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TITLE: Vascular Plaque Determination for Stroke Risk Assessment

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CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation, Cleveland, OH

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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 current research project is a pilot clinical study enrolled subjects who are scheduled for carotid endarterectomy (CEA). A research ultrasound exam of the carotid artery was performed prior to surgery. Following surgery, serial histology was applied to the excised plaque. This histology was used as the 'gold' standard when training the statistical classifiers for estimating plaque composition. During this research effort the key accomplishments are (1) reaching 100% enrollment; (2) demonstrating that spectral information extracted from the nonlinearly generated second harmonic is beneficial for plaque characterization; (3) demonstrating that the reference phantom approach is comparable to other more computationally intensive approaches. The best performing classifier had accuracies of 84%, 74%, and 81% for F, HNC, and Ca respectively.					
15. SUBJECT TERMS atherosclerosis, stroke, cerebrovascular accident, carotid endarterectomy, ultrasound, spectral analysis, carotid stenosis, tissue characterization, machine learning, noninvasive					
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1. INTRODUCTION:

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 to provide improved treatment guidance. 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 raw ultrasound backscatter data from the carotid artery prior to surgery and then creates histology slides of the excised tissue in order to train a statistical classifier for determining plaque composition. Candidate classifiers have been created and tested.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

○ What were the major goals of the project?

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

Major Task 1: Clinical Study Preparation

This task included the approvals and steps 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. For Year 3, actual enrollment was 28 with a targeted enrollment of 33. The remaining 19 were enrolled in Year 4 which was granted after

an extension. The original completion date for this task was June 2019 and was revised during the granted extension to **Mar 2020**.

Status of the subtasks are as follows:

Consent Subjects (goal 100%):	100% completed 26 Feb 2020
Research Ultrasound Imaging: (goal 100%):	100% completed 26 Feb 2020
Collection of Plaque from Carotid Endarterectomy (goal 100%):	100% completed 27 Feb 2020

Major task 2 was completed one month prior to the revised completion date of Mar 2020 (original completion date of Jun 2019).

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 the status at the end of this research effort are as follows:

Prepare Serial Histology of Carotid Plaque	100% completed 14 Sep 2020
Ultrasound Signal Processing Development and Testing	Final Candidates Tested
Match Slides to Ultrasound Grayscale <i>In Vivo</i> Data	87% completed

The Task 3 milestone is the extraction of the ultrasound spectral parameters from all regions of interest (ROI) with matched histology. Original targeted completion was August 2019. Revised completion date as stated in no-cost extension was June 2020. Interim analyses indicated that sufficient number of ROI's were obtained using 87% of the subject data. This permitted additional time and effort to be applied to the signal processing and statistical algorithm. The remaining subject data will be used for future development, testing, and validation.

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.

This was completed in Sep 2020 and the results are discussed below.

Major Task 5: Validation of Statistical Classifiers

Original target start in Aug 2019 with a revised start as stated in extension is Aug 2020.
Milestone: Validated final statistical classifiers – original target of Sep 2019 with revised target Sep 2020 following no-cost extension. This was **completed in Sep 2020**.

○ **What was accomplished under these goals?**

Enrollment

The first objective of this effort was the enrollment of 100 subjects for the project (see Table 1). Enrollment is at the heart of this study and for the first year the enrollment either met or exceeded expectations and then in Year 2 (Q6 and Q7) a number of the top enrolling surgeons left and the number of carotid endarterectomies dropped significantly. The limited enrollment at times during the study is the primary reason for the 12 month extension. Each enrolled subject had RF backscatter ultrasound collected prior to their carotid endarterectomy (CEA).

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	Q13*	Q14*	Q15*	Q16*
Projected	12	12	9	0	10*	8*	1*	0*
Actual	14	5	4	5	10	9	0	0
Actual Cumulative	67	72	76	81	91	100	100	100

*New projection for 12 month no-cost extension

Carotid Plaque Tissue Processing

Data collected under this grant was combined with data collected under a similar protocol with American Heart Association grant prior to the start of this research grant. The combined number of subjects enrolled with comparable data acquisition settings is 134 (100 under this grant). Of these 121 have been reviewed to obtain matched slides. No matches between the in vivo grayscale data collected prior to CEA and the serial histology were found for 14 cases. Thus there are matched slides from 107 subjects. A total of 737 homogenous ROI's were found in matched frames from 92 of these subjects with the following distribution:

F	Fibrous/Fibro-Fatty:	125 ROI's
H/NC	Hemorrhagic and/or Necrotic Core:	477 ROI's
Ca	Calcium:	135 ROI's

In order to form the training and testing subsets, a random subsample of 125 H/NC ROI's was selected to balance the dataset. A balanced set minimizes bias that can occur during training. Stratified random sampling was used to partition this smaller dataset into a training set and test set with the same relative class frequencies in each. The test set comprised approximately one-third of the data (F = 37, H/NC = 37 and Ca = 41), while the remaining was used for training (F = 88, H/NC = 88 and Ca = 94). Outliers were removed from the training set by keeping only data within two standard deviations of the mean of integrated backscatter and slope for each tissue type. The final training set is as follows: 80 F, 79 H/NC and 89 C ROI's. This removal of outliers prior to training is based on our expectation of some error in the matching steps and in placing ROI's within homogenous regions.

Statistical Classifier Creation and Optimization

A draft manuscript for submission to the journal *Ultrasonics* is attached in the Appendices titled: Spectral Analysis of Ultrasound Backscatter for Non-invasive Measurement of Plaque Composition. This manuscript provides a detailed description of the following investigations: 1) carotid plaque composition produced by the nonlinearly generated second harmonic portion of the backscattered signal; 2) testing of attenuation compensation approaches; 3) optimization of machine learning algorithms; and 4) validation of the best machine learning algorithms. Both random forest approaches and support vector machines (SVM) were applied to classify carotid plaque into the three tissue types: fibrous and/or fibro-fatty (F), hemorrhagic and/or necrotic core (HNC), and calcium (Ca). The input data is described in the preceding paragraph.

This manuscript discusses twelve different signal processing approaches which arise from combinations of four different attenuation compensation approaches and three frequency band choices. The attenuation compensation choices are

- Reference Phantom: depending on the reference phantom normalization to solely compensate for attenuation arising from propagation through overlying layers. Each of the other steps begins with this compensation.
- One Step Adventitia: Linear fit to the normalized power spectrum for each frequency as a function of depth for data obtained from the adventitia of normal subjects.
- Two Step Adventitia: Similar to the One Step Adventitia approach but now the propagation path is separated into two parts: skin/fat layer and muscle/connective tissue layer.
- OPSSE: Optimum Power Spectral Shift Estimation This approach estimates the attenuation along the propagation path using the shift of the center frequency that arises from the fact that the higher frequencies attenuate at a greater rate than the lower frequencies.

The three frequency band approaches are: only use parameters from the fundamental; only use parameters from the nonlinearly generated harmonic, or use parameters from both.

TABLE 2 Out-of-bag error for Random Forest classification models created with the training dataset and used to determine optimal minimum leaf size, maximum number of splits and number of predictors

ATTENUATION COMPENSATION	SPECTRUM TYPE	MODEL NO.	OOB ERROR	OPTIMIZED HYPERPARAMETERS		
				MIN LEAF SIZE	MAX NO. SPLITS	NO. PREDICTORS
Reference Phantom	Fundamental	1	0.29	6	27	1
	Harmonic	2	0.27	5	27	1
	Combined	3	0.25	9	27	2
One Step Adventitia	Fundamental	4	0.28	1	81	3
	Harmonic	5	0.29	16	9	1
	Combined	6	0.27	5	9	9
Two Step Adventitia	Fundamental	7	0.29	7	3	2
	Harmonic	8	0.30	17	9	1
	Combined	9	0.26	5	27	8
OPSSE	Fundamental	10	0.29	2	81	6
	Harmonic	11	0.28	6	27	1
	Combined	12	0.25	3	81	5

Minimum leaf size = Positive values in the range [1, 20]

Maximum number of splits = Positive values base-3 log-scaled in the range [3, 243]

Number of predictors = Positive values in the range [1, 8] for fundamental and harmonic spectra; [1, 16] for combined spectra

Table 2 displays the results of optimization of the following hyperparameters associated with construction of random forests: minimum leaf size, maximum number of splits, and number of randomly chosen predictors available to split each node. The key piece to focus on is the Out Of Bag (OOB) error. The OOB error is produced during the training of the algorithm and provides a reasonable estimate of the expected testing error. Minimization of the OOB error was used to determine the hyperparameter setting for each signal processing approach (Model No.). The OOB ranges from 0.25 to 0.3. For each type of attenuation compensation, the OOB error was minimized using a combination of fundamental and harmonic input data. Thus even though we are treating the nonlinearly generated second harmonic portion of the backscatter as a linearly propagating signal, the classification is improved with the use of

the harmonic content. In addition, the harmonic results alone appear quite comparable to the fundamental results for the same attenuation compensation.

The test data was then applied to the three best Random Forest classifiers: Reference Phantom, Two Step Adventitia, and OPSSE all using the combined frequency band parameters. The results from applying the test set are shown in Table 3. The result is that the OPPSE has the highest accuracy but all three are comparable. This is important to note since the OPPSE has a significantly greater computation burden compared to the other three attenuation approaches. These results support the use of only the reference phantom which simplifies the implementation on a clinical system.

The test data results were a bit worse than the OOB error which is not surprising since the training data on which the OOB error is based had the outliers removed. The key question are accuracies of 84%, 74%, and 81% for F, HNC, and Ca respectively sufficient for positively impacting clinical care cannot be answered with this data. Thus we are working on the awarded extension of this research effort to address the clinical significance of this algorithm.

TABLE 3

Overall Accuracy, H/NC accuracy, sensitivity and specificity, and kappa statistic for select independently evaluated classification models with cross-validations errors < 0.30 using their optimal set of parameters

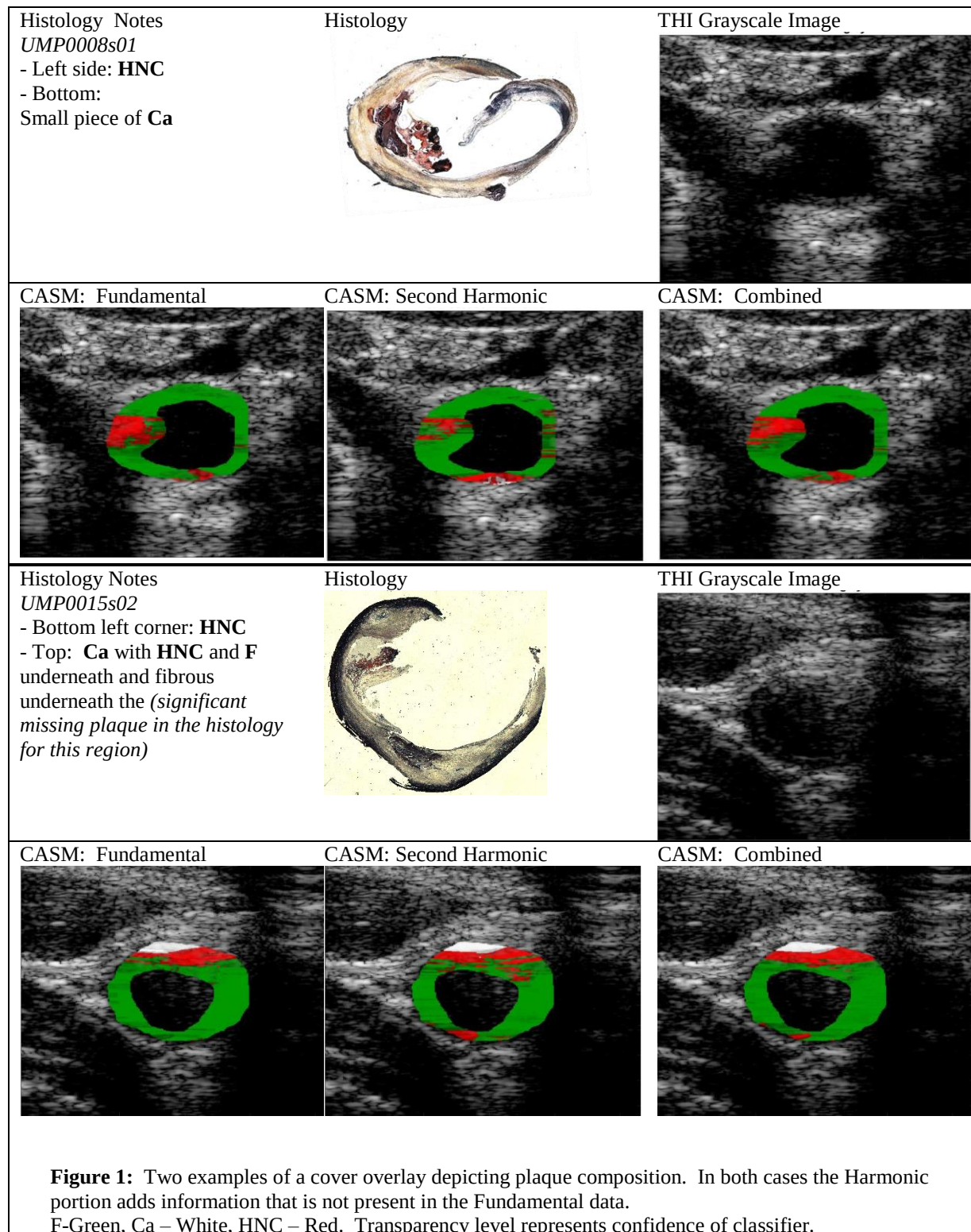
STATISTIC [†]	COMBINED PARAMETERS		
	Random Forest		
	Reference Phantom	Two-Step Adventitia	OPSSE
Overall Accuracy	0.68 ± 0.09	0.68 ± 0.09	0.69 ± 0.08
Fibrous	Accuracy	0.84 ± 0.07	0.87 ± 0.06
	Sensitivity	0.83 ± 0.12	0.81 ± 0.13
	Specificity	0.85 ± 0.08	0.90 ± 0.07
Hemorrhagic and/or necrotic core	Accuracy	0.71 ± 0.08	0.70 ± 0.08
	Sensitivity	0.43 ± 0.16	0.51 ± 0.16
	Specificity	0.85 ± 0.09	0.79 ± 0.10
Calcium	Accuracy	0.80 ± 0.07	0.78 ± 0.08
	Sensitivity	0.76 ± 0.13	0.71 ± 0.14
	Specificity	0.82 ± 0.09	0.82 ± 0.09
Kappa Statistic [‡]	0.52 ± 0.07	0.52 ± 0.07	0.54 ± 0.06

[†] $\pm$ margin of error based on 95% confidence interval

[‡] $\pm$ Standard error

Example of Color Overlay Results

We presented results comparing the use of fundamental and harmonic frequency band at the 2019 IEEE IUS conferences (manuscript is attached as an appendix). In Figure 1, we demonstrate that using the parameters obtained from the harmonic band appear to improve on detecting plaque structure as compared to using only the fundamental portion of the backscatter. This is the first known use of the nonlinearly generated second harmonic portion of the backscattered signal for tissue characterization based on spectral analysis.



Test of Interim Statistical Classifier on Distinguishing Between Symptomatic and Asymptomatic Subjects

The long term goal is to produce a statistical classifier capable of providing clinically useful information. As a test of whether our approach shows promise for this end goal, we performed the following test:

1. For the first 20 subjects in this study, one researcher chose the in vivo grayscale frame corresponding to roughly the center of the plaque. This researcher was blinded to symptomatic state of the subject (i.e. whether the patient has experienced either a stroke or a transient ischemic attack associated with the carotid plaque).
2. Borders were drawn for these 20 frames to segment the plaque from the surrounding tissue and blood.
3. A classifier using both fundamental and harmonic frequency content with a one-step adventitia based attenuation compensation from Year 3 was used to compute the color overlays for the segmented regions.
4. The percent of the plaque area comprised of HNC was computed.

Percent HNC was investigated, since hemorrhagic and necrotic core are associated with an increased risk of stroke. For these 20 subjects, 14 were asymptomatic and 6 were symptomatic. The average (standard deviation) of the measurement was 44% (11%) and 39% (11%) for the symptomatic and asymptomatic groups respectively. This is a small sample study and did not show statistical significance when applying a t-test with a p-value of 0.17. A significantly better test would be to systematically insonify the entire plaque and produce a three-dimensional measure of the plaque composition and plaque burden. Taking a two-dimensional slice such as this analysis could very well miss the portion of the plaque that is creating the greatest risk for the subject. *This is being done as part of the extension award of this initial research effort.*

Stated Goals Not Met

Two goals were not met during the execution of this research project:

1. Data from all 100 subjects enrolled under this grant were not fully processed. Data collection and histology were performed for all data sets and 87 sets of the data were fully processed. As we were performing interim analysis, it was observed that adding more training was not having an impact on the classification error. Thus we chose to keep these final data sets for future validation efforts.
2. Inter-pathologist comparison of reading histology was not compared to the output of the chosen statistical classifier. The onset of COVID 19 disrupted our ability to work with the pathologists and thus we were unable to perform the inter-pathologist comparison with the output of the chosen classifier. We do plan on pushing this forward once through the COVID 19 crisis.

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

Nothing to report

○ How were the results disseminated to communities of interest?

Conference presentations as described in the Bibliography included as an appendix to this report.

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

Nothing to Report

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
 - The backscatter of the nonlinearly generated second harmonic appears to provide additional information to complement tissue classification based solely on the fundamental bandwidth.
 - This work is the first use of the nonlinearly generated second harmonic for tissue characterization based on spectral analysis of ultrasonic backscattered signals.
 - Further analysis of the attenuation compensation demonstrates that, statistically, the benefit from the adventitious based attenuation compensation approaches or estimates based on center frequency shift are minimal at best. Thus the use of a 0.5 dB/cm-MHz attenuation coefficient slope to compensate for attenuation in all patients seems to be a valid choice and has low computational requirements (in contrast to the center frequency shift approaches).
- **What was the impact on other disciplines?**

A statistical classifier was developed that provides a measure of the plaque composition which may provide clinically useful information. Further testing is needed to determine the relationship between the classifier measurements and clinical outcomes.
- **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:

- **Changes in approach and reasons for change**

Year 1	Nothing to Report
Year 2	Nothing to Report
Year 3	Nothing to Report
Year 4	Nothing to Report
- **Actual or anticipated problems or delays and actions or plans to resolve them**

Year 1
It took five months to recruit a suitably qualified Research Engineer. This delayed progress on signal processing efforts, but these delays did not impact hitting milestones for the project in part due to the quick start to enrollment in Year 1.

In Year 1, we anticipated that consistently recruiting 12 subjects in each quarter during peak recruitment would be difficult based on recruitment rates in Year 1.

Year 2
Enrollment decline during Q5 and Q6 (first half of Year 2) 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. This enrollment was expected to recover as the new surgeons came on board.

Year 3

Enrollment rebounded in Quarter 1 of Year 3 (Quarter 9) for our best enrollment numbers of the study (14 enrolled) but then declined for the remainder of Year 3. We applied and received a no-cost extension in order to complete enrollment and the subsequent study actions that follow.

Year 4

Enrollment was completed prior to disruptions from COVID 19. However, the planned inter-pathologist review and comparison with the produced color overlays from the statistical classifier is delayed until hospital operations return to normal.

- **Changes that had a significant impact on expenditures**

Year 1

Salary for Research Engineer was budgeted for 12 months and only 7 months were used.

Year 2

The reduced enrollment had a comparable reduction in histological processing costs and sonographer charges since there were not as many cases to process.

Year 3

Dr. Heather Gornik left the Cleveland Clinic in the fall 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). Similar to Year 2 the reduced in enrollment led to reduced costs with respect to histological processing and sonographer charges.

Year 4

Nothing to Report

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

Cleveland Clinic IRB Study Number 12-797

Title: "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 human subjects

Year 1 Nothing to Report

Year 2 Nothing to Report

Year 3 Nothing to Report

Year 4 Nothing to Report

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

Report only the major publication(s) resulting from the work under this award.

Journal publications

Manuscript to be submitted to the journal Ultrasonics in Quarter 1 of 2021 is located in the appendix.

Books or other non-periodical, one-time publications

Nothing to Report

Other publications, conference papers, and presentations. (*manuscript)

*James SL, Fedewa RJ, Lyden S, Vince D. *Attenuation Compensation Comparison for Human Carotid Plaque Characterization Using Spectral Analysis of Backscattered Ultrasound*. 2019 IEEE International Ultrasonics Symposium. 2019 October.

*Fedewa RJ, James SL, Lyden S, Vince D. *Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque*. 2019 IEEE International Ultrasonics Symposium. 2019 October; pp. 48-51.

James SL, Fedewa RJ, Gornik H, Lyden S, Vince D. *Attenuation Compensation of Ultrasound Backscatter from Human Carotid Plaque*. The Journal of the Acoustical Society of America. 176th Meeting of the Acoustical Society of America; 2018 November 5; Victoria, BC, Canada. JASA 144, 1888; c2018.

Fedewa RJ, James SL, Lyden S, Vince DG. *Non-invasive Measurement Of Carotid Plaque Composition To Improve Risk Assessment Of Stroke*. 2019 Military Health System Research Symposium, Orlando, Florida, 19-22 August 2019.

