Ex vivo validation of 45 MHz intravascular ultrasound backscatter tissue characterization

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Aims
The objectives of the present study are to describe the algorithm for VH® IVUS using the 45-MHz rotational IVUS catheter and the associated ex vivo validation in comparison to the gold standard histology.

Methods and results
The first phase of the present study was to construct the 45 MHz VH IVUS algorithm by using a total of 55 human coronary artery specimens [111 independent coronary lesions and 510 homogenous regions of interest (ROIs)], obtained at autopsy. Regions were selected from histology and matched with their corresponding IVUS data to build the plaque classification system using spectral analysis and statistical random forests. In the second phase, the ex vivo validation of the VH IVUS algorithm assessed a total of 1060 ROIs (120 lesions from 60 coronary arteries) in comparison with histology. In an independent manner, two interventional cardiologists also classified a randomly selected subset of the ROIs for assessment of inter- and intra-observer reproducibility of VH IVUS image interpretation.

When including all ROIs, the predictive accuracies were 90.8% for fibrous tissue, 85.8% for fibro fatty tissue, 88.3% for necrotic core, and 88.0% for dense calcium. The exclusion of ROIs in the acoustically attenuated areas improved the predictive accuracies, ranging from 91.9 to 96.8%. The independent analysis of randomly selected 253 ROIs showed substantial agreement for inter-observer (κ = 0.66) and intra-observer (κ = 0.88) reproducibility.

Conclusion
Tissue classification by 45 MHz VH IVUS technology, when not influenced by calcium-induced acoustic attenuation, provided combined tissue accuracy >88% to identify tissue types compared with the gold standard histologic assessment, with high inter- and intra-observer reproducibility.

Keywords
atherosclerosis • coronary disease • spectral analysis • vulnerable plaque • VH IVUS • IVUS

Introduction
One of the biggest challenges the cardiologist has currently is to identify lesions precursor of acute coronary events, which sometimes manifest as sudden death. In patients under optimal medical treatment, it has been shown that the occurrence of death and acute coronary syndromes may be associated with the total number of segments with significant disease rather than the ischaemic burden.1 This suggests that plaque disruption might be the main cause of major cardiac outcomes rather than the ischaemia induced by obstructive plaques. Therefore, assessment of plaque composition, for identifying high-risk plaques, and the effectiveness of a treatment to alter plaque composition are fields of significant clinical relevance.

In recent years, cardiovascular imaging research has sought potential strategies for detecting high-risk plaques. A number of intravascular ultrasound (IVUS) backscatter analysis techniques are promising and multiple approaches have been developed to produce colour-coded tissue maps, such as spectral analysis,2,3 spectral analysis combined with statistical learning,4–6 and radiofrequency (RF) elastography analysis.7

IVUS RF backscatter analyses technologies, such as VH® IVUS (Volcano Corporation, San Diego, CA, USA), are aimed at identification
of patients at risk, plaque monitoring, and eventually to guide targeted therapy. High-risk plaque interpretation via VH IVUS analysis has been shown to be correlated with high-risk clinical features as defined by the Framingham Risk Score and to have the ability to predict cardiac events. Preceding published in vivo and ex vivo studies on VH IVUS accuracy have reported on plaque characterization with the 20 MHz VH IVUS algorithm using the digital 20-MHz IVUS catheter (Eagle Eye Catheter; Volcano Corporation), but this algorithm is restricted in detecting thin-caps associated with vulnerable fibroatheromas. IVUS of higher frequency has higher axial resolution, which could provide improved visualization of a thin-cap, albeit with loss of ultrasound penetration depth. Herewith, we report a new higher frequency IVUS backscatter analysis algorithm for a 45-MHz rotational IVUS catheter and the associated ex vivo validation in comparison to the gold standard histology.

**Methods**

**Subjects**
Similar to the development of the 20 MHz VH-IVUS algorithm, data were collected from 55 coronary artery specimens obtained at autopsy with IRB approval from the Cleveland Clinic, Cleveland, OH, USA. The study sample was limited to those without prior cardiac percutaneous interventions or surgical revascularization. Additionally, data were not acquired from alcohol and drug abuse cases or those with known blood-borne pathogen diseases (HIV, hepatitis, etc.). All vessels were excised within 24 h of death, and data were collected within 24 h of vessel procurement.

**Data acquisition**
Each vessel was pressure perfused using phosphate-buffered saline (PBS) solution at systolic pressure (~120 mmHg) and submerged within PBS to minimize any PBS–air interface reflections in the ultrasound data. AnS™ IVUS imaging system (Volcano Corporation) capable of saving the in-phase and quadrature (IQ) backscattered signals, and the Revolution™ 45-MHz rotational catheter (Volcano Corporation) were used for data collection and for visually locating ROI. Sections of interest were identified on the greyscale IVUS images as having ≥30% plaque burden, and IQ data were collected at the 8-mm field-of-view setting. A suture was attached to the surrounding tissue of the vessel to mark the location. Adjacent lesions were separated by at least 1 cm to permit histology processing. In addition to placing the catheter at these specific sites, an automated IVUS pullback data collection was performed over the length of the artery specimen from the distal to the proximal site. The automated pullback rate was set to 0.5 mm/s with a 60 bpm simulated heart rate, to allow the ECG-gated IQ data acquisition. This triggers the imaging system to save an IVUS backscatter data set once per simulated heartbeat, ~0.5 mm apart.

