removed, or the confidence in the match is too poor to trust. The plaque is then fixed in 10% buffered formalin and de-calcified (Cal-Rite, Richard-Allan Scientific, Kalamazoo, MI). The plaque is marked at each of the *in vivo* sites prior to paraffin embedding and cut to obtain two slides at 1mm steps prior to the marked site and 0.5mm steps through the marked region with 1 mm steps after the marked region.

The slide that best matches the ultrasound data from each *in vivo* site is found and a sample of the ultrasound data, histology, and plaque sample that form the basis of our carotid plaque study are shown in Fig. 1. Then regions of homogenous plaque tissue types are located within this matched slide and the slides proximal and distal to the matched slide. Based on these three-dimensional homogenous regions, regions of interest (ROI's) are drawn using a customized tool in the Matlab environment (MathWorks, Inc., Natick, MA). This tool provides the location of the RF data corresponding to the location of the ROI drawn on the grayscale image. Each ROI is drawn to be 64 RF data points by 15 lines (1.2 mm by 1.2 mm). Each of these ROI's corresponds to a homogenous region representing one of the following tissue types:

- F – Fibrous or Fibro-Fatty
- H/NC – Hemorrhagic and/or Necrotic Core with or without micro-calcifications
- Ca – Calcified plaque
- Adv – Adventitia (obtained from the normal group)

All normal subjects receive a research ultrasound exam for both carotid arteries with five sites per artery centered around the carotid bulb just prior to the bifurcation into the internal and external carotid arteries. These data are collected to obtain adventitial scattering data at varying depths in order to perform attenuation compensation.

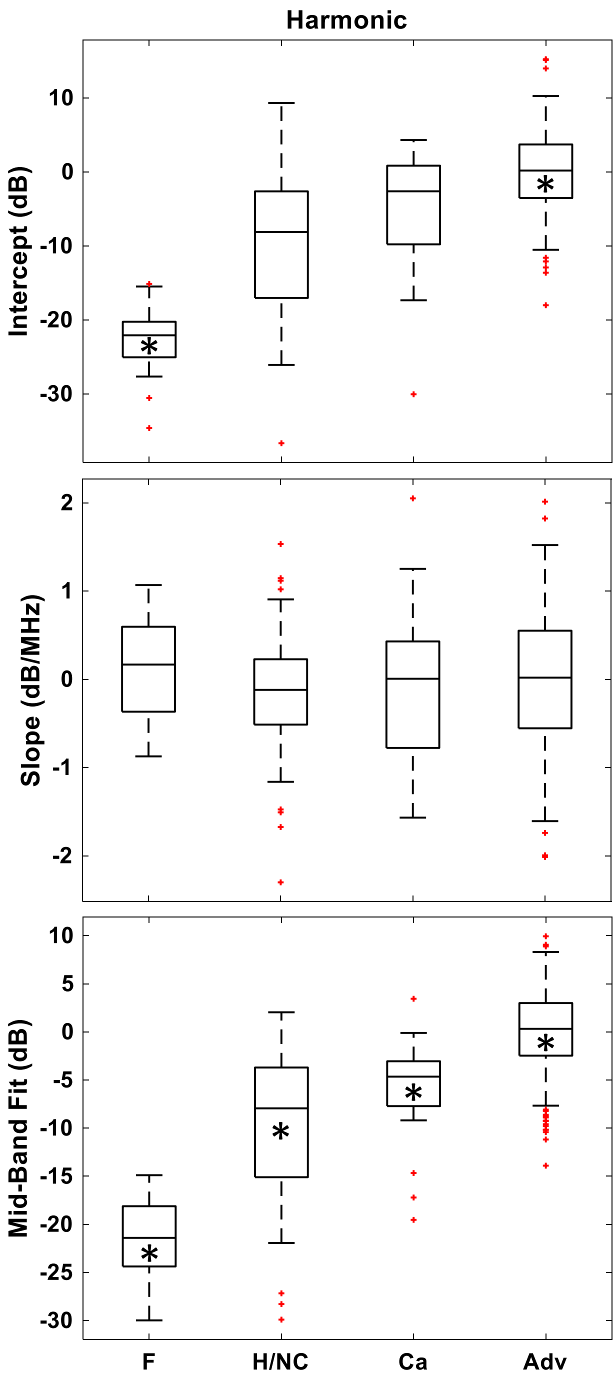

Fig 2. Boxplots depicting the linear fit parameters from the *eBTF* of the harmonic spectrum. The box spans from the 25th to the 75th percentile. Asterisk (*****) denotes that the mean of the tissue type is significantly different than the means of the other three types.