*Fedewa RJ, James SL, Gornik HL, Lyden S, Vince D. *Nonlinearly Generated Second Harmonic Ultrasonic Backscatter for Determining Composition of Human Carotid Plaque*. 2018 IEEE International Ultrasonics Symposium (IUS). 2018 October; Kobe, Japan.

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. (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.

Inventions, patent applications, and/or licenses

Fedewa RJ, Vince D, Elsharkawy H, inventors. Cleveland Clinic, assignee. *Advanced Ultrasonic Detection of Different Tissue Types*. Application Number 16/754414. USPTO. Application Date: 2018 October 15. Provisional Application: 2017 October. *Copy of application in Appendix*

NOTE: *The patent disclosure for this patent application had the original title of Title: Noninvasive Method for Determination of Carotid Plaque Composition.*

- **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 to 1 Aug 2021.

In vivo data from 100 human carotid plaques for subjects scheduled for a CEA 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
- 413 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)
- 100 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?**

Year 1: 15 Sep 2016 – 14 Sep 2017

Name:	D. Geoffrey Vince
Project Role:	Principal Investigator
Researcher 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
Researcher 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 L. James
Project Role:	Research Engineer
Researcher Identifier (e.g. ORCID ID):	0000-0002-5647-1106
Nearest person month worked:	7
Contribution to Project:	Ms. James dedicated time to signal processing development to account for effects of attenuation and diffraction, data collection, and data processing. In addition, Ms. James provided support for the implementation and execution of subject recruitment and enrollment.
Funding Support:	NA

Year 2: 15 Sep 2017 – 14 Sep 2018

D. Geoffrey Vince: No Change

Russell J. Fedewa: No Change

Name:	Sheronica L. James
Project Role:	Research Engineer
Researcher Identifier (e.g. ORCID ID):	0000-0002-5647-1106
Nearest person month worked:	12
Contribution to Project:	Ms. James dedicated time to signal processing development to account for effects of attenuation and diffraction, data collection, and data processing. In addition, Ms. James provided support for the implementation and execution of subject recruitment and enrollment.
Funding Support:	NA

Year 3: 15 Sep 2018 – 14 Sep 2019

D. Geoffrey Vince: No Change

Russell J. Fedewa: No Change

Sheronica L. James: No Change

Name:	Anna Phillips
Project Role:	Summer Intern
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	2
Contribution to Project:	Ms. Phillips worked on signal processing efforts and extension of the spectral analysis approach.
Funding Support:	NA

Year 4: 15 Sep 2019 – 14 Sep 2020

D. Geoffrey Vince: No Change

Russell J. Fedewa: No Change

Sheronica L. James: No Change

Name:	Jacqueline Loftis
Project Role:	Research Engineer
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	2
Contribution to Project:	Ms. Loftis has performed work matching histology to ultrasound images of carotid plaque and image segmentation
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 (for all four years)

In-kind support: Equipment loan of the following

- S3000 HELX Ultrasound System
- 9L4 Transducer
- Axius Direct Research Interface
- Matlab Software to Access RF Data

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES:

- A. Bibliography of Publications and Meeting Abstracts
- B. List of Personnel Receiving Pay From the Research Effort
- C. Manuscript Prepared for Submission to the journal *Ultrasonics*
- D. James SL, Fedewa RJ, Lyden S, Vince D. *Attenuation Compensation Comparison for Human Carotid Plaque Characterization Using Spectral Analysis of Backscattered Ultrasound*. 2019 IEEE International Ultrasonics Symposium. 2019 October.
- E. Fedewa RJ, James SL, Lyden S, Vince D. *Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque*. 2019 IEEE International Ultrasonics Symposium. 2019 October.
- F. USPTO Patent Application 16/754414

Appendix A:

Bibliography of Publications and Meeting Abstracts Associated with Research Award

Conference Proceedings

James SL, Fedewa RJ, Lyden S, Vince D. *Attenuation Compensation Comparison for Human Carotid Plaque Characterization Using Spectral Analysis of Backscattered Ultrasound*. 2019 IEEE International Ultrasonics Symposium. 2019 October.

Fedewa RJ, James SL, Lyden S, Vince D. *Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque*. 2019 IEEE International Ultrasonics Symposium. 2019 October; pp. 48-51.

Fedewa RJ, James SL, Gornik HL, Lyden S, Vince D. *Nonlinearly Generated Second Harmonic Ultrasonic Backscatter for Determining Composition of Human Carotid Plaque*. 2018 IEEE International Ultrasonics Symposium (IUS). 2018 October; Kobe, Japan.

Meeting Abstracts

James SL, Fedewa RJ, Gornik H, Lyden S, Vince D. *Attenuation Compensation of Ultrasound Backscatter from Human Carotid Plaque*. The Journal of the Acoustical Society of America. 176th Meeting of the Acoustical Society of America; 2018 November 5; Victoria, BC, Canada. JASA 144, 1888; c2018.

Fedewa RJ, James SL, Lyden S, Vince DG. *Non-invasive Measurement Of Carotid Plaque Composition To Improve Risk Assessment Of Stroke*. 2019 Military Health System Research Symposium, Orlando, Florida, 19-22 August 2019.

Appendix B:

List of Personnel Receiving Pay from the Research Effort

All personnel were employed by the Cleveland Clinic while working on this research effort.

D. Geoffrey Vince

Russell J. Fedewa

Sheronica L. James

Jacqueline Loftis

Anna Phillips

Sean Lyden

Heather Gornik

Alia Grattan

Kate McDonald

Anyi Liu

Dmitry Mitsyuk

Stefanie Beckner

Appendix C:

Manuscript Prepared for Submission to the journal *Ultrasonics*

Title: Spectral Analysis of Ultrasound Backscatter for Non-invasive Measurement of Plaque Composition

INTRODUCTION

Carotid artery plaque composition is a known predictor of future cerebrovascular accidents (CVAs) in patients with carotid atherosclerosis [1], [2]. The vast majority of CVA's (87%) involve ischemic stroke. Carotid plaque rupture and extra-cranial atherothrombosis are the predominant causes of stroke and transient ischemic attacks [3], [4]. Current clinical practice relies heavily on degree of stenosis and the presentation of symptoms for risk stratification of patients with carotid atherosclerosis. These criteria are the primary determining factors for selecting patients for surgical intervention, such as carotid endarterectomy (CEA). However, the benefit of a CEA in asymptomatic patients with high grade stenosis and symptomatic patients with a non-significant degree of stenosis is unclear. A large number of CVAs are, in fact, caused by rupture-prone plaques with specific morphological features, regardless of degree of stenosis [5], [6]. It has been hypothesized that plaque comprising fibrous elements and smooth muscle cells may be less prone to rupture, whereas plaque with a greater lipid content and inflammation may be more susceptible to rupture and distal embolization [7]. More specifically, the type of rupture-prone plaque most often associated with stroke is characterized by ulceration, intraplaque hemorrhage, a lipid-rich necrotic core, and active inflammation [3], [7]–[9]. Thus, the presence of intra-plaque hemorrhage with a necrotic core may distinguish unstable, rupture-prone lesions from more stable ones. Determination of plaque composition could help accurately identify plaques prone to rupture, and therefore constitutes an important prognostic marker of lesions at high risk for initiating a CVA.

While plaque composition matters more than degree of stenosis in plaque rupture, it is generally unavailable at the point of care, as current diagnostic imaging tools are unable to establish plaque composition in a consistent and cost effective manner. Non-contrast CT and CT angiography (CTA) can quantify calcium burden as well as detect presence of lipid and ulceration. However, CT approaches cannot discern areas of hemorrhage and carry the risk of exposure to ionizing radiation [10]. Heavy calcium burden further limits the assessment of plaque characteristics due to acoustic shadowing. Magnetic resonance imaging (MRI) has the ability to distinguish between tissue types (fibrous cap, intraplaque lipids, hemorrhage, and necrotic core regions) [11], [12][13]. However, MRI is expensive and not readily available to all patient populations. Intravascular ultrasound (IVUS) employing VH-IVUS® technology [8] [14] [15] can be used to assess plaque composition, but is invasive and thus not applicable.

Duplex ultrasound (DUS) is excellent with respect to assessing degree of stenosis, and is routinely used to monitor the severity of carotid stenosis [16]. However, ultrasound grayscale-based image analysis struggles at distinguishing hemorrhagic and necrotic core from other tissue types [17], [18]. While, videodensitometry, a computer-automated method of analyzing ultrasound grayscale images, has shown the ability to distinguish between symptomatic and asymptomatic plaque [19], [20] and to predict the degree of instability of asymptomatic plaque [21], grayscale image-based ultrasound characterization remains too unreliable to be consistently implemented clinically [22].

Spectral based analysis of the backscattered ultrasound can improve upon grayscale image based analysis by providing additional information. Multiple efforts have leveraged spectral analysis of ultrasound backscatter

for tissue characterization involving atherosclerotic plaque [14], [23]–[33]. For coronary plaque characterization, the VH-IVUS approach has been found to be clinically significant [34]. More recent work has been done with non-invasive approaches for carotid plaque [35]–[39].

This study examines the effects of a variety of attenuation compensation techniques and the use of the nonlinearly generated second harmonic bandwidth for characterizing human carotid plaque using spectral analysis and machine learning. Specific focus is placed on identifying hemorrhagic and/or necrotic core (H/NC) tissue, since this type of plaque tissue is most often associated with vulnerable or rupture-prone atherosclerotic lesions. This effort is a part of the longer term effort to provide a clinically useful tool to improve risk stratification for patients with carotid stenosis.

DATA ACQUISITION AND SIGNAL PROCESSING

Clinical Study

The goal of pilot clinical study was to collect ultrasound backscatter RF data prior to subjects receiving a carotid endarterectomy (CEA) and then collecting the excised plaque in order to use as a ‘gold’ standard for creating a tissue classification algorithm. Inclusion was limited to patients with atherosclerotic plaque within the distal common carotid artery, the carotid bulb, or the proximal internal carotid artery. Patients with a prior intervention with the ipsilateral carotid artery were excluded as were patients who could not provide consent in English.

The *in vivo* ultrasound employed a Siemens S3000 HELX ultrasound system with Aixius Direct Ultrasound Research Interface (URI) software enabling the acquisition of pulse inversion beamformed RF data at 40 MHz sampling. Using a 9L4 linear array transducer, two sets of data were acquired while imaging the carotid artery in a transverse orientation. The first set of data was acquired while holding the transducer still (i.e. static sites), this data were collected at approximately 1 cm separation from the proximal end to the distal end of the plaque and ending at the distal. Following these sites where the transducer was held still (i.e. static sites), RF data loop of 54 frames were collected while moving the transducer from the proximal end of the plaque to the distal end. This loop data served as a backup to the static sites and provided context for matching *in vivo* frames to histology. Reference RF data were acquired from a 0.5 dB/cm-MHz phantom (model 044, Computerized Imaging Reference Systems, Inc., Norfolk, VA, USA). Identical system settings were used for both the *in vivo* data collection and the phantom data collection. The depth was set to 4 cm with a transmit focus of 2cm and 456 lines per frame. The analysis in this paper is obtained from the first 119 subjects enrolled following this arrangement (the first 6 subjects enrolled in the study are not included since the acquisition did not employ pulse inversion). In addition to the CEA subjects, six normal subjects had ultrasound backscatter from both of their carotid arteries collected as well and used for attenuation compensation adjustments.

Histology

On the same day as the CEA, the excised plaque tissue is placed in saline and ultrasound images are collected in order to locate regions of the plaque corresponding to the *in vivo* static sites. The tissue was then placed in 10% buffered formalin for at least 24 hours followed by two rounds of de-calcification (Cal-Rite, Thermo Fisher Scientific, Runcorn, UK) for at least 24 hours. Prior to paraffin embedding the static sites were marked on the tissue with dye. Serial histology was performed with slide pairs taken at 1 mm steps before and after the marked region and 0.5 mm steps through the target region. One slide of each pair received hematoxylin and eosin stain while the other slide received Movat pentachrome stain. The best matching slide to each *in vivo* grayscale site was found by one member of the research team and reviewed by a different member.

Discrepancies were discussed and resolved. A pathology expert then read the matched slide and the nearest neighbor slides defining regions of homogenous tissue across all three slides. Three tissue types were defined: calcified regions (Ca), fibrous and/or fibro-fatty regions (F), and hemorrhagic and/or necrotic core regions (H/NC). Next, regions of interest (ROI's) were drawn within the grayscale *in vivo* images corresponding to these homogenous regions in histology (see Figure 2). Customized Matlab software (The Mathworks Inc.,

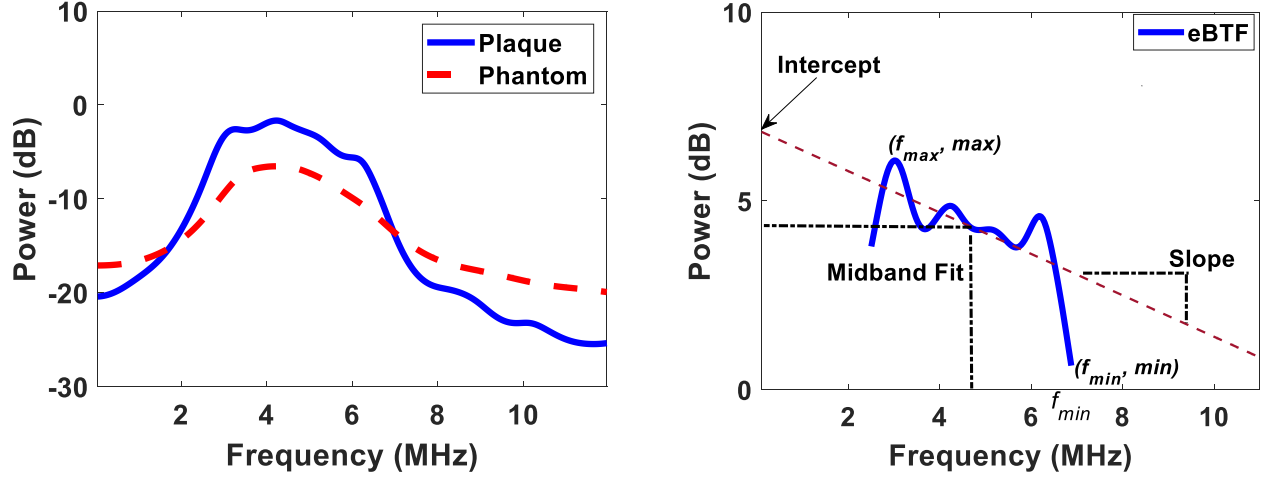


Fig. 1. Diagram illustrating computation of spectral parameters. Left - Sample average region-of-interest (ROI) power spectrum from plaque tissue and average reference phantom power spectrum. Right - Estimated backscatter transform function (eBTF) within the usable 20dB down bandwidth of 2.5 -6.9 MHz. Each short-time segment of radiofrequency data (RF) that passes through the ROI is extracted from the raw data. An autoregressive model is used to compute the power spectrum of each of the A-lines and then the resulting power spectra are averaged across the scanlines in the ROI. A power spectrum from a tissue phantom, from the same depth as the ROI, is used to normalize the average ROI power spectrum to get the eBTF. Spectral parameters are computed from the eBTF over a limited bandwidth of 2.5 -6.9 MHz as determined from the reference phantom. A regression line is fit to the eBTF, and the mid-band fit, slope and y-intercept of the regression line are determined. Four extreme points are obtained from the eBTF: maximum, minimum and corresponding frequencies. IB, a mean intensity parameter, was also calculated, but is not shown here.

Natick MA, USA) was used to extract the RF data corresponding to these ROI's. Each ROI was 64 points by 15 lines which corresponds to 1.2mm by 1.2 mm.

Spectral Analysis

The power spectrum of each line within each ROI was computed using a Yule-Walker Method of order 24 and converted to dB scale. The average over all lines was computed. Under the assumption of linearity and the Born approximation in a homogenous propagation medium, the power spectrum $S(f, d)$ of a backscattered RF signal can be expressed as the product of the transmit and receive transfer function $G(f)$, diffraction $D(f, d)$, attenuation from overlying tissue $A(f, d)$, and backscatter transfer function $B(f)$:

$$S(f, d) = G(f) D(f, d) A(f, d) B(f, d). \quad (1)$$

Implementing spectral analysis to determine tissue composition requires isolating the scattering properties of the target tissue (i.e. B). For *in vivo* applications in soft tissue, the reference phantom method has been demonstrated to effectively compensate for attenuation and diffraction effects, as well as the system transfer function [40]. A stable power spectrum was obtained from the reference phantom for each ROI by analyzing RF data collected from a homogenous region of the phantom. Data from 10 frames were extracted for the same start and stop depth of the tissue ROI's. The same spectral analysis approach was used to estimate the power spectrum and an average over all lines and frames was computed. This average formed the reference

power spectrum, $S_{ref}(f, d)$, corresponding to the target ROI. An illustration of this normalization step to obtain the estimate of the backscatter transfer function ($eBTF$) is shown in figure 3.

$$eBTF(f, d) = \frac{S_{sam}(f, d)}{S_{ref}(f, d)} \quad (2)$$

where the subscripts *sam* and *ref* represent the tissue sample and reference phantom, respectively. In a homogenous medium with uniform attenuation properties where the attenuation coefficient is assumed to be linear with frequency, the cumulative overlying attenuation can be written as follows,

$$A(f, d) = e^{\{-4\beta f d\}}, \quad (3)$$

where β is the slope of the attenuation coefficient. Substituting (1) and (3) into (2), we get

$$eBTF(f, d) = \frac{B_{sam}(f, d)}{B_{ref}(f, d)} e^{4(\beta_s - \beta_R) f d}. \quad (4)$$

Approaches for compensating for the remaining attenuation following reference phantom normalization ($\beta_s - \beta_R$) are investigated below. For each ROI, two $eBTF$ curves were computed: one for the fundamental portion of the backscatter obtained from subjecting the pulse inversion pairs and one for the nonlinearly generated second harmonic (i.e. harmonic) obtained by adding the pulse inversion pairs.

Spectral Parameters

Spectral parameters were extracted from both the fundamental and harmonic $eBTF$ of each ROI corresponding to a homogenous tissue type. These parameters along with the histology determined tissue type form the basis for training and testing the machine learning based classifiers. The operational bandwidths for the parameters was defined by the 20dB down locations obtained from the phantom data: 2.5 MHz to 6.9 MHz for the fundamental and 4.9 MHz to 10.1 MHz for the harmonic. Three parameters are obtained through a linear fit to the $eBTF$ for each band: slope, intercept, and mid-band fit. The area under the $eBTF$ for each band was computed to obtain the integrated backscatter (IB). The final four parameters are obtained by locating the maximum (P_{max}) and minimum (P_{min}) values within each band and the corresponding frequencies (F_{max} and F_{min}). The computation of these parameters for the fundamental spectrum are illustrated in figure 1.

ATTENUATION COMPENSATION

From equation 4 we see that the difference between the reference phantom attenuation and the attenuation of overlying tissue layers may still have an impact on the backscattered signals. Below we describe three additional approaches for compensating for this difference in attenuation. Thus a total of four attenuation compensation approaches were investigated and all four were applied to both the fundamental and harmonic bands.

Optimum power spectral shift estimation (OPSSE)

The optimum power spectral shift estimator (OPSSE) [41], is a correlation-based centroid shift estimator used to approximate local attenuation coefficients in soft tissue. It is a variation of the hybrid method [42] developed by Kim and Varghese that estimates the slope of attenuation using a combination of the reference phantom method and a spectral cross-correlation algorithm that best minimizes the variance in the estimation.

While the approach was investigated to obtain an intensity insensitive measure of the attenuation properties of the target tissue [41], we apply it here as a method to compensate for attenuation arising from the tissue path to and from the target tissue.

To calculate the attenuation of overlying tissue layers, RF data from a large window of interest ($\approx 10\text{mm} \times 5.5\text{mm}$) was divided into 5 axially consecutive blocks with 75% overlap. An average power spectrum was calculated for each block and normalized to a reference phantom. Following normalization, a Gaussian filter ($G(f)$) with center frequency (f_c) and bandwidth (σ) comparable to the transmit pulse,

$$G(f) = e^{\left\{ -\frac{(f-f_c)^2}{2\sigma^2} \right\}}, \quad (5)$$

was applied to yield the Gaussian filtered normalized spectrum ($GS(f, d)$)

$$GS(f, d) = e^{\left\{ -\frac{(f-f_c)^2}{2\sigma^2} \right\}} \frac{B_{sam}(f, d)}{B_{ref}(f, d)} e^{4(\beta_s - \beta_R) f d}. \quad (6)$$

We estimated f_c and σ by fitting a 2-term Gaussian model to the received signal from the reference spectrum at the depth of the axial transmit focus. The attenuation of the phantom (0.5 dB/cm-MHz) was compensated prior to this estimate.

Steps in rearranging Eq. (6), shown in work by others [42], lead to the following relation between the center frequency and the slope of the attenuation coefficient,

$$f_c = f - 4(\beta_s - \beta_R) d \sigma^2. \quad (7)$$

The OPSSE approach correlates the Gaussian filtered normalized spectrum with the following weight to estimate the shift in center frequency.

$$B_{opt} = \frac{d}{df} \left(\frac{1}{G(f)} \right) \quad (8)$$

A linear least-squares fit of consecutive center frequency shift estimates along depth was used to calculate the attenuation coefficient of the tissue layers overlying the carotid artery and thus provide an additional attenuation compensation for propagation beyond the reference phantom.

One-Step and Two-Step Adventitial Based Approaches

Adventitia has been used as a reference for tissue characterization [43]. Our adventitia-based attenuation compensation approaches use backscatter from the adventitia of normal subjects as a reference to compensate for attenuation differences between the reference phantom and tissue layers overlying the ROI's. The adventitia of 6 normal subjects was analyzed and 209 ROI's were obtained. An average power spectrum was estimated for each ROI and normalized by the reference phantom as described above to obtain the corresponding *eBTF*. A linear least-squares regression model was obtained for each frequency bin within the bandwidth by fitting a line to the graph of *eBTF* versus depth for all 209 ROI's.

The one-step approach defined a single depth as the distance from the surface of the transducer to the starting edge of the ROI. The 2-step approach breaks this path into two pieces based on the user defined boundary between the skin/fat layer (section 1) and the underlying muscle layer (section 2) as shown in Figure 2. An additional attenuation compensation was applied to each plaque ROI by inserting the ROI depth into the regression equations and subtracting the output from the $eBTF$:

$$\text{One Step Correction} = -[m_{adv}(f) \cdot d_{sam} + b_{adv}(f)] \quad (9)$$

$$\text{Two Step Correction} = -[m1_{adv}(f) \cdot d1_{sam}(f) + m2_{adv}(f) \cdot d2_{sam} + b_{adv}(f)] \quad (10)$$

where m_{adv} and b_{adv} are the slope and intercept, respectively, obtained from the linear fit of the adventitial $eBTF(f)$ and d_{sam} is the depth of the start of the tissue sample ROI from the transducer.

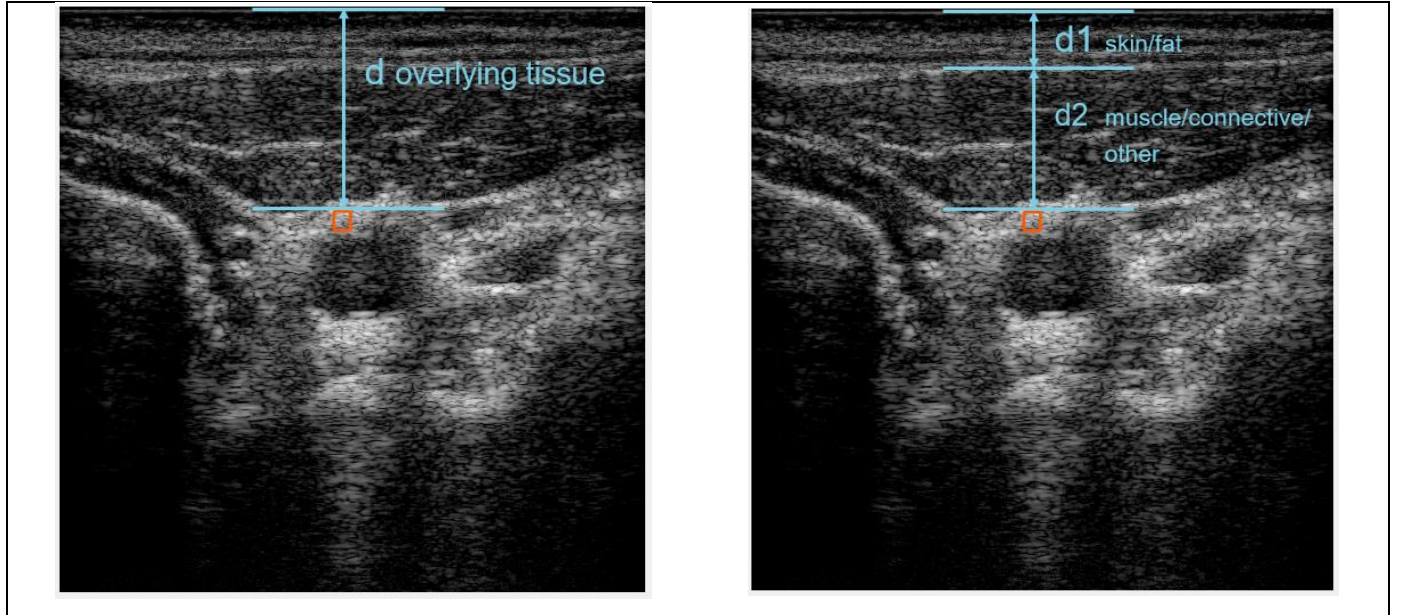


Figure 2: Sample grayscale images of carotid artery. Adventitia ROI in orange. Schematic on the left shows the total depth of the sample ROI from the transducer surface, while the schematic on the right shows the total depth divided into two layers: m(1) skin/fat tissue and (2) muscle, connective and other tissue.

MACHINE LEARNING FOR CLASSIFICATION

Two machine learning classification techniques were explored; random forest classification and multi-class support vector machine classification. The random forest algorithm, introduced in its entirety by Brieman in 2001[44], uses an ensemble of decision trees on random subset of data samples, then combines the predictions from each of them to select the best output by means of voting. Random forest models used in this study were created using the Matlab *treebagger* [45] function. The function *fitcecoc* [46] was used to train a classification multiclass error-correcting output codes (ECOC) model using support vector machine (SVM) binary learners [47]. A support vector machine is a binary learning model used separate two classes of data points [48]. The objective is to find a hyperplane that maximizes the distance, or margin, between data points of both classes, therefore optimizing the classification of future data points. Since our data consisted of 3 classes, an error-correcting output code (ECOC) model with a one vs one coding design was used to reduce the 3-class problem to a set of binary classification problems. The multiclass ECOC model was then trained using support vector machine (SVM) binary learners.

Previous work in our lab has shown overlap in spectral parameter values of carotid plaque tissue types [38]. This presents an issue where SVM classification problems are not linearly separable, meaning they do not have a simple hyperplane as a useful separation criterion. In these cases, it is useful to apply transformations to the data, which map the data points from the original space into a higher dimensional feature space [49]. The goal is that the classes will become linearly separable after transformation to the higher dimensional space, enabling us to fit a decision boundary, or hyperplane, that separates the classes and make predictions [49], [50]. However, because this transformation is computationally expensive, kernels, or similarity measures between the original data and transformed data, are often used to compute the decision boundary. Kernel functions take the original data points as inputs and returns the dot product of the original data and the transformed data. This approach, called the kernel trick, allows us to compute this dot product without actually having to transform the data points to a higher-dimensional space. Thus, we can compute decision boundaries in a higher dimensional space without explicitly mapping the input points into this space. Three commonly used kernel functions were used in this study: linear, radial basis or Gaussian, and polynomial.

Our initial dataset contained a total of 737 ROI's ($F = 125$, $H/NC = 477$ and $Ca = 135$) from 92 subjects. In order to form the training and testing subsets, a random subsample of 125 H/NC ROI's was selected to balance the dataset. A balanced set minimizes bias that can occur during training. Stratified random sampling was used to partition this smaller dataset into a training set and test set with the same relative class frequencies in each. The test set is comprised approximately of one-third of the data ($F = 37$, $H/NC = 37$ and $C = 41$), while the remaining two-thirds was used for training. Outliers were removed from the training set by keeping only that data within two standard deviations of the mean of IB and slope for each tissue type. The final training set consisted of 80 F, 79 H/NC and 89 C ROI's. The test set did not have outliers removed.

Hyperparameter Optimization

Because a separate validation set was not feasible with our sample size, cross-validation was used to tune our hyperparameters prior to the creation of the statistical classifiers. Two common cross-validation methods were used in this study: out-of-bag error for the random forest classifier, and 5-fold cross validation for the SVM classifier.

Out-of-bag error

A key aspect of the random forest is the existence of out-of-bag (OOB) samples arising from the random sampling of the training data [51]. The OOB error is commonly considered as a nearly unbiased proxy of the performance estimation obtained through the use of an independent test dataset or through resampling-techniques such as cross-validation.

K-fold cross-validation

K-fold cross-validation involves randomly dividing a set of observations into k groups, or folds, of approximately equal size. The first fold is treated as a validation, or hold-out, set, and the model is trained on the remaining $k - 1$ folds. This is repeated with each of the folds being the hold-out.

Random Forest Optimization Hyperparameters

The OOB error was used to optimize the following hyperparameters:

1. Maximum number of decision splits per tree
2. Minimum leaf size , or minimum number of observations per leaf
3. Number of predictors to select at random for each split
4. Number of trees in the forest

Hyperparameters (3) and (4) typically have the strongest impact on predictive accuracy, therefore tuning of these is essential. By default, the number of predictors to select at random for each split is the square root of the total number of predictors. In our case, this would be 3 and 4 predictors for 8 and 16 parameters respectively. Although technically not a hyperparameter in this study, the number of trees needs to be sufficiently large to stabilize error. Inspecting the OOB error as a function of the number of trees provides visual evidence for when there are sufficient trees in the forest. Note, however, that each additional tree in a random forest adds to the computational cost of implementing the algorithm. Hyperparameters (1) and (2) usually have marginal impact on predictive accuracy but are still worth exploring. A high value for maximum number of decision splits may result in underperforming trees that, if numerous, could affect the overall performance of the model. Similarly, a smaller leaf will make individual decision trees, and thus the model, more prone to capturing noise in training data.

Support Vector Machine Optimization Hyperparameters

K-fold cross-validation was used to optimize the following hyperparameters:

1. C parameter, also called the box constraint or soft margin constant
2. Kernel scale

For linear and polynomial kernel functions, only box constraint needs to be optimized. The box constraint parameter, or C-parameter, controls the trade-off between maximizing the margin and minimizing the training error. This allows us to create a ‘soft-margin’ SVM, which allows for some points to be misclassified at the expense of penalizing the cost function proportionally to degree of misclassification. Box constraint and kernel scale must be optimized simultaneously for Gaussian kernels. The kernel scale, often called the gamma parameter, determines the radius of influence of a support vector chosen by the model, with low values meaning ‘far’ and high values meaning ‘close’. It essentially adjusts the curvature of the decision boundary. Low values of gamma will result in more data points being grouped together. For high values of gamma, the data points need to be very close to each other in order to be considered belonging to the same class. For large kernel scale values, the effect of box constraint becomes negligible. If gamma is small, box constraint affects the model just as it would a linear model. For this analysis, positive values log-scaled in the range [1e-3,1e3] were chosen for both box constraint and kernel scale. Polynomials of different degree, in the range [2, 6], were also explored.

Statistical Measures

Following hyperparameter optimization, two classification models were trained for each of the 12 input parameter datasets using the full training set: a random forest classifier and a multi-class SVM classifier. The predicted outcomes of the optimized models were compared to the known plaque types from histology to obtain the predictive accuracy, sensitivity and specificity:

$$\text{Predictive Accuracy} = \frac{\text{All Correct Decisions}}{\text{Total Cases}} \quad (11)$$

$$\text{Sensitivity} = \frac{\text{True Positive Decisions}}{\text{Decisions Actually Positive}} \quad (12)$$

$$\text{Specificity} = \frac{\text{True Negative Decisions}}{\text{Decisions Actually Negative}} \quad (13)$$

An overall measure of agreement between the predictions and histology interpretation was determined by the Kappa statistic κ ,

$$\kappa = \frac{n_a - n_\epsilon}{n - n_\epsilon}, \quad (14)$$

where n = number of samples, n_a = number of agreements and n_ϵ = number of agreements due to chance. The measure falls between 0 and 1, where zero indicates agreement due purely to chance and one is perfect agreement [52]. The most commonly used scale rates a kappa of 0.41 to 0.60 as moderate, 0.61 to 0.80 as substantial and 0.81 to 0.99 as almost perfect agreement with histology [53].

TABLE 1 Out-of-bag error for Random Forest classification models created with the training dataset and used to determine optimal minimum leaf size, maximum number of splits and number of predictors

ATTENUATION COMPENSATION	SPECTRUM TYPE	MODEL NO.	OOB ERROR	OPTIMIZED HYPERPARAMETERS		
				MIN LEAF SIZE	MAX NO. SPLITS	NO. PREDICTORS
Reference Phantom	Fundamental	1	0.29	6	27	1
	Harmonic	2	0.27	5	27	1
	Combined	3	0.25	9	27	2
One Step Adventitia	Fundamental	4	0.28	1	81	3
	Harmonic	5	0.29	16	9	1
	Combined	6	0.27	5	9	9
Two Step Adventitia	Fundamental	7	0.29	7	3	2
	Harmonic	8	0.30	17	9	1
	Combined	9	0.26	5	27	8
OPSSE	Fundamental	10	0.29	2	81	6
	Harmonic	11	0.28	6	27	1
	Combined	12	0.25	3	81	5

Minimum leaf size = Positive values in the range [1, 20]

Maximum number of splits = Positive values base-3 log-scaled in the range [3, 243]

Number of predictors = Positive values in the range [1, 8] for fundamental and harmonic spectra; [1, 16] for combined spectra

RESULTS

Random Forest

The grid-search method was used to tune the hyperparameters of each model. With this technique, we simply built cross-validated classification models for each possible combination of all of the hyperparameter values provided and selected the combination of parameters which produced the lowest cross-validation error. All random forest models were grown to 100 trees, and the average OOB classification error for the entire ensemble was used to determine the optimal values for minimum leaf size, maximum number of splits and number of predictors. OOB errors for each model with corresponding hyperparameter values are listed in Table 1.

All of the random forest models produced a maximum OOB error ≤ 0.3 with less than 5% difference among all 12. Models with combined parameters had the lowest OOB error for each attenuation compensation method, but there was no apparent trend in parameter values within this group. Harmonic models performed best when sampling just one predictor at each node, but this likely isn't the most feasible value in practice, as having only one feature to choose from at each node will almost certainly increase the correlation between

trees. The random forest models using combined parameters and either a reference phantom or OPSSE approach for determining the attenuation slope performed best, with an OOB error of 25%.

TABLE 2 For a **linear** kernel, 5-fold cross-validation classification error for Multiclass Support Vector Machine Classification models created with the training dataset and used to determine optimal box constraint and kernel scale hyperparameters.

Attenuation Compensation	Spectrum Type	Model No.	5-fold Error	Optimized Hyperparameters Box Constraint
Reference Phantom	Fundamental	1	0.31	0.01
	Harmonic	2	0.32	0.01
	Combined	3	0.29	0.01
One Step Adventitia	Fundamental	4	0.31	1000
	Harmonic	5	0.33	0.1
	Combined	6	0.30	0.01
Two Step Adventitia	Fundamental	7	0.30	100
	Harmonic	8	0.33	0.1
	Combined	9	0.31	0.01
OPSSE	Fundamental	10	0.31	0.01
	Harmonic	11	0.32	0.01
	Combined	12	0.29	0.01

Box constraint and Kernel scale = Positive values log-scaled in the range [1e-3,1e3]

TABLE 3 For a **Gaussian** kernel, 5-fold cross-validation classification error for Multiclass Support Vector Machine Classification models created with the training dataset and used to determine optimal box constraint and kernel scale hyperparameters.

Attenuation Compensation	Spectrum Type	Model No.	5-fold Error	Optimized Hyperparameters	
				Box Constraint	Kernel Scale
Reference Phantom	Fundamental	1	0.31	1	10
	Harmonic	2	0.32	10	10
	Combined	3	0.30	1	10
One Step Adventitia	Fundamental	4	0.33	1	1
	Harmonic	5	0.33	100	100
	Combined	6	0.30	1	10
Two Step Adventitia	Fundamental	7	0.32	1	1
	Harmonic	8	0.32	10	10
	Combined	9	0.31	0.1	10
OPSSE determined attenuation slope	Fundamental	10	0.31	1	10
	Harmonic	11	0.32	10	10
	Combined	12	0.30	1	10

Box constraint and Kernel scale = Positive values log-scaled in the range [1e-3,1e3]

Support Vector Machine

The results from the hyperparameter optimization for SVM using linear, Gaussian, and polynomial kernels are located in Tables 2, 3, and 4 respectively. Unsurprisingly, the models that combine all parameters almost always produce the lowest classification errors, regardless of hyperparameter values and attenuation compensation type.

TABLE 4 For a **polynomial** kernel, 5-fold cross-validation classification error for Multiclass Support Vector Machine Classification models created with the training dataset and used to determine optimal box constraint and kernel scale hyperparameters.

Attenuation Compensation	Spectrum Type	Model No.	5-fold Error	Optimized Hyperparameters	
				Box Constraint	Polynomial order
Reference Phantom	Fundamental	1	0.33	0.01	2
	Harmonic	2	0.33	0.01	3
	Combined	3	0.31	0.01	2
One Step Adventitia	Fundamental	4	0.32	0.01	2
	Harmonic	5	0.33	0.001	3
	Combined	6	0.30	0.01	2
Two Step Adventitia	Fundamental	7	0.31	0.01	2
	Harmonic	8	0.32	0.01	2
	Combined	9	0.34	0.01	2
OPSSE	Fundamental	10	0.33	0.01	2
	Harmonic	11	0.33	0.01	3
	Combined	12	0.32	0.01	2

Box constraint = Positive values log-scaled in the range [1e-3,1e3]

Polynomial Order = Positive values in the range [2, 4]

Evaluation of optimal classification systems

Classification error for each cross-validated model was used for selecting the best three random forest and best three multiclass SVM models. In the event of a tie, the simplest algorithm, i.e. the model with the lowest computational cost, was selected. Each model was trained using its optimal hyperparameters, and the accuracy assessment of each classification system was based on the confusion matrix constructed from the predicted and known values of the test data set. Plaque ROI's were classified into three types: Calcium (Ca); Fibrous/Fibro-Fatty (F); Hemorrhagic and/or Necrotic Core (H/NC). Predictive accuracy, sensitivity, and specificity for each class in the test data set is displayed in Figure 5. The overall accuracy and kappa statistic are also listed. In regard to overall performance and tissue-specific predictive power, all models produced similar results.

DISCUSSION

The results of our study demonstrate that the simplest approach for the attenuation compensation produces comparable results to more complicated approaches. The OPSSE approach appears to provide the best results (see Table 5) but the differences are not statistically significant and thus the higher computational costs of the OPSSE approach could be a valid reason to simply implement a reference phantom approach. Overall accuracy of roughly 70% demonstrates that there is information provided by the algorithm but it is not clear if there is sufficient information for a clinically useful tool. Also, the results indicate that either random forests or SVM approaches provide reasonable results and other factors such as computation costs should be considered when implementing an approach.

This study indicates that the statistical classifiers obtained from nonlinearly generated second harmonic spectral parameters provide comparable results as the statistical classifiers built from fundamental band parameters. This is despite the fact that the signal processing approach treats the nonlinearly generated second harmonic as a linearly propagating signal. This assumption is valid after scattering but is not prior

to scattering. In addition, combining the fundamental and harmonic parameters generally provides a reduced error in classification. The use of the harmonic band for tissue characterization likely works for two key reasons: 1) attenuation is roughly consistent across patients as shown by the limited benefit of using the OPSSE approach in this work; 2) the nonlinearity parameter does not vary greatly for soft tissues [54]. Attenuation has a large influence on the intensity of the nonlinearly generated harmonic portion at the target tissue arising from the amplitude of the generation of second harmonic signal being proportional to the square of the amplitude of the fundamental. This use of the harmonic band is most likely comparable to having an extended bandwidth for the fundamental since we are relying on the nonlinearity and attenuation of the reference phantom to account for the amplitude of the harmonic portion at the target tissue location. We would expect that the benefits of harmonic imaging would also benefit tissue characterization based on the harmonic band, namely improved resolution with reduced clutter and coherent noise.

TABLE 5 Overall Accuracy, H/NC accuracy, sensitivity and specificity, and kappa statistic for select independently evaluated classification models. Tested the top three of each type of classifier.

STATISTIC [†]	COMBINED PARAMETERS					
	Random Forest			Multiclass Support Vector Machine		
	Reference Phantom	Two-Step Adventitia	OPSSE	Reference Phantom Linear	One-Step Adventitia Gaussian	One-Step Adventitia 2 nd order Polynomial
Overall Accuracy	0.68 ± 0.09	0.68 ± 0.09	0.69 ± 0.08	0.65 ± 0.09	0.66 ± 0.09	0.66 ± 0.09
Accuracy	0.84 ± 0.07	0.87 ± 0.06	0.84 ± 0.07	0.84 ± 0.07	0.84 ± 0.07	0.88 ± 0.06
Fibrous Sensitivity	0.83 ± 0.12	0.81 ± 0.13	0.84 ± 0.12	0.73 ± 0.14	0.78 ± 0.13	0.89 ± 0.09
Specificity	0.85 ± 0.08	0.90 ± 0.07	0.85 ± 0.08	0.90 ± 0.07	0.87 ± 0.07	0.87 ± 0.08
Hemorrhagic Accuracy	0.71 ± 0.08	0.70 ± 0.08	0.74 ± 0.08	0.68 ± 0.09	0.69 ± 0.08	0.68 ± 0.09
and/or Sensitivity	0.43 ± 0.16	0.51 ± 0.16	0.49 ± 0.16	0.43 ± 0.16	0.43 ± 0.16	0.35 ± 0.18
necrotic core Specificity	0.85 ± 0.09	0.79 ± 0.10	0.86 ± 0.07	0.79 ± 0.09	0.81 ± 0.09	0.83 ± 0.09
Calcium Accuracy	0.80 ± 0.07	0.78 ± 0.08	0.81 ± 0.07	0.78 ± 0.08	0.79 ± 0.07	0.77 ± 0.08
Sensitivity	0.76 ± 0.13	0.71 ± 0.14	0.76 ± 0.13	0.78 ± 0.13	0.76 ± 0.13	0.73 ± 0.13
Specificity	0.82 ± 0.09	0.82 ± 0.09	0.84 ± 0.08	0.78 ± 0.09	0.81 ± 0.09	0.78 ± 0.11
Kappa Statistic [‡]	0.52 ± 0.07	0.52 ± 0.07	0.54 ± 0.06	0.49 ± 0.07	0.49 ± 0.07	0.49 ± 0.07

[†] ± margin of error based on 95% confidence interval

[‡] ± Standard error

An average kappa statistic of 0.5 suggests that the combination of parameters work moderately well at distinguishing between fibrous, hemorrhagic and/or necrotic core, and calcified ROI's as determined by histology.