B. *Signal and Spectral Processing*

The RF data is processed in order to obtain an estimate of the backscatter transfer function (*eBTF*). The first step is the formation of the fundamental and harmonic signals through finding the difference or summation of the pulse inversion RF signals respectively. Based on prior work [13], a Yule-Walker autoregressive spectral estimation algorithm was chosen and applied to each 64 point long line within the ROI's. These spectra are then log-averaged to compute the average power spectrum for each ROI in units of dB (PS_{ROI}).

A reference phantom approach is then applied to each of the fundamental and harmonic ROI power spectra in order to account for diffraction, transmit and receive effects, and attenuation from the overlying layers of tissue. The reference spectrum is obtained from ultrasound backscattered from a uniform region of a tissue mimicking phantom (CIRS Model 044, Norfolk, VA) with a 0.5 dB/cm-MHz attenuation. The reference data is obtained by moving the transducer over the surface of the phantom to insonify different scatterer arrangements for each frame. The RF data from the same depth as each ROI extracted from each line within 10 frames. Each of these lines is processed using the same spectral estimation approach used to process the ROI data. The ROI power spectrum is then normalized by the average reference phantom power spectrum (PS_{ref}).

Attenuation compensation, in addition to the 0.5 dB/cm-MHz attenuation of the phantom, was applied using the data from the 202 ROI's at varying depths were obtained from the 6 normal subjects. The RF data corresponding to these adventitial ROI's was processed and normalized by the reference phantom. Then a line was fit to each frequency bin within the bandwidth as a function of depth. For all processing 1024 points were used with a 40MS/sec digitization and thus each bin was approximately 39 kHz. This results in a slope (m) and intercept (b) for each frequency in order to provide compensation for attenuation. Thus the *eBTF* for both the harmonic and fundamental bands is computed as follows:

$$eBTF(f) = PS_{ROI}(f) - PS_{ref}(f) - [\, m(f)*d + b(f)\,] \qquad (1)$$

where, d, is the distance to the center of the ROI. The attenuation correction is implemented. The linear fit to the *eBTF* is then obtained for both fundamental and harmonic bands.

III. Spectral Analysis Results

A linear fit to the *eBTF* over the 20dB bandwidth was performed for each of the ROI's (number): Ca (20), F (18), H/NC (53), and Adv (202). The fundamental bandwidth is 2.5 - 6.9 MHz and the nonlinearly generated second harmonic bandwidth is 4.9 - 10.1 MHz. Fig. 2 displays the results for the harmonic bandwidth and demonstrates that for the slope parameter there is no significant difference between the means. For the intercept the fibrous (F) and adventitia (Adv) means are significantly different from the others and all the means are distinguishable for the mid-band fit. In Fig. 3, the results for the fundamental are shown and demonstrate similar but not identical results. The intercept means are significantly different for the fibrous and hemorrhagic/necrotic core (HNC) and slope for the mean of the calcium (Ca) is distinct. For the mid-band fit all means are significantly different. attenuation compensation derived from adventitial backscatter of normal subjects.

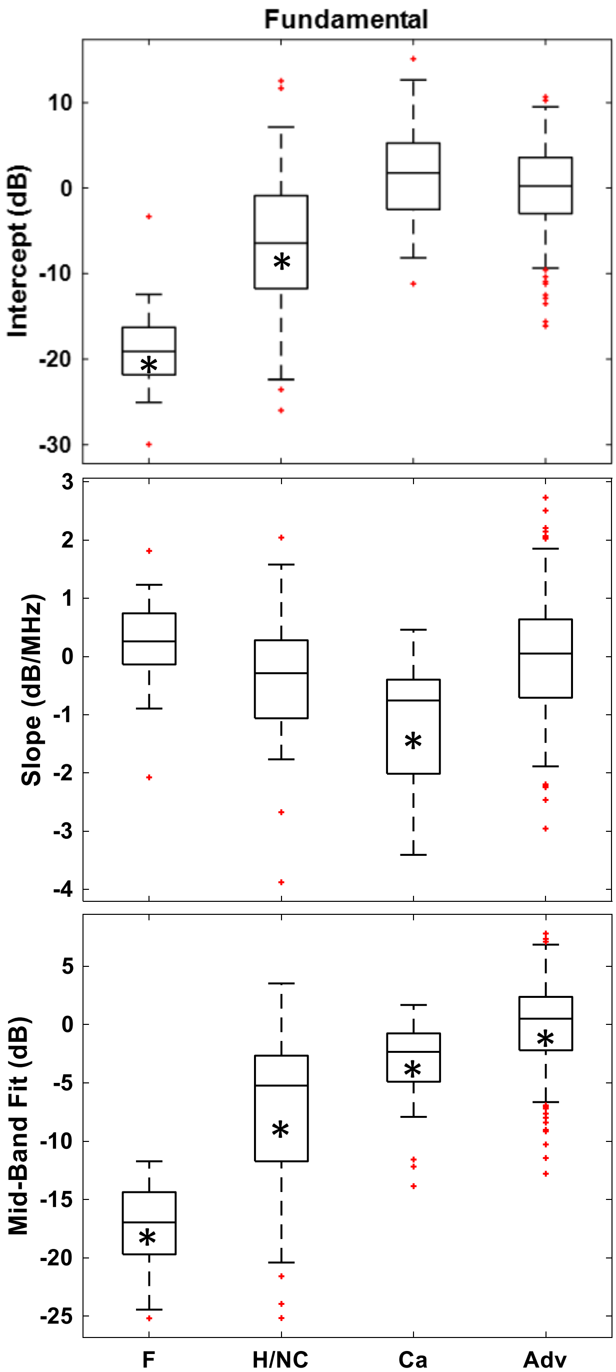

Fig 2. Boxplots depicting the linear fit parameters from the *eBTF* of the fundamental spectrum. Asterisk (*****) denotes that the mean of the tissue type is significantly different than the means of the other three types.

IV. Conclusion and Discussion

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 in Fig. 2 and Fig. 3 are all computed relative to adventitia since it was used as a baseline for attenuation compensation.

Acknowledgment

The authors thank the skilled contributions provided by the sonographers within the Heart and Vascular Institute Vascular Laboratory at the Cleveland Clinic: Alia Grattan, Kate McDonald, Stefanie Beckner, Anyi Liu, Dmitriy Mitsyuk, Susan Whitelaw, and Tammy Rubin. Study data were collected and managed using REDCap electronic data capture tools hosted at Cleveland Clinic.

References

[1] Centers for Disease Control and Prevention (CDC), "Prevalence and most common causes of disability among adults-United States, 2005," *MMWR Morb Mortal Wkly Rep,* vol. 58, pp. 421-426, 2009.

[2] A. S. Go, D. Mozaffarian, V. L. Roger, E. J. Benjamin, J. D. Berry, M. J. Blaha, S. Dai, E. S. Ford, *et al.*, "Heart disease and stroke statistics--2014 update: a report from the American Heart Association," *Circulation,* vol. 129, pp. e28-e292, Jan 21 2014, http://www.ncbi.nlm.nih.gov/pubmed/24352519.

[3] L. G. Spagnoli, A. Mauriello, G. Sangiorgi, S. Fratoni, E. Bonanno, R. S. Schwartz, D. G. Piepgras, R. Pistolese, *et al.*, "Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke," *JAMA,* vol. 292, pp. 1845-52, Oct 20 2004, http://www.ncbi.nlm.nih.gov/pubmed/15494582.