Conclusion

In general, spectral analysis based approaches show potential to noninvasively measure carotid plaque composition. Attenuation compensation based on 0.5 dB/cm-MHz attenuation coefficient slope is a reasonable choice for processing the backscattered signals, and the nonlinearly generated harmonic when treated as a linearly propagating signal provides useful information for tissue characterization. In summary, backscattered ultrasound may be able to locate regions of unstable plaque (i.e. hemorrhagic or necrotic core) to improve clinical care of patients with carotid stenosis. The clinical usefulness of this method will be determined by the ability to predict clinical outcome for patients presenting with carotid stenosis.

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Appendix D:

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Attenuation Compensation Comparison for Human Carotid Plaque Characterization Using Spectral Analysis of Backscattered Ultrasound

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Abstract— Carotid atherosclerotic plaque composition is a valuable predictor of stroke risk. Ultrasound spectral analysis has been successfully implemented clinically for determining plaque composition in coronary arteries via intravascular ultrasound. Noninvasive implementation for carotid plaque requires compensation for the attenuating 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 machine learning classification. Radiofrequency (RF) data was acquired from 20 subjects prior to carotid endarterectomy (CEA). 41 fibrous (F), 60 hemorrhagic and/or necrotic core (H/NC), and 54 calcified (Ca) regions of interest (ROI) were selected from the RF data corresponding to homogenous zones within the histology of the excised plaque tissue. 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; (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. While there were no statistically significant differences in the accuracy of the classification models based off each attenuation compensation approach, our work has shown that additional attenuation compensation may provide a benefit for characterizing carotid plaque.

Index Terms— Ultrasound, attenuation compensation, carotid plaque, tissue characterization

I. INTRODUCTION

The ability to predict future cerebrovascular accidents (CVAs), such as stroke, in patients with carotid atherosclerosis is currently limited. While a high degree of carotid stenosis is the primary determining factor for selecting patients for carotid endarterectomy (CEA) to prevent stroke, the benefit of CEA in asymptomatic patients with high grade stenosis and symptomatic patients with a non-significant degree of stenosis

is unclear.

Studies have shown that a large number of CVAs are caused by rupture-prone plaques with specific morphological features, regardless of degree of stenosis [1]–[3]. In carotid atherosclerosis, intra-plaque hemorrhage with a necrotic core distinguishes unstable, rupture-prone lesions from more stable ones. Thus, while plaque composition matters more than degree of stenosis in plaque rupture, it is generally unavailable at the point of care with current diagnostic tools.

While spectral analysis of intravascular ultrasound backscatter has been successfully implemented clinically for determining plaque composition in coronary arteries [4], [5], noninvasive implementation for carotid plaque characterization requires the ability to compensate the attenuation effects of overlying tissue in order to be sensitive to spectral parameters. This study examines the effects of four attenuation compensation techniques on characterizing human carotid plaque using spectral analysis and a random forest machine learning algorithm. Specific focus is placed on identifying hemorrhagic and/or necrotic core (H/NC) tissue, since this type of plaque tissue is most often associated with vulnerable or rupture-prone atherosclerotic lesions. An ultrasound-based technique that can noninvasively identify vulnerable lesions may aid in better risk stratification, particularly for asymptomatic patients and those with stenosis not considered clinically relevant.

II. HUMAN STUDY

A Siemens S3000 ultrasound system with Siemens Axius Direct Ultrasound Research Interface (URI) software was used to acquire beamformed RF data at a 40 MHz sampling rate from 20 subjects prior to carotid endarterectomy (CEA). Using a 9L4 linear array transducer, RF data was acquired in transverse slices approximately 1 cm apart throughout the plaque region. Histology slices of a portion of the excised plaque tissue obtained following removal during the CEA were prepared and matched to ultrasound frames. Regions of interest (ROI's), RF data points by 15 scanlines (~ 1.2 mm x 1.2 mm), were

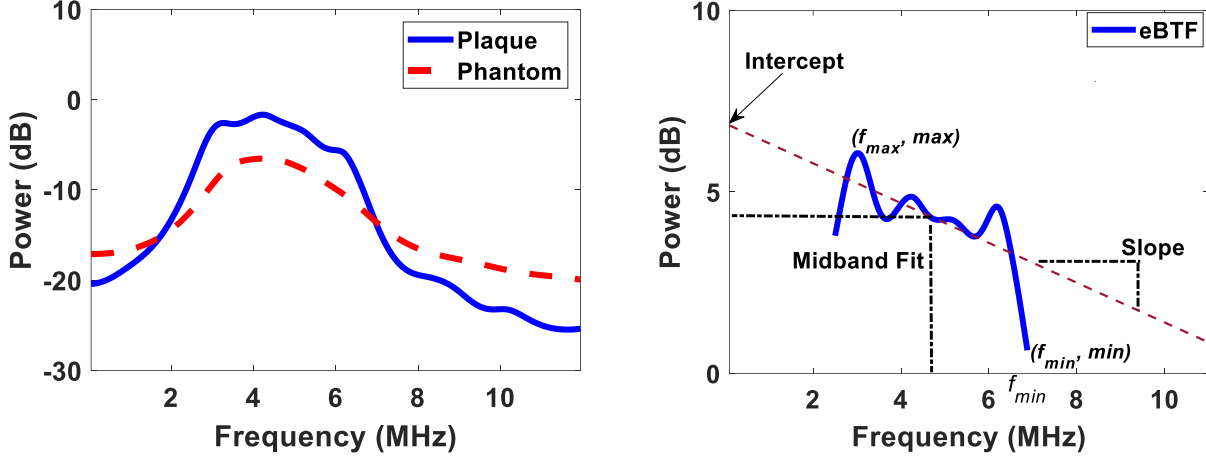


Fig. 1. Left – Sample average ROI power spectrum from plaque tissue and average reference phantom power spectrum. Right – eBTF within the usable 20dB down bandwidth of 2.5 -6.9 MHz. Linear fit parameters: slope, intercept and mid-band fit. Extreme points: maximum, minimum and corresponding frequencies. IB, a mean intensity parameter, was used

drawn in the RF data corresponding to homogenous zones within the matched histology slide. ROI's were categorized as F - fibrous ($n = 41$), H/NC - hemorrhagic and/or necrotic core ($n = 60$), or Ca - calcium ($n = 54$). Additionally, 209 ROI's were obtained from the adventitia (Adv) of six normal subjects.

III. SPECTRAL ANALYSIS

Under the assumptions of linear propagation, transmission and reception of the ultrasound wave, as well as single scattering (Born approximation) in a homogenous propagation medium, the power spectrum $S(f, d)$ of a backscattered RF signal can be expressed as the product of the transmit and receive transfer function $G(f)$, diffraction effects $D(f, d)$, attenuation from overlying tissue $A(f, d)$, and backscatter transfer function $B(f)$, represented as

$$S(f, d) = G(f) \cdot D(f, d) \cdot A(f, d) \cdot B(f, d). \quad (1)$$

Implementing spectral analysis to determine tissue composition requires separating the effects of the system, diffraction, and attenuation from the backscattered signal in order to be sensitive to the tissue properties. An autoregressive model (Yule-Walker, order = 24) was used to estimate an average power spectrum for each of the ROI's previously described. Below we discuss approaches to compensate for $G(f)$, $D(f, d)$, and $A(f, d)$ to obtain an estimate of the backscatter transfer function ($eBTF$) that is robust and sensitive to the scattering properties of the target tissue within each ROI.

IV. ATTENUATION COMPENSATION METHODS

A. Reference Phantom Method

For *in vivo* applications in soft tissue, the reference phantom method has been demonstrated to effectively compensate for attenuation and diffraction effects, as well as the system transfer function [1]. Reference RF data was acquired from a 0.5 dB/cm-

MHz phantom with identical transducer and system settings used to scan patients. A stable reference spectrum was obtained by averaging across 10 RF data frames and all scanlines. Each plaque ROI power spectrum was normalized to a reference power spectrum, $S_{ref}(f, d)$, corresponding to the same depth. An illustration of this normalization step is shown in figure 1. With the normalization step, the $eBTF$ is defined as

$$eBTF(f, d) = \frac{S_{sam}(f, d)}{S_{ref}(f, d)} \quad (2)$$

where the subscripts *sam* and *ref* represent the tissue sample and reference phantom, respectively. In a homogenous medium with uniform attenuation properties, the cumulative overlying attenuation is expressed as

$$A(f, d) = e^{\{-4\beta \cdot f \cdot d\}}, \quad (3)$$

where β is the slope of attenuation expressed in dB/cm-MHz. Substituting (1) and (3) into (2),

$$eBTF(f, d) = \frac{B_{sam}(f, d)}{B_{ref}(f, d)} e^{4(\beta_s - \beta_R)f \cdot d}, \quad (4)$$

we see that the difference between the reference phantom attenuation and the attenuation of overlying tissue layers may still have an impact on the backscattered signals. While the $eBTF$ does not need to be a perfect measure of the backscatter transfer function, it is desired that this robust approach permits the extraction of parameters sensitive to tissue properties. The following 3 attenuation compensation approaches were used to explore whether adding an additional attenuation compensation step to the phantom normalization improved our ability to characterize plaque tissue by enhancing separation of tissue properties.

B. Optimum power spectral shift estimation

The optimum power spectral shift estimator (OPSSE) [2], is

a correlation-based centroid shift estimator that improves upon the hybrid method [3] for estimating local attenuation coefficients in soft tissue. OPSSE provides an estimation of the center frequency shift with lower variance and higher stability compared to previous spectral shift methods [4], [5]. While the approach was initially used to isolate the scattering effects of the target tissue [2], we explore its use here as an attenuation compensation method to improve estimation of our power spectra by directly determining the attenuation of overlying tissue layers, β_R .

To calculate the attenuation of overlying tissue layers, RF data from a large window of interest ($\approx 10\text{mm} \times 5.5\text{mm}$) was divided into 5 axially consecutive blocks with 75% overlap. An average power spectrum was calculated for each block and normalized to a reference phantom. Following normalization, a Gaussian filter with center frequency (f_c) and bandwidth (σ) similar to the transmit pulse,

$$G(f) = e^{\left\{ -\frac{(f-f_c)^2}{2\sigma^2} \right\}}, \quad (5)$$

was applied to yield the Gaussian filtered normalized spectrum

$$GS(f, d) = e^{\left\{ -\frac{(f-f_c)^2}{2\sigma^2} \right\}} \cdot \frac{B_{sam}(f, d)}{B_{ref}(f, d)} e^{4(\beta_s - \beta_R)f \cdot d}. \quad (6)$$

We estimated f_c and σ by fitting a 2-term Gaussian model to the received signal from the reference spectrum at the depth of the axial transmit focus. The 0.5 dB/cm-MHz attenuation was added back into the spectrum prior to fitting. Steps in rearranging Eq. (6), shown in previous work [4], [5], ultimately suggest that the normalized and Gaussian filtered spectrum will have a Gaussian form centered at

$$f_c = f - (\beta_s - \beta_R)d\sigma^2. \quad (7)$$

OPSSE correlates the Gaussian filtered normalized spectrum with the following weight to estimate the shift in center frequency.

$$B_{opt} = \frac{d}{df} \left(\frac{1}{G(f)} \right) \quad (8)$$

A linear least-squares fit of consecutive center frequency shift estimates along depth was used to calculate the attenuation coefficient of the tissue layers overlying the carotid artery.

C. Adventitia-based attenuation compensation

Adventitia has been used as a reference for tissue characterization [6]. Our adventitia-based attenuation compensation approaches use backscatter from the adventitia of normal subjects as a reference to compensate for attenuation differences between the reference phantom and tissue layers overlying the ROI's. 209 ROI's were selected from the adventitia of 6 normal subjects. An average power spectrum was estimated for each ROI and normalized to a uniform reference phantom. A linear least-squares regression model was obtained for each frequency within the fundamental bandwidth by fitting a line to the graph of $eBTF(f)$ versus depth

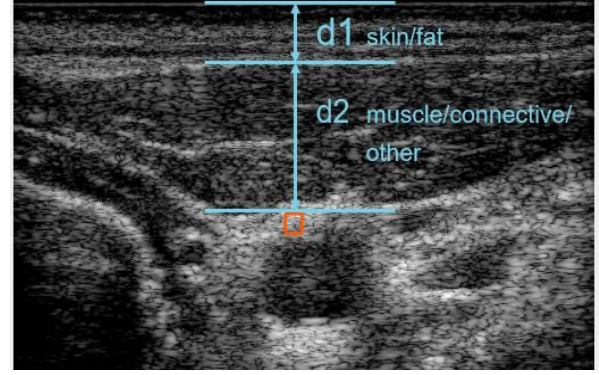


Fig 2. Sample grayscale image of a carotid artery from normal subject. Adventitia ROI in orange. The total depth of the ROI is the distance from the surface of the transducer to the starting edge of the ROI. Two layers of tissue comprise the total depth: (d1) skin/fat tissue and (d2) muscle, connective and other tissue.

for all 209 ROI's. For the one-step approach, depth was defined as the distance from the surface of the transducer to the starting edge of the ROI. The 2-step approach adds an additional parameter to the linear regression model by dividing the total depth into 2 layers, shown in figure 2: $d1$, skin and fat, and $d2$, the remaining muscle and other overlying tissue in the path to the ROI. An additional attenuation compensation was applied to each plaque ROI by inserting the ROI depth into the regression equations and subtracting the output from the $eBTF$.

D. Random Forest Classification

A linear least squares fit of the resulting $eBTF$ was performed over the fundamental bandwidth of 2.5 MHz – 6.9 MHz, and a set of eight spectral parameters, shown in figure 1, were used to classify ROI's. These are slope, y-intercept, mid-band fit, integrated backscatter and the maximum and minimum powers with their corresponding frequencies [7]–[11].

A classification model for each data set was created using the MATLAB® *treebagger* [7] function, which implements the random forest algorithm [8]. Two-thirds of the data was randomly selected for training the model, while the remaining one third was used for cross-validation. The predicted outcomes of the cross-validation sets were compared to the known plaque types from histology to obtain the predictive accuracy, sensitivity and specificity of the classification models:

$$\text{Predictive Accuracy} = \frac{\text{All Correct Decisions}}{\text{Total Cases}} \quad (9)$$

$$\text{Sensitivity} = \frac{\text{True Positive Decisions}}{\text{Decisions Actually Positive}} \quad (10)$$

$$\text{Specificity} = \frac{\text{True Negative Decisions}}{\text{Decisions Actually Negative}} \quad (11)$$

An overall measure of agreement between the predictions and histology interpretation was determined by the Kappa statistic κ ,

TABLE I
CLASSIFICATION SYSTEM ACCURACY

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

CI - 95% confidence interval

$$\kappa = \frac{n_a - n_e}{n - n_e}, \quad (12)$$

where n = number of samples, n_a = number of agreements and n_e = number of agreements due to chance. The measure should fall between 0 and 1. Generally, a result of zero indicates low agreement (i.e. low predictive accuracy), while a test with $0.70 \leq \kappa \leq 1$ indicates a substantial to almost perfect agreement, with histology [9].

V. RESULTS

The accuracy assessment of each classification system was based on the confusion matrix constructed from the predicted and known values of each cross-validation data set. Plaque ROI's were classified into three types: Calcium (Ca); Fibrous/Fibro-Fatty (F); Hemorrhagic and/or Necrotic Core (H/NC). Results in Table I focus primarily on H/NC versus not H/NC, since this tissue type is associated with vulnerable or rupture-prone plaques. Four measures were considered: overall Kappa statistic, H/NC accuracy, H/NC sensitivity and H/NC specificity. While there were no statistically significant differences found in any of the methods to accurately classify the tissue types, the 2-step adventitia-based compensation does trend toward being the best overall, with a Kappa statistic of 0.70, 80% accuracy, 85% sensitivity. Specificity was similar for all attenuation compensation methods, between 71% and 77%.

VI. DISCUSSION

This study combined ultrasound spectral analysis with a random forest machine learning algorithm to classify carotid plaque tissue. Four attenuation compensation methods were compared for their effects on the accuracy of our classification system. Specific focus was placed on H/NC. The results of this study demonstrate that a reference phantom alone may be sufficient for attenuation compensation. However, this was a preliminary analysis with a small sample size. Thus, trends in the data appear to suggest that additional compensation beyond the 0.5 dB/cm-MHz based phantom attenuation may be beneficial. Obtaining a larger sample size and more importantly, comparing directly to histology is needed for a better comparison between compensation approaches. While ROI's used here were taken from homogeneous areas, plaques are heterogeneous by nature, and overlap between spectral parameter values is a likely source of inaccuracy in classification.

VII. CONCLUSION

A 0.5 dB/cm-MHz attenuation coefficient slope is a reasonable choice for attenuation compensation. Our work has shown that additional attenuation compensation may provide a benefit. Specifically, using adventitia as a reference shows promise. The final determination of the best attenuation compensation approach to use will depend on comparison to histology.

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Appendix E

Fedewa RJ, James SL, Lyden S, Vince D. *Spectral Analysis of Nonlinearly Generated Second Harmonic Backscatter for Characterization of Human Carotid Plaque*. 2019 IEEE International Ultrasonics Symposium. 2019 October.

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

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Abstract— Plaque composition is generally unavailable for patients with carotid stenosis. 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. Human carotid plaque is collected following carotid endarterectomy from 46 subjects. Serial histology is performed and the slides are matched to grayscale images created from RF data collected *in vivo* prior to surgery. Regions of homogenous tissue (F-fibrous, HNC – hemorrhagic and/or necrotic core, Ca – calcium) are determined based on histology. The corresponding RF data is processed by computing an average power spectrum over a 1.2 mm by 1.2 mm region (15 lines by 64 points) and normalized 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. Random forests were created using eight spectral parameters for both fundamental and harmonic bands. The out of bag error is 0.26, 0.25, and 0.23 for forests based on fundamental data, harmonic data, and combined data sets.

Keywords—ultrasound, carotid, atherosclerosis, spectral analysis, carotid endarterectomy, tissue characterization, harmonic imaging

I. INTRODUCTION

Carotid plaque rupture and extra-cranial atherothrombosis are the predominant causes of cerebrovascular accidents (CVA) [1, 2]. The type of vulnerable or rupture-prone plaque most often associated with stroke and myocardial infarction is characterized by ulceration, intraplaque hemorrhage, necrotic core, and active inflammation [3], but knowledge of plaque composition is generally unavailable to clinicians considering the best course of treatment for patients with carotid stenosis. This is a greater concern for patients with diabetes since the diabetic sub-population in general presents with larger necrotic core regions

compared with non-diabetic patients for comparable stenosis [4].

Currently MRI is the only clinically available imaging modality able to provide compositional information, but MRI is unable to be a point-of-care tool for patients presenting with carotid stenosis arising from cost and availability limitations. Ultrasound contains the potential to be a point-of-care tool for obtaining compositional information regarding carotid plaque. Multiple ultrasound based approaches are being investigated to address the lack of compositional information available to clinicians in regards to carotid plaque. These include intravascular ultrasound [5], acoustic radiation force impulse (ARFI) [6], elastography combined with echogenicity and signal statistics [7], photo-acoustic measurements [8], and attenuation estimations [9]. In this work, we apply spectral analysis to the nonlinearly generated harmonic portion of the backscattered ultrasound signal to differentiate between carotid plaque tissue types. Preliminary work is shown for standard parameters extracted from normalized power spectra for both fundamental and harmonic bands.

II. METHODS

A. *In vivo* Ultrasonic Backscatter from Human Carotid

Two groups of subjects were recruited: normal subjects and subjects scheduled for a carotid endarterectomy (CEA). Both groups were age restricted to greater than 40 years since stenosis in younger patients is often due to other processes besides atherosclerosis. The CEA group inclusion criteria were that plaque must be located between the distal-end of the common carotid artery through the internal carotid artery including the common carotid artery bulb. Exclusion criteria were patients with prior ipsilateral carotid intervention. The normal group was defined by the absence of a past or upcoming carotid artery intervention on either side.

Each group underwent a research ultrasound exam that used either a Siemens S2000 or S3000 ultrasound system with a 9L4 probe (Siemens Medical Solutions USA, Inc., Malvern, PA). The sonographer chose sites separated by roughly 1 cm throughout the plaque. At each site location the transducer was

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

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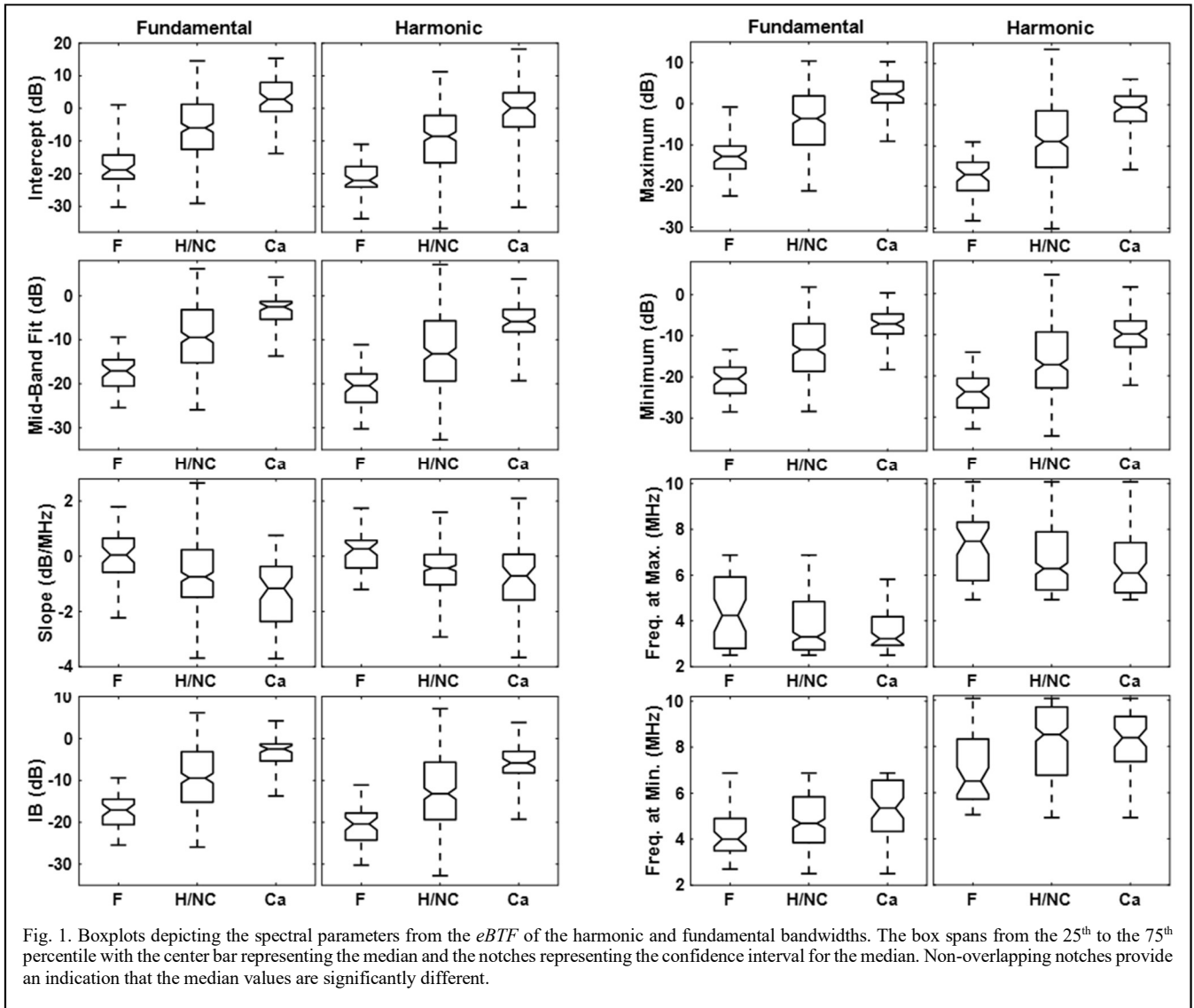
held still in a transverse orientation with respect to the carotid artery while 10 frames of RF data were collected using the Axius Direct software which records the post-beamformed RF signals prior to image processing. Following this acquisition, RF data was collected while slowly sliding the transducer along the skin and imaging the carotid artery in the transverse orientation. These cine loops were useful as backup data to the primary sites and more importantly provided the data necessary to locate the primary sites within the excised carotid plaque. For normal subjects, RF data was collected from both their right and left carotid arteries with 5 sites chosen centered on the bifurcation.

Each frame of RF data is composed of 456 lines by 2076 points digitized at 40 MHz. The ultrasound system settings were a 2 cm transmit focus, dynamic receive focus with a Hamming window, 4 cm depth, 9.00 MHz transmit setting, Tissue Harmonic Imaging enabled (pulse inversion), and mechanical index of 1.2. These settings and the TGC and overall gain were the same for all collections. The frame rate was 14 Hz in order to obtain pulse inversion data and maximize the number of lines per frame to aid the spectral averaging.

B. Histology Matching

For the CEA subjects, the excised plaque was collected following surgical removal. A total of 46 CEA subjects were enrolled and RF data was collected from 178 sites. The excised plaque (see bottom right panel in Fig. 2 and Fig. 3) were ultrasonically imaged in saline to locate the sites from the *in vivo* ultrasound data acquisition. The plaque tissue was fixed using formalin followed by de-calcification (Cal-Rite, Richard-Allan Scientific, Kalamazoo, MI). At this point, the plaque tissue is imaged again in water to locate the *in vivo* sites and the tissue is marked to indicate the matched region. These multiple imaging sessions with plaque insure that we maintain the *in vivo* site location through the fixation and decalcification steps. The tissue is then paraffin embedded and sliced in 1 mm steps proximal to distal before and after the marked site and 0.5 mm steps within the marked region. At each of these steps, a pair of slides are prepared with differing stains: Movat pentachrome and hematoxylin & eosin.

Each stack of slides is reviewed and compared to the fundamental and harmonic grayscale *in vivo* images using the ex



vivo imaging data as a guide along with notes containing the relative position of the cut within the *in vivo* images. The slide that best matches the *in vivo* site is located. Then an expert in atherosclerotic plaque reads the matched slide along with the slides immediately distal and proximal to the matched slide in order to locate homogenous tissue regions. Three categories of plaque were created: F- Fibrous or Fibro-Fatty; HNC – Hemorrhagic and/or Necrotic Core with or without microcalcifications; Ca – Calcified.

This process of data collection and matching began with 178 *in vivo* sites where RF data were collected. From these sites, matches with histology were found for 86 sites (48%). Inability to match slides was due to the following: plaque not being removed, plaque sent pathology, plaque removed in pieces and thus interfering with matching, and simply low or no confidence in locating a matched histology slide to the grayscale frame. The primary reason for failure to match in roughly half the cases is that either the tissue was not provided to research or was ripped or damaged in such a manner that a match could not be reliably made. Matched sites were found for 42 of the 46 CEA subjects.

Using customized code within the Matlab environment (Mathworks, Inc., Natick, MA), regions of interest (ROI's) are

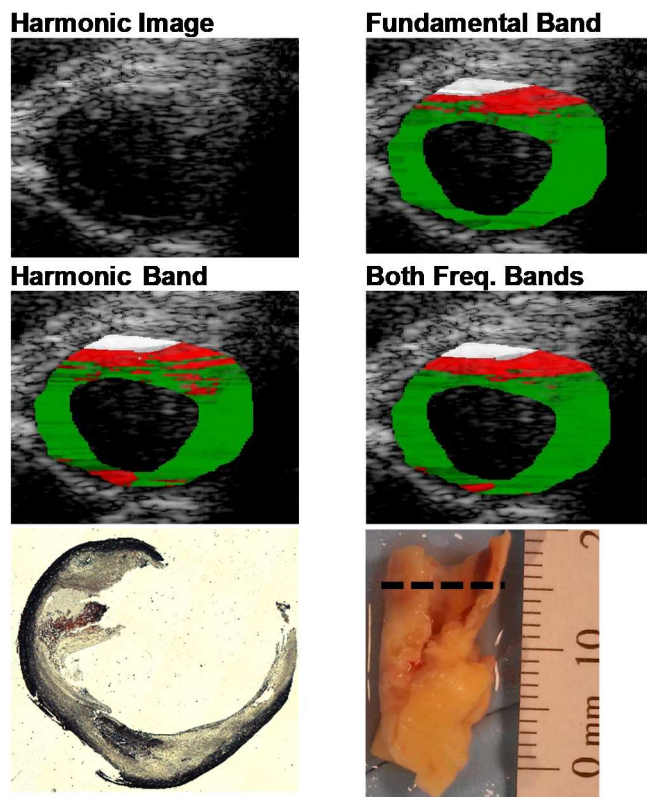


Fig. 2. Composition determined from spectral parameters for UMP0015s02 comparing the output from using parameters obtained from the Fundamental band, Harmonic band, or both frequency bands. Histology is shown in the lower left from a Movat pentachrome stain. The bottom left portion of the plaque contains a necrotic core region and the top of the plaque has calcified regions overlying a strip of hemorrhagic necrotic core with a fibrous region along the lumen however a portion of the plaque is missing on top. The bottom right image is the excised right carotid plaque with the dashed lined representing the position that matches the grayscale and histology images. Color Overlay Legend: F-Green, Ca – White, HNC – Red. Transparency level represents confidence of classifier.

defined within the RF data of 1.2 mm by 1.2 mm (64 points by 15 lines). Each ROI was placed in a region corresponding to a homogenous tissue type. A total of 378 ROI's were created with **62 Ca, 48 F, and 268 H/NC**.

C. Signal Processing

First step in the signal processing chain was to subtract the pulse inversion signals to obtain the fundamental signal and to add them together to isolate the harmonic spectrum. An average power spectrum was obtained from each ROI for each band through use of Yule-Walker autoregressive spectral estimation approach of order 24. The -20dB bandwidths were obtained from the phantom data: 2.5 MHz to 6.9 MHz for the fundamental and 4.9 MHz to 10.1 MHz for the harmonic. These signals were normalized using a reference phantom approach (Ultrasound Phantom Model 044, Computerized Imaging Reference Systems, Inc., Norfolk, VA) [10]. In addition, the one step adventitia based attenuation compensation [11] was implemented for both harmonic and fundamental signals to obtain an estimate of the backscatter transfer function (*eBTF*).

Eight spectral parameters were extracted from the *eBTF*: integrated backscatter (IB), linear fit parameters (slope, intercept, and mid-band fit), and the minimum and maximum values along with their corresponding frequencies. These served as input parameters for three random forests based on the

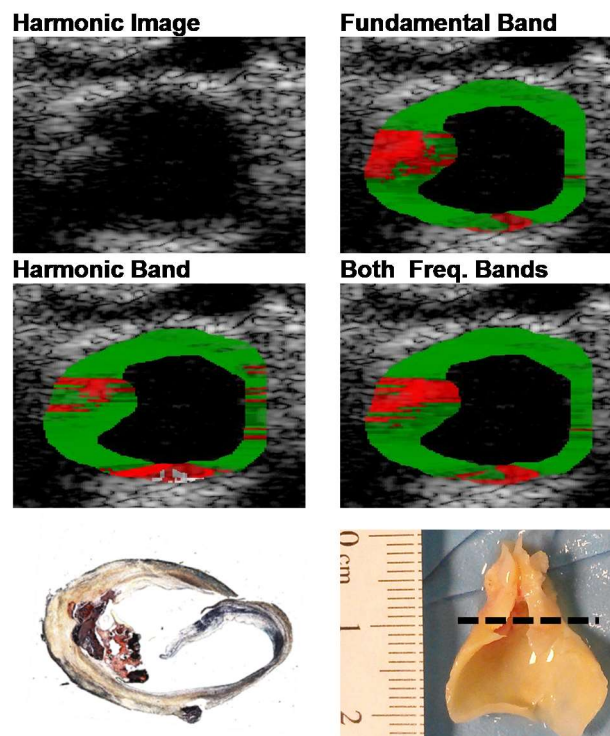


Fig. 3. Composition determined from spectral parameters for UMP0008s01 comparing the output from using parameters obtained from the Fundamental band, Harmonic band, or both frequency bands. Histology is shown in the lower left from a Movat pentachrome stain. The left side is dominated by a hemorrhagic necrotic core while the bottom nodule contains calcium. The bottom right image is the excised left carotid plaque with the dashed lined representing the position that matches the grayscale and histology images. Color Overlay Legend: F-Green, Ca – White, HNC – Red. Transparency level represents confidence of classifier.

following parameter sets: fundamental, harmonic, and both combined.

Random forests for each frequency band (and combined) were created within Matlab using 100 trees, 3 split parameters (4 for the combined), and 48 randomly chosen ROI's of each type for a balanced training set. Color overlays were produced using a 75% overlap of regions.

III. RESULTS AND DISCUSSION

The boxplots in Fig. 1 depict the parameters obtained from the fundamental and harmonic bandwidths. At a first glance, the general trends for each parameter are comparable for both the fundamental and harmonic. The intensity-based parameters (mid-band fit, integrated backscatter, minimum, maximum, and intercept) all provide similar outcomes and trends with F corresponding to the weakest intensity and Ca corresponding to the strongest. For all of these intensity-based parameters the median values are significantly different for both fundamental and harmonic data sets.

The slope box plots in Fig. 1 depict the F category as being closest to zero and thus closest to adventitia for this measure (even though the intensity of backscatter from adventitia is closest to Ca. The H/NC and Ca are indistinguishable for the slope for both fundamental and harmonic data. Similar results are seen with the frequency corresponding to the minimum value of the *eBTF*. Results of the frequency of the maximum for the harmonic do not exhibit statistical significance for the median values though the F median value for the fundamental is significantly different. When looking at all eight spectral parameters the harmonic data does provide useful information for distinguishing between tissue types and has the potential to be beneficial for tissue characterization.

The three random forests were produced to provide a preliminary evaluation of the use of harmonic data. The out of bag error is 0.26, 0.25, and 0.23 for forests based on fundamental, harmonic, and both bandwidths combined respectively. This change in error is slight which is not surprising when seeing the example compositional color overlays in Fig. 2 and Fig. 3. Specifically, in Fig. 2, the necrotic core region on the bottom of the plaque is missed by the fundamental version of the random forest and is reasonably obtained in the harmonic version with the combined providing a smoother top region and still retaining the bottom necrotic region. Similarly, in Fig. 3, the harmonic image identifies Ca along the bottom but appears to underestimate the H/NC region on the left side where the fundamental image better represents the histology.

IV. CONCLUSION AND LIMITATIONS

In this effort, we have applied spectral analysis for tissue characterization to the harmonic backscatter. Harmonic content demonstrates the ability to distinguish tissue types in carotid plaque and may be beneficial when combined with fundamental based input parameters. The benefits of harmonic imaging in clinical practice (reduced clutter, reduction in reverberations, improved out-of-plane mainlobe to sidelobe ratio, better resolution arising from higher frequency, etc.) are also likely to be a benefit for the use of harmonics in tissue characterization.

Limitations of this study include the following: No validation of the machine learning algorithms were performed in order to systematically compare the color overlay results with histology. No attempt was made to eliminate or weed out superfluous input parameters. Applying the reference phantom normalization approach to harmonic data is potentially flawed since the intensity of the harmonic signal has a greater dependence on the attenuation and aberration arising from the overlying tissue as compared to the fundamental band.

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Appendix F:

USPTO Patent Application 16/754414



US 20200364854A1

(19) **United States**(12) **Patent Application Publication**
Fedewa et al.(10) **Pub. No.: US 2020/0364854 A1**(43) **Pub. Date: Nov. 19, 2020**(54) **ADVANCED ULTRASONIC DETECTION OF
DIFFERENT TISSUE TYPES***A61B 8/00* (2006.01)*A61B 8/14* (2006.01)*A61B 8/08* (2006.01)(71) Applicant: **THE CLEVELAND CLINIC
FOUNDATION**, Cleveland, OH (US)(52) **U.S. Cl.**CPC *G06T 7/0012* (2013.01); *G06K 9/46*
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7/52036 (2013.01); *G01S 13/89* (2013.01);
A61B 8/469 (2013.01); *A61B 8/14* (2013.01);
A61B 8/0891 (2013.01); *A61B 8/463*
(2013.01); *A61B 8/5223* (2013.01); *G06K*
2209/05 (2013.01); *G06T 2207/30101*
(2013.01); *G06T 2207/10132* (2013.01); *G06K*
9/6276 (2013.01); *G06K 9/6269* (2013.01);
G06K 9/6282 (2013.01)(72) Inventors: **Russell J. Fedewa**, Cleveland, OH
(US); **David Geoffrey Vince**,
Cleveland, OH (US); **Hesham A.**
Elsharkawy, Cleveland, OH (US)(21) Appl. No.: **16/754,414**(22) PCT Filed: **Oct. 15, 2018**(86) PCT No.: **PCT/US2018/055949**

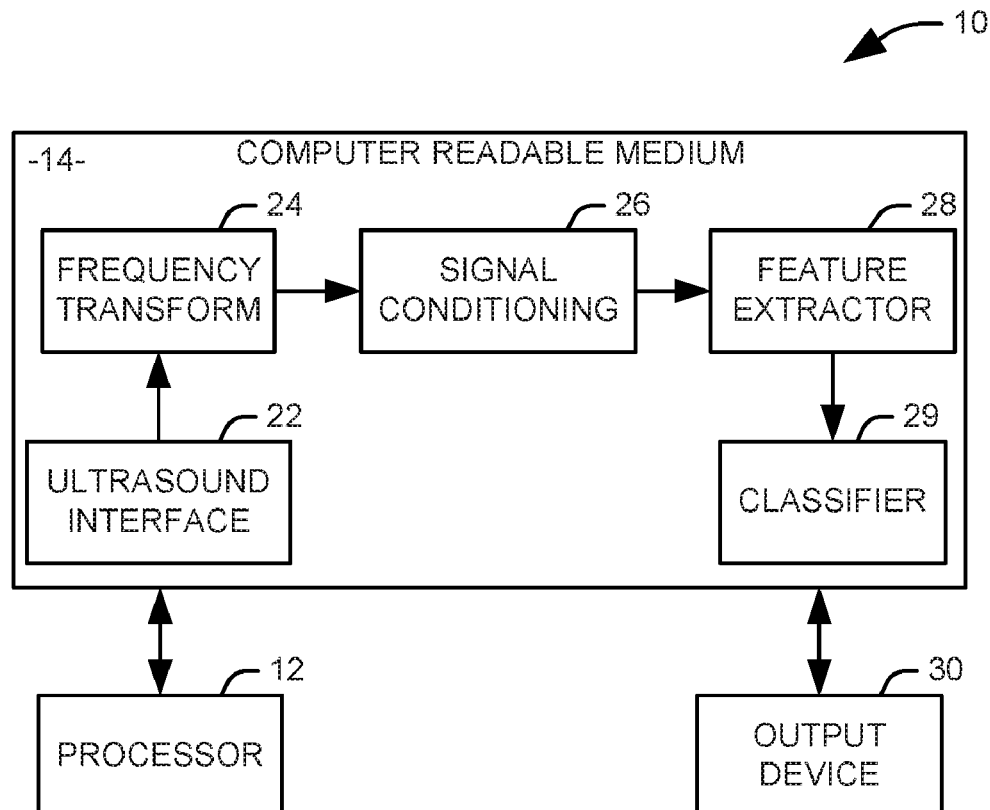
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(57)

ABSTRACT

A system includes a processor and, a computer-readable tangible storage device storing program instructions for execution by the processor. The program instructions include instructions for receiving ultrasonic derived data comprising an ultrasound image, representing a region of interest, and radio frequency “(RF)” data associated with the ultrasound image, and instructions for analyzing the RF data to identify at least one feature associated with a region of interest, including either or both of a feature representing a two-dimensional spectrum feature and a feature based on a cepstrum determined from the RF data. The program instructions further include instructions for classifying the region of interest as one of a plurality of anatomical classes from the identified at least one feature and causing a display to display the anatomical class.