[4] R. C. Pasternak, M. H. Criqui, E. J. Benjamin, F. G. Fowkes, E. M. Isselbacher, P. A. McCullough, P. A. Wolf, Z. J. Zheng, and A. American Heart, "Atherosclerotic Vascular Disease Conference: Writing Group I: epidemiology," *Circulation,* vol. 109, pp. 2605-12, Jun 1 2004, http://www.ncbi.nlm.nih.gov/pubmed/15173042.

[5] H. J. Barnett, R. W. Gunton, M. Eliasziw, L. Fleming, B. Sharpe, P. Gates, and H. Meldrum, "Causes and severity of ischemic stroke in patients with internal carotid artery stenosis," *JAMA,* vol. 283, pp. 1429-36, Mar 15 2000, http://www.ncbi.nlm.nih.gov/pubmed/10732932.

[6] J. L. Tuttle, R. D. Nachreiner, A. S. Bhuller, K. W. Condict, B. A. Connors, B. P. Herring, M. C. Dalsing, and J. L. Unthank, "Shear level influences resistance artery remodeling: wall dimensions, cell density, and eNOS expression," *Am J Physiol Heart Circ Physiol,* vol. 281, pp. H1380-9, Sep 2001, http://www.ncbi.nlm.nih.gov/pubmed/11514310.

[7] D. R. Owen, A. C. Lindsay, R. P. Choudhury, and Z. A. Fayad, "Imaging of atherosclerosis," *Annu Rev Med,* vol. 62, pp. 25-40, 2011, http://www.ncbi.nlm.nih.gov/pubmed/21226610.

[8] T. Saam, T. S. Hatsukami, N. Takaya, B. Chu, H. Underhill, W. S. Kerwin, J. Cai, M. S. Ferguson, and C. Yuan, "The vulnerable, or high-risk, atherosclerotic plaque: noninvasive MR imaging for characterization and assessment," *Radiology,* vol. 244, pp. 64-77, Jul 2007, http://www.ncbi.nlm.nih.gov/pubmed/17581895.

[9] A. Nair, M. P. Margolis, B. D. Kuban, and D. G. Vince, "Automated coronary plaque characterisation with intravascular ultrasound backscatter: ex vivo validation," *EuroIntervention,* vol. 3, pp. 113-20, May 2007, http://www.ncbi.nlm.nih.gov/pubmed/19737694.

[10] E. B. Diethrich, M. Pauliina Margolis, D. B. Reid, A. Burke, V. Ramaiah, J. A. Rodriguez-Lopez, G. Wheatley, D. Olsen, and R. Virmani, "Virtual histology intravascular ultrasound assessment of carotid artery disease: the Carotid Artery Plaque Virtual Histology Evaluation (CAPITAL) study," *J Endovasc Ther,* vol. 14, pp. 676-86, Oct 2007, http://www.ncbi.nlm.nih.gov/pubmed/17924734.

[11] C. M. Campos, R. J. Fedewa, H. M. Garcia-Garcia, D. G. Vince, M. P. Margolis, P. A. Lemos, G. W. Stone, P. W. Serruys, and A. Nair, "Ex vivo validation of 45 MHz intravascular ultrasound backscatter tissue characterization," *Eur Heart J Cardiovasc Imaging,* Mar 1 2015, http://www.ncbi.nlm.nih.gov/pubmed/25733210.

[12] R. H. Silverman, R. Muratore, J. A. Ketterling, J. Mamou, D. J. Coleman, and E. J. Feleppa, "Improved visualization of high-intensity focused ultrasound lesions," *Ultrasound Med Biol,* vol. 32, pp. 1743-51, Nov 2006, https://www.ncbi.nlm.nih.gov/pubmed/17112960.

[13] R. J. Fedewa, H. L. Gornik, and D. G. Vince, "Spectral Analysis of Ultrasonic Backscatter from Human Carotid Plaque," in *2016 IEEE International Ultrasonics Symposium*, Tours, France, 2016.