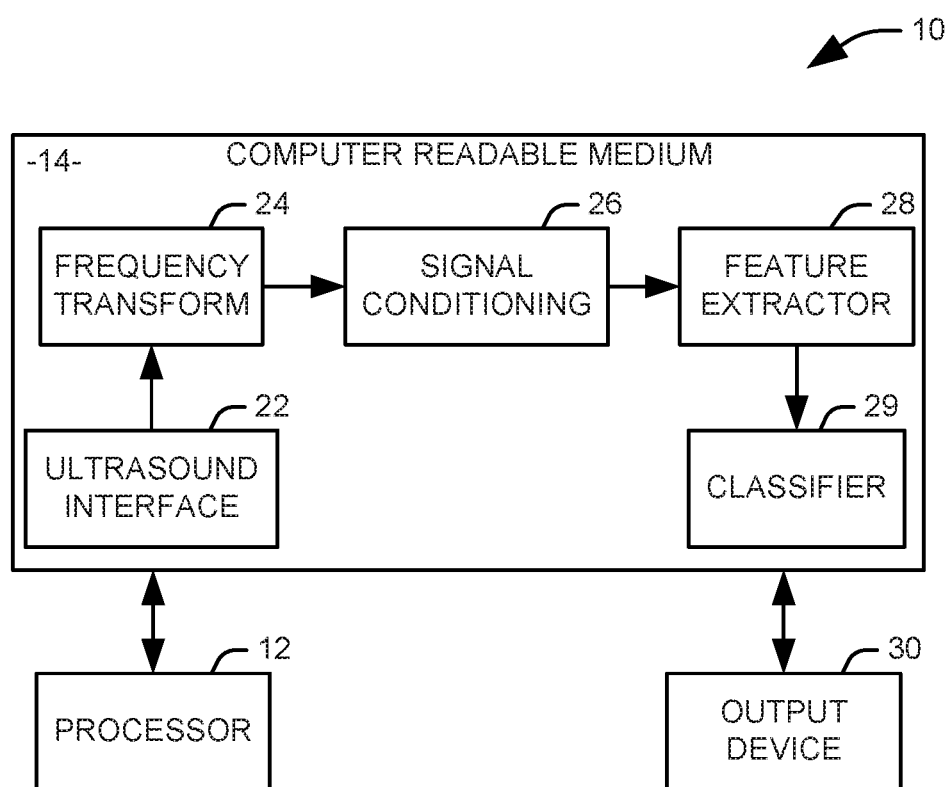


FIG. 1

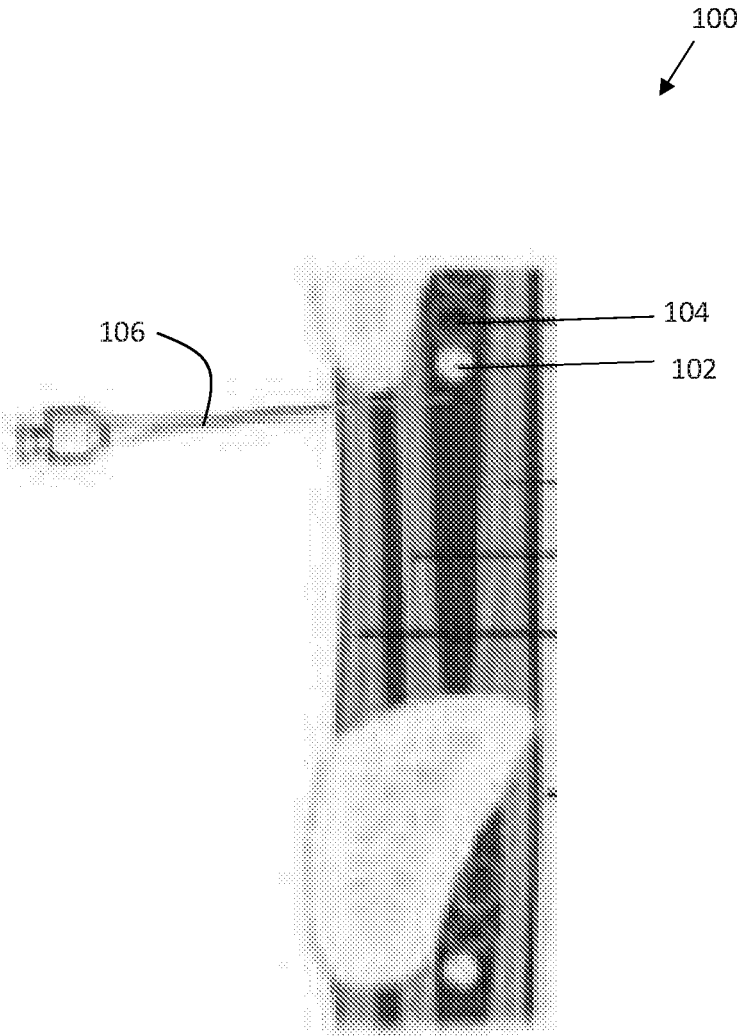


FIG. 2

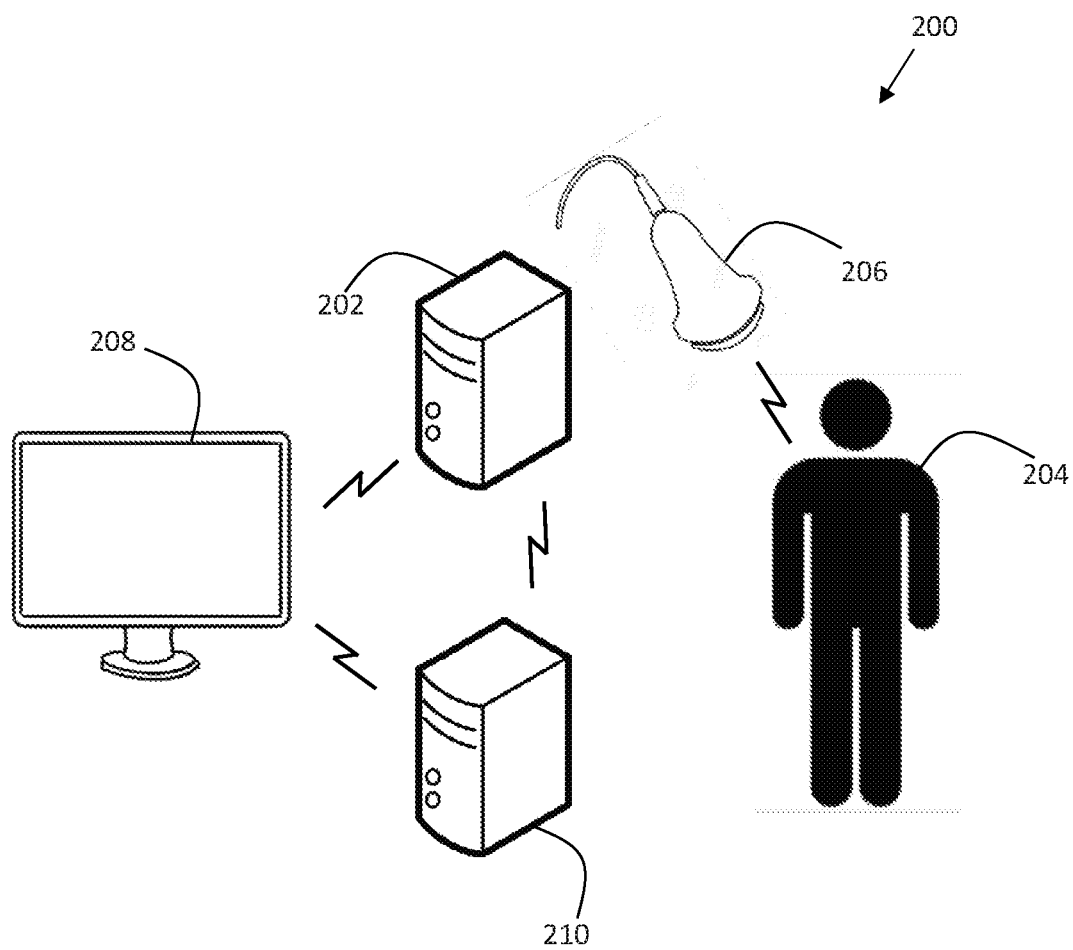


FIG. 3

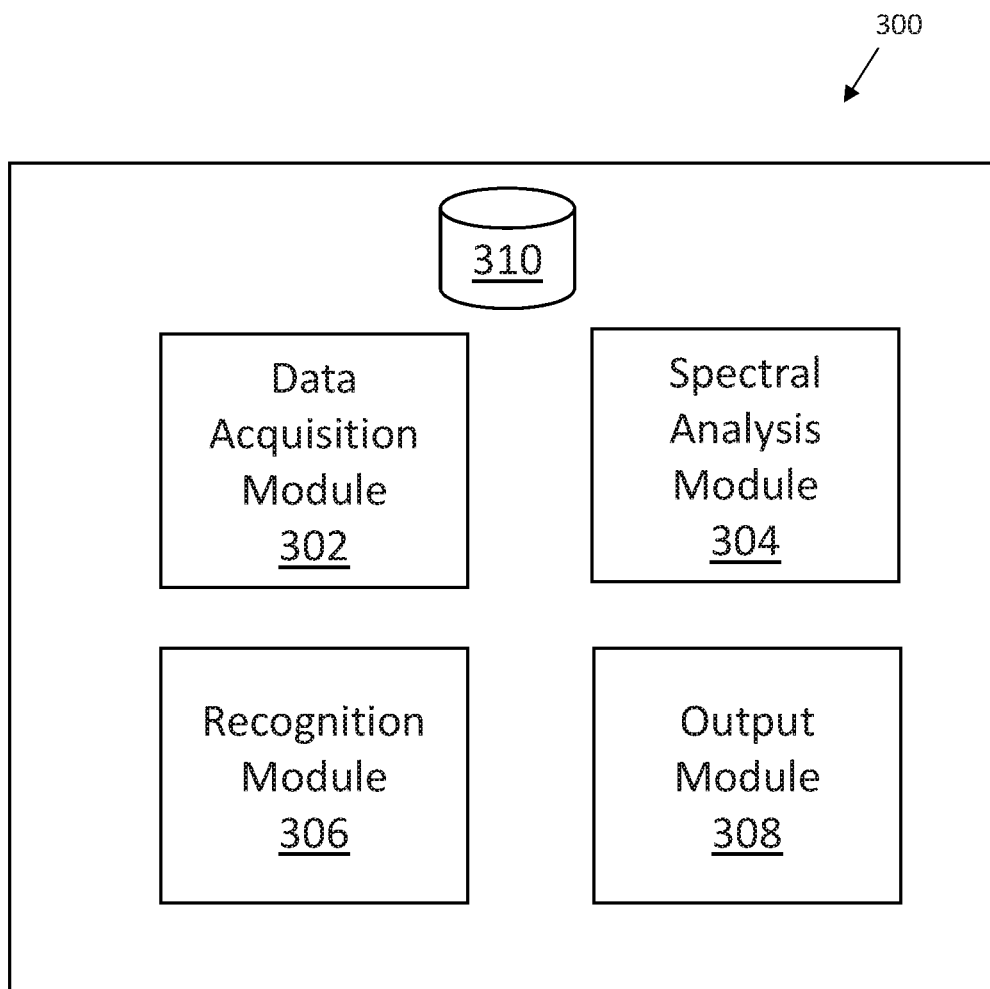


FIG. 4

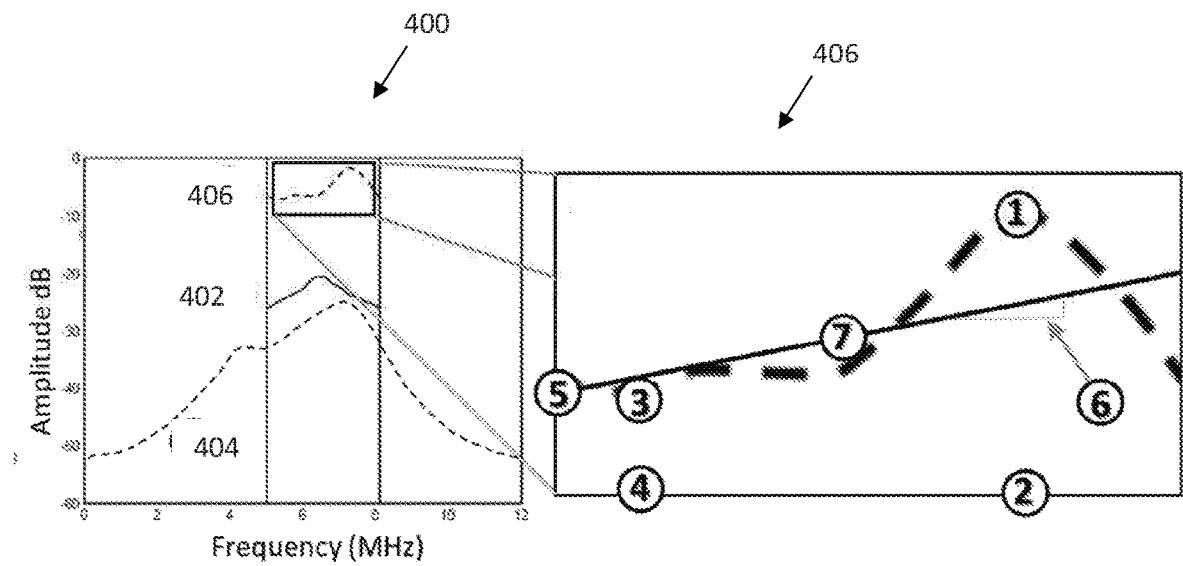


FIG. 5A

FIG. 5B

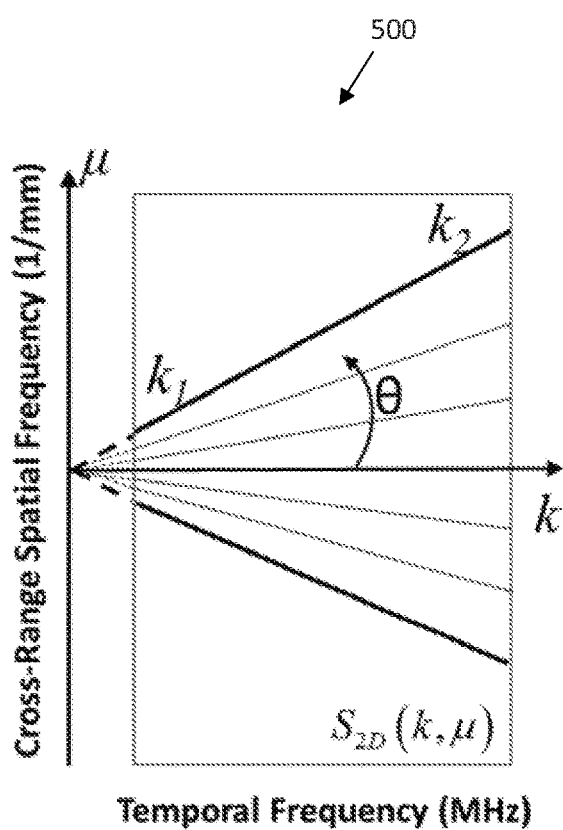


FIG. 6

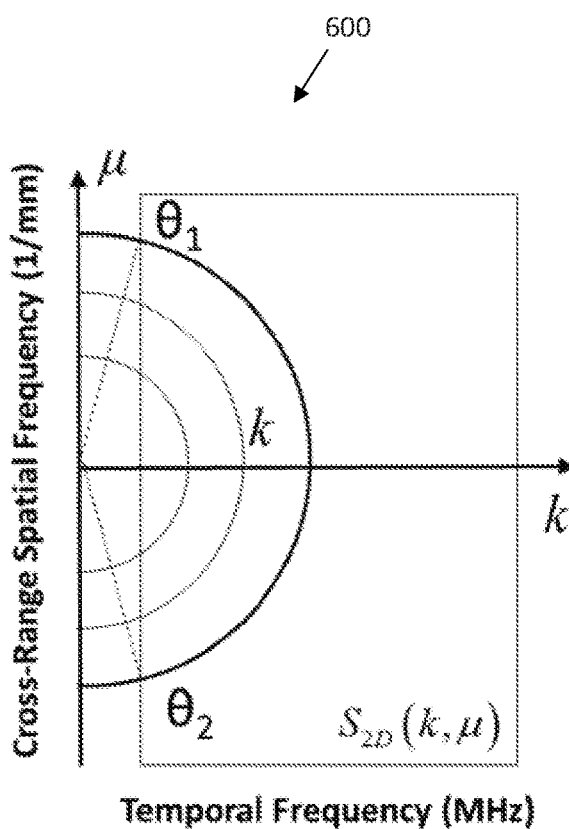


FIG. 7

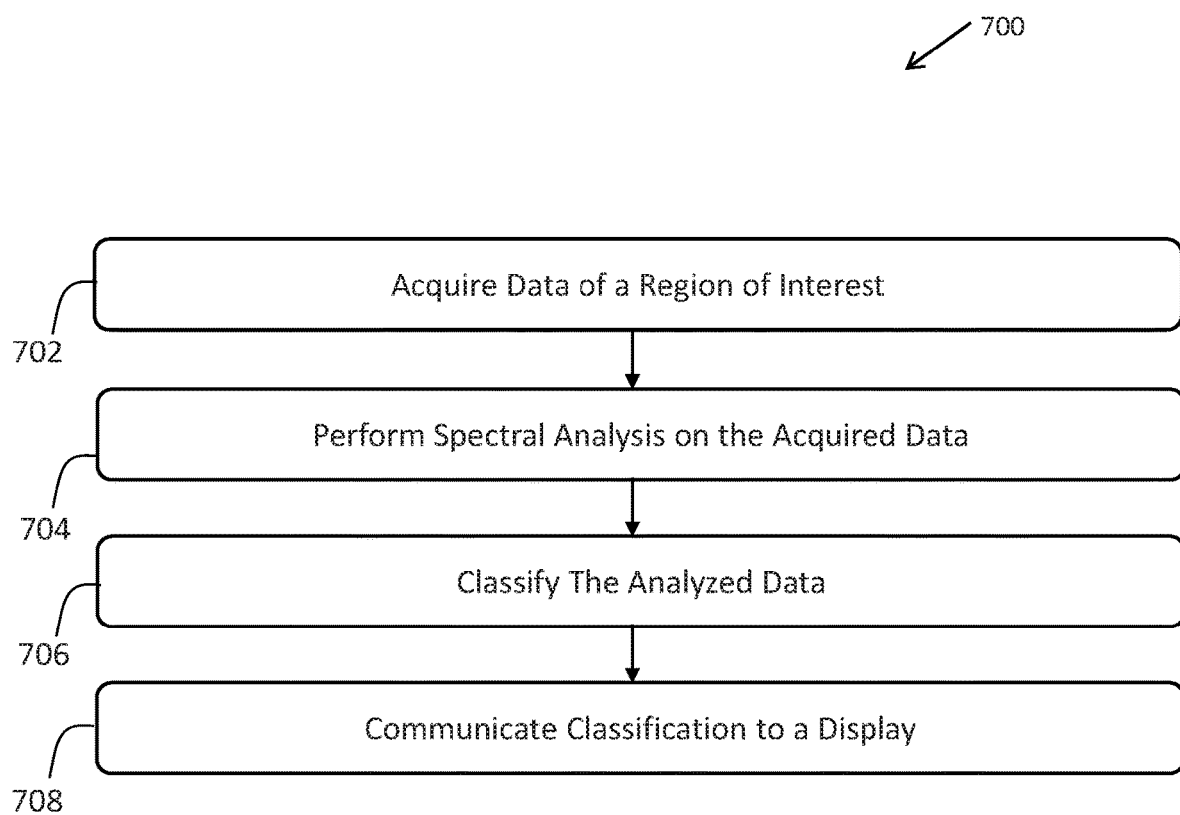


FIG. 8

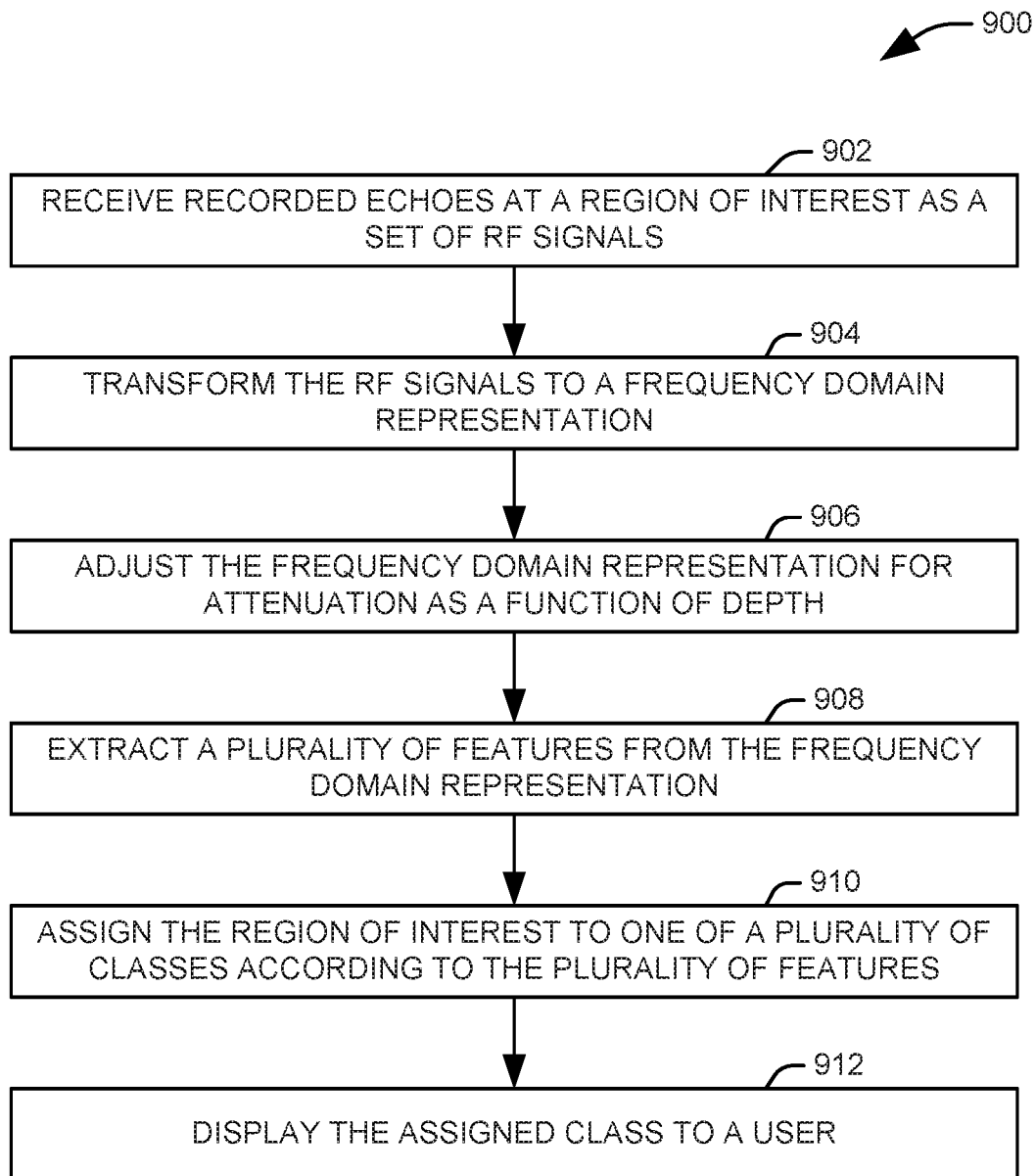


FIG. 9

ADVANCED ULTRASONIC DETECTION OF DIFFERENT TISSUE TYPES

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Ser. No. 62/571,850 (“the ‘850 application”), filed Oct. 13, 2017 under Attorney Docket Number CCF-027057 and entitled ULTRASONIC SPECTRAL PARAMETER DETECTION OF NEUROVASCULAR BUNDLES. The entirety of the ‘850 application is hereby incorporated by reference.

TECHNICAL FIELD

[0002] This disclosure relates to medical imaging and, more particularly, to ultrasonic spectral parameter detection of different tissue types, machine learning for merging input data from multiple ultrasonic sources, and use of cepstral parameters to aid in differentiating between tissue types.

BACKGROUND

[0003] Paravertebral nerve block (“PVB”) is a technique involving the injection of anesthetic in a space immediately lateral to where the spinal nerves emerge from the intervertebral foramina. PVB is commonly used for treatment of acute and chronic pain and as analgesia for various surgical procedures such as thoracic and abdominal procedures. The paravertebral space is wedge-shaped and lies on either side of the vertebral column. It is defined posteriorly by the superior costotransverse ligament (one side of the wedge), anterolaterally by the pleura (the other side of the wedge), and at the base of the wedge by the vertebral body and intervertebral disk. It extends laterally, in a continuous fashion, to the intercostal space. The intercostal artery, intercostal vein, and intercostal nerve are all contained in this space, originating medially at the spine, traversing through the paravertebral space, and extending laterally between the ribs into the intercostal space. Injection of a local anesthetic to the paravertebral space can result in ipsilateral somatic block in multiple contiguous dermatomes.

[0004] PVB is commonly performed using various modes of guidance, including landmark recognition, loss of resistance, nerve stimulation, or live fluoroscopic or ultrasound image guidance. However, using landmark recognition techniques may result in less than desirable levels of analgesia. Image guidance and live visualization of the injection typically results in more appropriate levels of analgesia and fewer complications. However, fluoroscopy subjects the patient and the anesthesiologist to a radiation dose and requires the use of contrast agents, which may not be desirable.

[0005] Ultrasound imaging is non-ionizing and non-invasive and therefore may be more desirable. Moreover, due to the real-time visualization of the needle, using ultrasound guided PVB may result in improved outcomes, reduced complications, and higher success rates. However, while visualization using ultrasound can reduce the risk of some complications, the intercostal vessels and intercostal nerve are difficult to identify during the needle injection because peripheral nerves and their associated blood vessels tend to have similar acoustic impedance as the surrounding tissue. Moreover, Doppler ultrasound may fail to locate the blood vessels arising from the need for a non-normal angle of

incidence which is often difficult to achieve. Thus, a user such as a physician performing a PVB may rely on physical landmarks and relative resistance from inserting a needle in order to find a correct injection location, which may not be effective or accurate. This may result in other complications.

[0006] For carotid plaque, duplex ultrasound is commonly used for determining the position and extent of plaque within the carotid arteries. Specifically, Doppler ultrasound is used as a surrogate for the degree of stenosis. Other imaging modalities can also provide this measure: computed tomography and magnetic resonance angiography. However these are both significantly more expensive and CT exposes the patient to ionizing radiation. The degree of carotid stenosis is a primary measure to determine how best to treat the patient, but it is known plaque composition is a better approach for determining the risk of future stroke and thus composition is more important for determining how best to treat the patient.

[0007] Composition of carotid plaque is not available through any imaging modality used clinically. Magnetic resonance struggles to distinguish between fibrous and hemorrhagic plaque (former is stable while the latter is unstable). CT is very good at determining the calcium burden but struggles with distinguishing between soft tissue types. VH-IVUS is available but requires an invasive procedure to place an intravascular ultrasound probe within the carotid artery.

SUMMARY

[0008] In one example, a system includes a processor and, a computer-readable tangible storage device storing program instructions for execution by the processor. The program instructions include instructions for receiving ultrasonic derived data comprising an ultrasound image, representing a region of interest, and radio frequency (“RF”) data associated with the ultrasound image, and instructions for analyzing the RF data to identify at least one feature associated with a region of interest, including either or both of a feature representing a two-dimensional spectrum feature and a feature based on a cepstrum determined from the RF data. The program instructions further include instructions for classifying the region of interest as one of a plurality of anatomical classes from the identified at least one feature and causing a display to display the anatomical class.

[0009] In another example, a system for classifying a region of interest as an anatomical feature includes a processor and a non-transitory computer readable medium storing executable instructions executable by the processor. The instructions include an ultrasound interface that receives, from an associated ultrasound system, recorded echoes from the region of interest as a set of radio frequency (RF) signals and constructs an ultrasound image from the RF signals. The RF signals including a set of harmonic signals being extracted from a frequency range associated with second harmonics of the recorded echoes. A frequency transform component transforms the RF signals from a time domain representation to a frequency domain representation. A feature extractor extracts a plurality of features from the frequency domain representation, with at least one of the plurality of features representing the set of harmonic signals. A classifier assigns the region of interest to one of a plurality of classes representing anatomical features according to the extracted plurality of features.

[0010] In yet another example, a method is provided. Recorded echoes from a region of interest are received as a set of radio frequency (RF) signals. The RF signals are transformed from a time domain representation to a frequency domain representation. The frequency domain representation is adjusted for attenuation as a function of the depth of the region of interest. A plurality of features are extracted from the frequency domain representation. The region of interest is assigned to one of a plurality of classes representing anatomical feature according to the extracted plurality of features at a classifier model.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 illustrates a system for classifying a region of interest as one of a plurality of anatomical features.

[0012] FIG. 2 illustrates an example in which the system is used in the paravertebral space.

[0013] FIG. 3 illustrates an example system for identifying and classifying anatomical features.

[0014] FIG. 4 illustrates a block diagram of an example classification computer used by the system in FIG. 3.

[0015] FIG. 5A is a graph illustrating an average spectrum from a single region-of-interest including a normalized spectrum.

[0016] FIG. 5B is a graph illustrating a zoomed in view of the normalized spectrum of FIG. 5A.

[0017] FIG. 6 is a graph illustrating example geometry for computing radially-integrated spectral power.

[0018] FIG. 7 is a graph illustrating angularly-integrated spectral power.

[0019] FIG. 8 illustrates an example method for identifying and classifying a region of interest in an anatomy of a patient.

[0020] FIG. 9 illustrates a method for classifying a region of interest into one of a plurality of classes representing anatomical features.

DETAILED DESCRIPTION

[0021] This disclosure relates to medical imaging and, more particularly, to systems and method for identifying anatomical features within a region of interest. An anatomical feature can include any of a type of tissue, an absence of tissue (e.g., a cavity), or a known artifact caused by the presence of a certain tissue type (e.g., acoustic shadowing at the selected region of interest), or purposely caused changes in the tissue properties (e.g. changes in acoustic backscatter from heating tissue). FIG. 1 illustrates a system 10 for classifying a region of interest as one of a plurality of anatomical features. The system 10 includes a processor 12 and a non-transitory computer readable medium 14 storing executable instructions executable by the processor.

[0022] The computer readable medium includes an ultrasound interface 22 that receives, from an associated ultrasound system (not shown), recorded echoes from the region of interest as a set of radio frequency (RF) signals and constructs an ultrasound image from the RF signals. The RF signals include both signals representing a fundamental frequency associated with the ultrasound device at the time of transmitting the ultrasound from the transducer, as well as a set of harmonic signals representing a frequency range associated with nonlinearly generated second harmonic that was created during propagation or scattering of the ultra-

sound. In one implementation, the fundamental frequency range is between 3 and 7 MHz, with second harmonics between 5.3 and 8.7 MHz.

[0023] The inventors have determined that conditioning of the signal and extraction of appropriate classification features is simplified by converting the received signals to a frequency domain representation. Accordingly, the instructions include a frequency transform component 24 that transforms the RF signals from a time domain representation to a frequency domain representation. In one implementation, the frequency transform component 24 uses an autoregressive spectral estimation technique to the signal to provide a power spectrum. In this example, a given region of interest can be represented as fifteen sets of sixty-four measurements. Each line can be converted separately, and then averaged, after a transformation into decibels, to provide the frequency domain representation.

[0024] The resulting frequency domain representation is then provided to a signal conditioning component 26. The signal conditioning component 26 deconvolves the frequency domain representation of the RF signals to remove a transfer function of the ultrasound system and adjusts the frequency domain representation of the RF signals for attenuation. For a homogenous propagation medium, the power spectrum ($S(f,d)$) of received signals scattered at given depth (d) within the focal zone of the ultrasound system can be represented as $S(f,d)=T(f)R(f,d)D(f,d)A(f,d)B(f)$

[0025] where f is the frequency, $T(f)$ and $R(f,d)$ represents the transmit and receive transfer functions of the transducer and system electronics of the ultrasound system through beam formation, $D(f,d)$ includes the effects of diffraction, $A(f,d)$ accounts for the overlying attenuation, and $B(f)$ is the backscatter transfer function.

[0026] In one implementation, an ideal reflector can be used to determine $T(f)$, $R(f, d)$, and $D(f,d)$ in a water tank. In another implementation, simulation of transmit and receive aperture beam information, as well as receive beam information (with gain stages) to obtain the spectral compensation for these items. A third implementation could use a hydrophone to determine both $T(f)$ and $D(f,d)$ while introducing a point source of ultrasound to directly measure $R(f,d)$ as a function of distance and frequency. Regardless of how estimates of $T(f)$, $R(f, d)$, and $D(f,d)$ are obtained, they can be used to compensate the frequency representation of the RF signals for these effects.

[0027] Analysis of backscattered RF data from a uniform reference phantom may also be performed to obtain an estimate of determine $T(f)$, $R(f, d)$, and $D(f,d)$ and thus compensate the received signal. The RF backscattered from the phantom is able to account for diffraction effects, transmit transfer function, and receive transfer function. It also performs a partial compensation for attenuation. The phantom needs to be a phantom with uniform scattering and acoustic properties. Ideally, this phantom should exhibit a comparable speed of sound as the tissue. For example, the CIRS model 044 phantom has a speed of sound of 1540 m/s and a uniform material with an attenuation of 0.5 dB/cm-MHz. Data is obtained from the phantom by collecting multiple frames from different regions in the phantom and then averaging the estimated power spectra across all lines and frames for the same depth as the in vivo region of interest, thus creating a reference power spectrum. Dividing the region of interest power spectrum by the reference power

spectrum (or subtracting the reference power spectrum from the region of interest power spectrum if both are in decibels) removes the effects of the transmit and receive electronics and transducer, diffraction, and part of the effects of attenuation.

[0028] The signal conditioning component **26** can further provide compensation for attenuation to obtain an estimate of the backscatter transfer function (eBTF). In one implementation, a standard attenuation compensation (e.g. 0.5 dB/cm-MHz) can be used. If a reference phantom approach is used, the inherent attenuation of the phantom may suffice to obtain a useful eBTF for tissue characterization. In another implementation, attenuation compensation based on backscatter from tissue within the patient having known properties can be used. For example, in an implementation for identifying arterial plaque within an artery, adventitial tissue surrounding artery can be used. Alternatively, values for the known tissue in a normal subject or ex vivo data can be used. In either example, backscatter from the known tissue can be used to compute an estimated attenuation compensation as a function of depth for each of separate frequencies spanning the useable bandwidth. Further, the attenuation value can be adjusted based on the relative thickness of the overlying skin and fat regions as compared to the overlying muscle in the path for each patient, allowing an attenuation compensation that is adaptable to each specific patient. Finally, a spectral based approach, such as an Optimum Power-Spectral Shift Estimator (OPSSE) approach can be used.

[0029] Both the fundamental and nonlinearly-generated second harmonic portions of the backscattered data will require attenuation compensation and may require different approaches for these two types of signals. Specifically, the best attenuation compensation approach for the fundamental may likely not be the same as for the nonlinearly-generated second harmonic and vice versa, requiring the application of multiple methods for compensation.

[0030] The compensation approach to obtain an estimate of the backscatter transfer function (eBTF) using the reference phantom may be written as follows with dependence on both depth (d) and frequency (f):

$$eBTF(f) = \frac{S(f, d)}{S_{ref}(f, d)} e^{A_{\Delta} d} \quad (2)$$

[0031] where $S(f, d)$ is the measured signal from the carotid plaque, $S_{ref}(f, d)$ is the backscattered signal from the reference phantom and $e^{A_{\Delta} d}$ is the attenuation coefficient to compensate for the difference between the reference phantom attenuation and the attenuation of the overlying layers of tissue between the transducer and adventitia. This compensation attenuation is determined through one of the attenuation estimation approaches listed above, and thus each of the fundamental frequencies and the harmonic frequencies can be represented by a backscatter transfer function.

[0032] A feature extractor **28** extracts at least one feature from the RF signal. In one example, at least one feature is extracted from the set of harmonic signals. Accordingly, for each of the eBTF for the fundamental frequencies and the harmonic frequencies, exemplary features can include the slope, mid-band fit, and intercept values for a regression line fit to the eBTF as a function of frequencies, an integrated

backscatter over each of the upper half of the bandwidth, the lower half of the bandwidth, and the entire bandwidth, maximum and minima values in the eBTF, frequencies corresponding the maximum and minimum values, and two values extracted from a cepstrum of the eBTF, a peak cepstral value and a frequency associated with the peak cepstral value.

[0033] A classifier **29** assigns the region of interest to one of a plurality of classes representing anatomical features according to the extracted at least one feature. In one example, the classifier **29** can include one or more of a random forest model, a support vector machine, and a K nearest neighbor model. The assigned class can be stored at the non-transitory medium **20** and provided to a user at an associated output device **30**, such as a display.

[0034] FIG. 2 illustrates identification of nerves **102** and blood vessels **104** using a machine learning algorithm to perform spectral analysis of data obtained from ultrasound imaging of a region of interest such as a paravertebral or intercostal space **100**. Identifying the nerves **102** enables visualization of the nerves while performing a medical procedure such as a paravertebral nerve block (“PVB”) **106**, which may result in a reduction in complications or adverse events from occurring during the procedure. In addition, identifying the blood vessels **104** enables visualization of the blood vessels **104** while performing a medical procedure, such as a PVB **106**, may permit robust pain management since vessels can act as conduits for the anesthetic and reduce efficacy. This may improve patient comfort and outcomes. Although the example systems and methods described herein may refer specifically to PVB **106** procedures, the systems and methods described may similarly be used in other applications for locating nerves **102** and blood vessels **104**, which may lead to the ability to minimize or avoid damage to nerve tissue and to improve outcomes. Further, it will be appreciated that the system can be used to identify other tissue and non-tissue content on the image. In one implementation, the system can differentiate between ligament tissue, intercostals muscle, other muscle tissue, pleura, shadow from the ribs or other bone, and paravertebral space, as well as nerves and blood vessels. In another implementation, it can differentiate between ablated tissue and normal tissue in the absence of microbubbles. In still another implementation, the system can differentiate between different types of arterial plaque.

[0035] FIG. 3 illustrates an example system for identifying and classifying tissue and other anatomical structures (hereinafter referred to as “system”) **200** in a region of interest, for example, located within a paravertebral space. The system **200** includes an ultrasound computer **202** for collecting ultrasonic derived data of a patient **204** via a probe **206**. The probe **206** may be directed towards any region of interest of the patient **204**. In addition to capturing ultrasound images, the probe **206** also enables capturing of radio frequency (“RF”) signals corresponding to the ultrasound images. In particular, the probe **206** transmits pressure waves or pulses into the tissue. Depending on the tissue density, the pulses scatter and reflect, some of which reflect and scatter back towards the probe **206**. The probe **206** converts these pulses into a received electrical signal, or an RF signal.

[0036] The system **200** also includes a display **208** for displaying a display generated by the ultrasound computer **202** based on the collected ultrasonic derived data. For

example, the display 208 may display ultrasound images relating to a region of interest captured by the ultrasound computer 202.

[0037] The system 200 further includes a region of interest classification computer (hereinafter referred to as “classification computer”) 210. Although the classification computer 210 and the ultrasound computer 202 are illustrated as two distinct computers, the classification computer 210 and the ultrasound 202 computer may be combined into a single computing system (not shown). For example, the ultrasound computer 202 may be configured to provide the features and functionality of the classification computer 210 described herein.

[0038] The classification computer 210 is configured to receive ultrasonic derived data collected by the ultrasound computer and classify a region of interest into one of a plurality of anatomical classes. A given anatomical class can represent, for example, a type of tissue, an absence of tissue, or a known artifact caused by the presence of a certain tissue type (e.g., acoustic shadowing). In one example, the classification computer 210 is configured to receive from the ultrasound computer 202 ultrasonic derived data including acoustic radiation force impulse (hereinafter “ARFI”) derived data. ARFI data may include ARFI relative stiffness data which measures the relative tissue stiffness arising from how much displacement follows the application of an ARFI push pulse. ARFI data may further include shear wave elasticity imaging (hereinafter “SWEI”), which uses an ARFI push pulse to generate shear waves propagating orthogonal to the direction of the insonifying ultrasound and ARFI data may include derivatives of the displacement measurement (e.g. velocity and acceleration). Imaging is then performed to track the shear wave propagation speed, which is directly related to Young’s modulus of the tissue.

[0039] The classification computer 210 is further configured to receive from the ultrasound computer 202 ultrasonic derived data including spectral parameters of ultrasonic backscatter which is obtained from an estimate of a backscatter transfer function (“eBTF”). The eBTF may be obtained by deconvolving the effects arising from the transmit and receive transfer functions of ultrasound computer 202, the effects of diffraction, and the effects of attenuation. The eBTF is a normalized power spectrum from which parameters are obtained from the useable bandwidth. The obtained parameters include three linear fit parameters including slope, intercept, and mid-band fit. The obtained parameters further include a root mean square (“RMS”) deviation of the eBTF from the linear fit. The obtained parameters further include three integrated backscatter (“IB”) parameters, or summation over defined bandwidths of the area under the eBTF, including full bandwidth, lower half of the bandwidth, and upper half of the bandwidth. The obtained parameters further include four maximum and minimum values and the corresponding frequencies over useable bandwidth of the eBTF. The obtained parameters further include two cepstral parameters, including peak value and the frequency of the peak value. A cepstrum is obtained by computing the magnitude of the Fourier transform of the power spectrum or computing the Fourier transform of the magnitude of the eBTF.

[0040] In one example, classification computer 210 receives only spectral parameters. In another example, the classification computer 210 receives both ARFI derived parameters as well as spectral parameters. The ARFI derived

parameters are sensitive to the stiffness and mechanical properties of the tissues while the spectral parameters are sensitive to scatterer distribution, size, and strength of backscatter. Thus, spectral parameters and ARFI derived parameters may provide complementary information regarding tissue. Accordingly, in one example, the two ARFI data points described and the thirteen spectral parameters described are combined into a single determination of tissue type by using them as combined input to the classification computer 210. Two examples of generation and application of these parameters can be found in *Spectral Analysis of Ultrasound Radiofrequency Backscatter for the Identification of Five Tissue Types Found In and Around the Paravertebral Space*, by Haggard et al. in *Ultrasonic Imaging and Tomography*. SPIE, Vol. 10530, 1058016.

[0041] The classification computer 210 is further configured to identify and classify the region of interest based on the received ultrasonic derived data. In particular, the classification computer 210 is configured to use a supervised machine learning model to identify and classify the region of interest based on at least a portion of the received ultrasonic derived data. In one example, the classification computer 210 is configured to use at least a portion of the received ultrasonic derived data as training input in order to learn to identify and classify the region of interest. For example, the classification model can be implemented as a classification tree, a random forest method, a support vector machine, or a K nearest neighbor model.

[0042] Regardless of the supervised machine learning model implemented, the classification computer 210 is configured to learn (i.e. to be trained) based on defined standard for identifying tissue types (also referred to as a “gold standard”). In one example, the gold standard may be user interpretation of images acquired from the ultrasound computer 202. Images may be selected, for example, for use by the classification computer 210 for training based on a degree of user confidence. In another example, the gold standard reference may be based on histology or other suitable imaging modalities. Although the intercostal space provides a very limited acoustic window and thus there is often no Doppler signal from blood vessels, stiffness and scattering does not depend on the insonification angle with respect to blood flow, and therefore the data provided by the ultrasound computer 202 remains a robust measure of tissue type.

[0043] The classification computer 210 is further configured to (subsequently after being trained) perform statistical analysis on the received ultrasonic derived data in order to identify and classify the region of interest. The classification computer 210 is further configured to communicate to the display 208 the identified region of interest. For example, the classification computer 210 causes the display 208 to augment an ultrasound image provided by the ultrasound computer 202 with the identified nerve and vessel information in order to provide a user such as a physician the ability to visualize nerves and vessels while performing a medical procedure such as a PVB.

[0044] FIG. 4 illustrates a block diagram of an example classification computer 300 (e.g. the classification computer 210 of FIG. 3). The classification computer 300 includes a data acquisition module 302 for receiving ultrasonic derived data from an ultrasound computer (i.e. the ultrasound computer 202 of FIG. 3). In one example, the data acquisition module 302 stores the ultrasonic derived data in a database

310 for further processing. The ultrasonic derived data includes ultrasound images as well as underlying RF data associated with the ultrasound images. In one example, the ultrasonic derived data further includes color Doppler loops for use a reference for identification of intercostal arteries and/or veins and to aid in selection of regions of interest for analysis.

[0045] The classification computer **300** includes a spectral analysis module **304** for analyzing RF signals for selected regions of interest in order to identify features for the selected regions of interest. In one example, the spectral analysis module **304** is configured to identify or calculate three feature categories for each region of interest, including spectral features based on a frequency spectrum of one dimensional signals traversing the regions of interest, two-dimensional spectral features, and cepstrum-based features.

[0046] In one example, the spectral analysis module **304** is configured to perform spectral analysis of the RF signals using an autoregressive (“AR”) model. The AR model of spectral estimation does not require zero-padding and may have better frequency resolution than alternative approaches. The AR model may also excel for short time series analysis as compared to alternative approaches. Thus, the AR model may be a desirable choice for implementation by the spectral analysis module **304**. However, in other examples, the spectral analysis module **204** may implement alternative models for performing spectral analysis.

[0047] An AR model of a random process can be described by the equation:

$$x[n] = -\sum_{k=1}^p a[k] \times [n-k] + e[n] \quad (3)$$

[0048] where p is defined as the AR order, $a[k]$ are valued as the AR coefficients, and $e[n]$ is a white noise random process. The present value is modeled as a weighted sum of past values with the additive noise term $e[n]$ that has the white noise variance σ^2 .

[0049] The power spectral density (PSD) can be estimated from the AR model as shown in the following equation:

$$PSD_{AR}(f) = \frac{\sigma^2}{\left| 1 + \sum_{k=1}^p a[k] \exp(-j2\pi f k) \right|^2} \quad (4)$$

[0050] where PSD is the power spectral density as a function of frequency (f), σ^2 is the variance of the white noise, $a[k]$ are the AR coefficients, and p is the AR order of the model. The Yule-Walker system of equations are obtained through a relationship between the AR coefficients and the autocorrelation function that can be used to generate the AR-based PSD and leads to the following form:

$$\begin{bmatrix} R_{XX}(0) & R_{XX}(-1) & L & R_{XX}(-p) \\ R_{XX}(1) & R_{XX}(0) & L & R_{XX}(-p+1) \\ M & M & M & M \\ R_{XX}(p) & R_{XX}(p-1) & L & R_{XX}(0) \end{bmatrix} \begin{bmatrix} 1 \\ a_1 \\ M \\ a_p \end{bmatrix} = \begin{bmatrix} \sigma^2 \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad (5)$$

[0051] where σ^2 is the variance of the white noise, and R_{XX} is the autocorrelation calculated from the signal. These equations can be solved recursively using the Levinson-Durbin algorithm, incrementally computing the AR parameters $\{a_1, a_2, \dots, a_p, \sigma^2\}$. The variance of the noise, σ^2 , is equal to the modeling error in the spectral estimate for this model. Because of this, the AR parameters can be computed recursively, with increasing order p until reducing the error to an applicant dependent level.

[0052] Some of the noise introduced by increasing the order of the AR model may aid in the process tissue characterization when dealing with diffuse scattering of ultrasound waves from heterogeneous tissue. Therefore, an optimal range of AR order may exist, where it is large enough to create an accurate spectral estimate and include useful noise for tissue characterization, but not so large that the noise obfuscates the data. To select appropriate AR-orders to investigate, the spectral analysis module **304** is configured to observe mean squared error (MSE) using an order penalizing cost function. In one example, the spectral analysis module **304** is configured to use one of three order-penalizing cost functions, including: Final Prediction Error (FPE), Akaike’s Information Criterion (AIC), and Minimum Description Length (MDL). The cost functions for each of these techniques can be defined by the given equations, respectively:

$$FPE = \frac{N+p+1}{N-p-1} \sigma(p)^2 \quad (6)$$

$$AIC = N \ln[\sigma(p)]^2 + 2p \quad (7)$$

$$MDL = N \ln[\sigma(p)]^2 + p \ln N \quad (8)$$

[0053] where p is the AR order, $\sigma(p)^2$ is the error as a function of p order, and N is the number of samples used for analysis.

[0054] The spectral analysis module **304** is further configured to use the RF signal for each A-line in a region of interest to compute a PSD (also referred to as “spectra”) using the AR model. In one example, the spectral analysis module **304** is configured to compute multiple spectra for different region of interest sizes, order, and at different bandwidths. In such an example, the spectral analysis module **304** is further configured to average and normalize the spectra via spectral subtraction using acquired calibration data.

[0055] In one example, the spectral analysis module **304** is configured to generate a linear regression of amplitude on frequency from the normalized data and compute eight spectral parameters from the regression line and the normalized data, including: (1) maximum power and (2) its corresponding frequency, (3) minimum power and (4) its corresponding frequency, (5) a Y-intercept of the regression line and (6) slope of the regression line, (7) mid-band fit, and (8) an integrated backscatter parameter.

[0056] FIG. 5A is a graph **400** illustrating the average spectrum from a single region-of-interest is shown **402**, along with the normalization spectrum from the same depth as the selected region of interest **404**, and the resulting normalized spectrum **406**. FIG. 5B illustrates a zoomed in view of the normalized spectrum **406** and associated regression line, including the eight spectral parameters: maximum

power 1, its corresponding frequency 2, minimum power 3, its corresponding frequency 4, Y-intercept 5, slope of the regression line 6, and mid-band fit 7. Integrated backscatter is not illustrated but can be defined by the equation:

$$IBS = \frac{1}{f_{max} - f_{min}} \int_{f_{min}}^{f_{max}} S(f) df \quad (9)$$

[0057] where $S(f)$ is the normalized power spectral density (PSD) computed by the AR-model-based method.

[0058] Referring again to FIG. 4, the one-dimensional (“1-D”) parameters calculated for each ROI contain frequency content only in the range direction, parallel with the beam. Thus, in one example, to supplement the 1-D spectral information, the spectral analysis module 304 is further configured to compute and include two-dimensional (“2-D”) spectral parameters in the feature set for classification. A two-dimensional spectral feature, as the term is used herein, represents frequency content in both a range direction of the ultrasound beam as well as a cross-range direction. In one example, the spectral analysis module 304 is configured to compute the 2-D spectral parameters using a Hamming window and zero padding the samples in the region of interest to 1024 in each direction. The spectral analysis module 304 is further configured to compute the Fourier transform in the range direction, to normalize the range-oriented magnitude spectra, and to compute the Fourier transform in the cross-range direction, yielding the 2-D PSD, designated as $S_{2D}(k, \mu)$.

[0059] The geometry for computing radially-integrated spectral power (RISP) is illustrated by the graph 500 in FIG. 6. RISP is a function of angle, as illustrated by the graph 500 in FIG. 6, and can be defined in Equation:

$$RISP(\theta) = \frac{\int_{k_1}^{k_2} S'_{2D}(k, \mu) dk}{\int_{k_1}^{k_2} dk} \quad (10)$$

[0060] where the spatial frequency is effectively replaced using the definition of the radial coordinate angle

$$\mu = k \tan(\theta) \quad (11)$$

and

$$S'_{2D}(k, \mu) = 10 \log S_{2D}(k, \mu) \quad (12)$$

[0061] Integration is performed along radial lines over the temporal frequency range from k_1 , the low end of the frequency band, to k_2 , the upper end of the frequency band. Angularly-integrated spectral power (AISP) is illustrated by the graph 600 in FIG. 7. AISP is a function of radius (K) and is defined below in Equation:

$$AISP(K) = \frac{\int_{\theta_1}^{\theta_2} S'_{2D}(k, \mu) K d\theta}{\int_{\theta_1}^{\theta_2} K d\theta} \quad (13)$$

where

$$K = \sqrt{k^2 + \mu^2} \quad (14)$$

[0062] and the range of the angles is constrained by the usable bandwidth. In one example, four 2-D spectral parameters are derived from the RISP and AISP functions, and included the peak value of RISP, the 3 dB bandwidth of RISP, the slope of a regression line fit to AISP, and the intercept of a regression line fit to AISP.

[0063] Referring again to FIG. 4, in one example, in addition to 1-D and 2-D spectral parameters, a feature based on the cepstrum may be included for classification. Accordingly, the spectral analysis module 304 is further configured to calculate a power cepstrum of a signal segment in a region of interest according to the Equation:

$$\text{Power Cepstrum} = \mathfrak{S}^{-1} \{ \log(| \mathfrak{S} \{ f(t) \} |^2) \}^2 \quad (15)$$

[0064] In particular, the power cepstrum of a signal is defined as the squared magnitude of the inverse Fourier transform of the logarithm of the squared magnitude of the Fourier transform of a signal. The spectral analysis module 304 is further configured to calculate an average cepstrum for the region of interest and to extract the peak of the average cepstrum to use in classification.

[0065] The classification computer 300 further includes a recognition module 306 for recognizing or classifying region of interest in a region of interest based on the features or spectral parameters provided by the spectral analysis module 304. In one example, the recognition module 306 classifies region of interest based on a combination of spectral parameters provided by the spectral analysis module 304 and acoustic radiation force impulse (“ARFI”) derived information, as previously described.

[0066] In one implementation, the recognition module 306 can comprise one or more pattern recognition classifiers, each of which utilize the extracted features or a subset of the extracted features to determine an appropriate class. Where multiple classifiers are used, an arbitration element can be utilized to provide a coherent result from the plurality of classifiers. Each classifier is trained on a plurality of training images representing various classes of interest. The training process of the a given classifier will vary with its implementation, but the training generally involves a statistical aggregation of training data from a plurality of training images into one or more parameters associated with the output class. Any of a variety of optimization techniques can be utilized for the classification algorithm, including support vector machines, self-organized maps, fuzzy logic systems, data fusion processes, boosting and bagging methods, rule based systems, or artificial neural networks.

[0067] For example, a SVM classifier can utilize a plurality of functions, referred to as hyperplanes, to conceptually divide boundaries in the N-dimensional feature space, where each of the N dimensions represents one associated feature of the feature vector. The boundaries define a range of feature values associated with each class. Accordingly, an output class and an associated confidence value can be determined for a given input feature vector according to its position in feature space relative to the boundaries. A rule-based classifier applies a set of logical rules to the extracted features to select an output class. Generally, the rules are applied in order, with the logical result at each step influencing the analysis at later steps. In one implementation, the SVM can be implemented via a kernel method using a linear or non-linear kernel.

[0068] An ANN classifier comprises a plurality of nodes having a plurality of interconnections. The values from the

feature vector are provided to a plurality of input nodes. The input nodes each provide these input values to layers of one or more intermediate nodes. A given intermediate node receives one or more output values from previous nodes. The received values are weighted according to a series of weights established during the training of the classifier. An intermediate node translates its received values into a single output according to a transfer function at the node. For example, the intermediate node can sum the received values and subject the sum to a binary step function. A final layer of nodes provides the confidence values for the output classes of the ANN, with each node having an associated value representing a confidence for one of the associated output classes of the classifier.

[0069] A rule-based classifier applies a set of logical rules to the extracted features to select an output class. Generally, the rules are applied in order, with the logical result at each step influencing the analysis at later steps. The specific rules and their sequence can be determined from any or all of training data, analogical reasoning from previous cases, or existing domain knowledge. One example of a rule-based classifier is a decision tree algorithm, in which the values of features in a feature set are compared to corresponding threshold in a hierarchical tree structure to select a class for the feature vector. A random forest classifier is a modification of the decision tree algorithm using a bootstrap aggregating, or “bagging” approach. In this approach, multiple decision trees are trained on random samples of the training set, and an average (e.g., mean, median, or mode) result across the plurality of decision trees is returned. For a classification task, the result from each tree would be categorical, and thus a modal outcome can be used.

[0070] The classification computer **300** further includes an output module **308** for communicating with a display (e.g. the display **208** of FIG. **3**) and for causing changes to the display, and in particular for causing the display to display data provided by the recognition module **306**. The output module **308** may be configured to communicate with the display via either a wired or wireless connection. In one example, the output module **308** is configured to overlay or merge data provided by the recognition module **306** with other data provided to the display, such as images provided to the display by an ultrasound computer (e.g. the ultrasound computer **202** of FIG. **3**).

[0071] In view of the foregoing features described above, an example method will be better appreciated with reference to FIGS. **8** and **9**. While, for purposes of simplicity of explanation, the method is shown and described as executing serially, it is to be understood and appreciated that the method is not limited by the illustrated order, as parts of the method could occur in different orders and/or concurrently from that shown and described herein.

[0072] FIG. **8** illustrates a method for identifying and classifying a region of interest in an anatomy of a patient. At **702**, ultrasound images, including RF and Doppler data, are acquired by an ultrasound imaging system (e.g. the ultrasound computer **202** of FIG. **3**) from an anatomy of a patient. In one example, the ultrasound images are taken of a paravertebral space and an intercostal space. In one example, ultrasound images are taken on both side of an area on the anatomy. In one example, both acoustic radiation force impulse derived data and spectral parameters of ultrasonic backscatter is received. The ultrasound images are stored for further retrieval and analysis.

[0073] At **704**, spectral analysis is performed by a computer (e.g. the classification computer **210** of FIG. **3**) on the acquired ultrasound images to identify at least one feature associated with the region of interest. In one example, a plurality of features in three categories are identified, including one dimensional spectral features, two-dimensional spectral features, and cepstrum-based features. In one example, the spectral analysis is performed using an autoregressive (“AR”) model. In one example, a plurality of spectra are computed, based on the AR model, for different region of interest sizes, order, and at different bandwidths, and the plurality of spectra are averaged and normalized via spectral subtraction using acquired calibration data. In one example, eight spectral parameters are computed from the normalized data, including: maximum power, frequency corresponding to maximum power, minimum power, frequency corresponding to maximum power, Y-intercept, slope of a regression line, mid-band fit, and integrated backscatter.

[0074] At **706**, the region of interest is classified using supervised machine learning based on the at least one identified feature. In one example, one of single classification tree, random forest, support vector, and K nearest neighbor supervised machine learning is used to classify the region of interest as a particular type of tissue or anatomical feature. At **708**, a display is caused to alter its output and to display the classification for the region of interest.

[0075] FIG. **9** illustrates a method **900** for classifying a region of interest into one of a plurality of classes representing anatomical features. At **902**, recorded echoes from an ultrasound system are received from a region of interest as a set of radio frequency (RF) signals. In one implementation, the RF signals including a set of harmonic signals being extracted from a frequency range associated with second harmonics of the recorded echoes. At **904**, the RF signals are transformed from a time domain representation to a frequency domain representation. In one example, the signals can be transformed to provide a first frequency domain representation, representing fundamental frequencies of the ultrasound device, and a second frequency domain representation, representing second harmonic frequencies of the fundamental frequencies.

[0076] At **906**, the frequency domain representation is adjusted for attenuation as a function of the depth of the region of interest. In one example, this function is patient specific, such the attenuation is adjusted as a function of each of the depth of the region of interest and a ratio of a thickness of skin and fat to a thickness of muscle within the patient. In another example, the attenuation function can be determined from a subject other than a subject associated with the region of interest, using measured backscatter from known tissue. This can also be performed with a phantom or an ex vivo tissue sample. The attenuation function can also be frequency specific, such that a first function is associated with a first frequency band and a second function is associated with a second frequency band, and the frequency domain representation is adjusted for attenuation with the first function for signals within the first frequency band and with the second function for signals within the second frequency band.

[0077] At **908**, a plurality of features are extracted from the frequency domain representation. In one implementation, at least one feature is extracted from a frequency domain representation of the set of harmonic signals. Alter-

natively or additionally, the features can include at least one of a two-dimensional spectral feature and a feature based on a cepstrum determined from the set of harmonic data. At 910, the region of interest is assigned to one of a plurality of classes representing anatomical feature according to the extracted plurality of features at a classifier model, and the assigned class is displayed to a user at 912.

[0078] What have been described above are examples. It is, of course, not possible to describe every conceivable combination of components or methodologies, but one of ordinary skill in the art will recognize that many further combinations and permutations are possible. Accordingly, the disclosure is intended to embrace all such alterations, modifications, and variations that fall within the scope of this application, including the appended claims. As used herein, the term “includes” means includes but not limited to, the term “including” means including but not limited to. Additionally, where the disclosure or claims recite “a,” “an,” “a first,” or “another” element, or the equivalent thereof, it should be interpreted to include one or more than one such element, neither requiring nor excluding two or more such elements.

What is claimed is:

1. A system comprising:
 - at least one processor; and
 - at least one computer-readable tangible storage device storing program instructions for execution by the at least one processor, the program instructions comprising:
 - first program instructions for receiving ultrasonic derived data comprising an ultrasound image, representing a region of interest, and radio frequency “(RF)” data associated with the ultrasound image;
 - second program instructions for analyzing the RF data to identify at least one feature associated with the region of interest, the at least one feature including one of a two-dimensional spectral feature and a feature representing a cepstrum determined from the RF data;
 - third program instructions for classifying the region of interest as one of a plurality of anatomical classes from the identified at least one feature using a machine learning model; and
 - fourth program instructions for causing a display to display the one of the plurality of anatomical classes.
2. The system, of claim 1, wherein the at least one feature is a two-dimensional spectral feature.
3. The system of claim 1, wherein the at least one feature represents the cepstrum.
4. The system of claim 1, wherein the RF signals including a set of harmonic signals being extracted from a frequency range associated with second harmonics of the recorded echoes, and the at least one feature includes a feature representing the set of harmonic signals.
5. The system of claim 1, wherein the plurality of anatomical classes each represent a type of arterial plaque.
6. The system of claim 1, wherein the second program instructions are configured to perform a linear regression of amplitude on frequency from the RF data to provide a regression line and calculate eight spectral parameters from the normalized data, comprising: a maximum power, a frequency corresponding to the maximum power, a minimum power, a frequency corresponding to the maximum

power, a Y-intercept of the regression line, a slope of the regression line, mid-band fit, and an integrated backscatter parameter.

7. The system of claim 1, wherein the third program instructions use, as the machine model, one of a random forest model, a support vector machine, and a K nearest neighbor model.

8. A system for classifying a region of interest as an anatomical feature comprising:

- a processor; and
 - a non-transitory computer readable medium storing executable instructions executable by the processor comprising:
 - an ultrasound interface that receives, from an associated ultrasound system, recorded echoes from the region of interest as a set of radio frequency (RF) signals and constructs an ultrasound image from the RF signals, the RF signals including a set of harmonic signals being extracted from a frequency range associated with second harmonics of the recorded echoes;
 - a frequency transform component that transforms the RF signals from a time domain representation to a frequency domain representation;
 - a feature extractor that extracts a plurality of features from the frequency domain representation, at least one of the plurality of features representing the set of harmonic signals; and
 - a classifier that assigns the region of interest to one of a plurality of classes representing anatomical features according to the extracted plurality of features.
9. The system of claim 8, further comprising a signal conditioning element that adjusts the frequency domain representation of the RF signals according to an attenuation function.

10. The system of claim 9, wherein the signal conditioning element deconvolves the frequency domain representation of the RF signals to remove a transfer function of the ultrasound system.

11. The system of claim 8, wherein plurality of features includes at least one of a two-dimensional spectral feature and a feature based on a cepstrum determined from the set of harmonic data.

12. The system of claim 8, wherein the frequency range associated with second harmonics is the frequency range between 5.3 megahertz and 8.7 megahertz.

13. The system of claim 8, wherein one of the plurality of classes represent ablated tissue and one of the plurality of classes represent tissue that has not been ablated

14. A method comprising:

- receiving recorded echoes from a region of interest as a set of radio frequency (RF) signals;
- transforming the RF signals from a time domain representation to a frequency domain representation;
- adjusting the frequency domain representation for attenuation as a function of the depth of the region of interest;
- extracting a plurality of features from the frequency domain representation; and
- assigning the region of interest to one of a plurality of classes representing anatomical feature according to the extracted plurality of features at a classifier model.

15. The method of claim 14, wherein the RF signals including a set of harmonic signals being extracted from a frequency range associated with second harmonics of the

recorded echoes, and extracting a plurality of features from the a frequency domain representation comprising extracting at least one feature representing the set of harmonic signals.

16. The method of claim **15**, wherein the extracted at least one feature includes at least one of a two-dimensional spectral feature and a feature based on a cepstrum determined from the set of harmonic data.

17. The method of claim **14**, wherein the region of interest is in a first subject and adjusting the frequency domain representation for attenuation as the function of the depth of the region of interest comprises determining the function using measured backscatter from known tissue in a second subject.

18. The method of claim **15**, wherein the function of the depth of the region of interest is a first function, associated with a first frequency band, the method further comprising

determining a second function associated with a second frequency band, such that adjusting the frequency domain representation for attenuation comprises adjusting the frequency domain representation for attenuation with the first function for signals within the first frequency band and using the second function for signals within the second frequency band.

19. The method of claim **15**, wherein determining the function comprises determining a function of each of the depth of the region of interest and a ratio of a thickness of skin and fat to a thickness of muscle within the patient.

20. The method of claim **14**, wherein adjusting the frequency domain representation for attenuation as the function of the depth of the region of interest comprises determining the function using measured backscatter from known tissue in an ex vivo sample.

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