Following imaging and IVUS data collection, the artery specimens were pressure fixed using 10% buffered formalin at systolic pressure for at least 4 h. The vessels were then sectioned into 1 cm lengths so that the sutures representing the location of the imaged lesions were centred within each section. The sections of artery specimens were then sent for histology processing, which included a decalcification step, if needed. Following paraffin embedding, pairs of histology slides were prepared at multiple locations (100 μm apart) proximal, at, and distal to the suture location. Each pair of histology slides was stained using haematoxylin and eosin (H&E) and the Movat pentachrome stains, respectively.

**Image correlation**
Following data acquisition, matches between corresponding histology slides and greyscale IVUS images were determined by observing plaque burden and orientation and the surrounding tissue structures, such as side branch location, veins, location of the myocardium and pericardium, etc. The matching process with histology has been described previously. The slides were reviewed by an expert to identify regions-of-interest (ROIs) within the plaque that represent homogenous areas for each of the four tissue types, as described in previous work: FT—fibrous, FF—fibro-fatty, NC—necrotic core, and DC—dense calcium. Slides were reviewed both proximally and distally to the matched slide, to insure that the ROIs were consistent over a length along the vessel comparable to the out-of-plane resolution of the 45-MHz IVUS catheter. It was critical to have homogenous regions with respect to the ultrasound resolution both within the imaging plane and beyond the imaging plane, to successfully train the statistical classifier.

The ROIs obtained from the histology review were then translated onto regions within the matched greyscale IVUS image by a second expert (different than the expert used for the histology review). Sectors of an annulus were drawn on the reconstructed greyscale image using customized software run with MATLAB (Mathworks, Natick, MA, USA). The software calculates the ultrasound backscatter signal location in the de-convolved IQ data for each ROI. Each of these homogenous ROIs comprised 64 digitized IVUS samples in depth and 10 IVUS scan lines in width out of the total 256 scan lines that are used to construct one greyscale IVUS image.

**45 MHz VH IVUS algorithm**
A total of 111 independent lesions and 510 homogenous ROIs were selected to train the 45 MHz VH IVUS algorithm. The homogenous ROIs comprised 153 fibrous, 61 fibro-fatty, 112 necrotic core, and 184 dense calcium regions. The IQ signals representing each ROI were converted back to RF data, and the signals were processed to remove system effects comparable to previously described efforts. In addition, an adjustment factor was applied to the data to compensate for acoustic attenuation due to blood in the in vivo environment. This was to compensate for the higher attenuation of IVUS at 45 MHz in vivo, an environment with blood compared with the attenuation in the PBS ex vivo environment.

The power spectra were then computed, and spectral parameters were obtained. These included mid-band fit, intercept, slope, integrated backscatter, maximum power, frequency at maximum power, minimum power, and frequency at minimum power. These spectral parameters were calculated from the normalized power spectra for each homogenous ROI. The spectral parameters and corresponding homogenous tissue-type categorization from histology formed the data set for further statistical classification using a Random Forest as an ensemble classification method. The statistical random forests technique is an extension of tree classification schemes and is known for improved predictive power for a diagnostic test with multiple outcomes. Fifty classification trees were used in the 45 MHz VH IVUS algorithm with randomly chosen spectral parameters and a sub-sample of data to build each tree. The VH IVUS classification is a result of a voting scheme from all 50 trees in the forest, resulting in a robust and statistically stable algorithm. The ‘randomForest’ package was used within the R software environment to implement this approach.

**45-MHz VH IVUS image construction**
The 45-MHz VH IVUS algorithm was applied to multiple backscatter data sets after user-defined plaque lumen and medial–adventitial boundaries were obtained. Each lesion data set was analysed using a finite sized data
window that was $0.0736 \text{ radians}$ in the lateral or circumferential direction ($\approx 147 \mu \text{m}$ at 2 mm depth) and $\approx 250 \mu \text{m}$ in depth. This window of analysis was translated along each scan line one digitized sample at a time to construct a VH colourized tissue map out of the 256 scan lines that represent each IVUS backscattered image data set.

**45 MHz VH IVUS algorithm ex vivo accuracy assessment**

The data utilized for creating the 45 MHz VH algorithm are based on relatively ‘homogenous’ regions representing the four VH IVUS plaque types compared with the majority of plaque composition. In contrast, most plaques are not as homogenous as these ROIs. Thus, to validate the accuracy of the VH IVUS algorithm, a systematic approach was used to blindly and randomly choose heterogeneous regions and compare the results from histology to the results from the reconstructed VH IVUS images; similar to the approach described for the 20 MHz VH IVUS ex vivo validation.$^5$ VH tissue maps were constructed from 45-MHz IVUS data collected from a total of 120 lesions from 60 coronary artery specimens. Majority of these data were also used in training the VH algorithm, although an independent and different cohort of ROIs was used for training than the cohort of ROIs used for the accuracy assessment.

A team of four investigators analysed the data in a manner that minimizes potential bias. The first step was performed by two of the investigators and involved selecting random ROIs within the histology slides. A square grid pattern was printed on clear plastic overhead projection sheets. These were positioned over scaled printouts of the matched histology Movat pentachrome stained slide images so that each square was $1/3 \text{ mm} \times 1/3 \text{ mm}$, or $\approx a 333-\mu \text{m}-\text{ sized square}$. The two investigators then traced the boundary of the plaque on the overhead projection sheet for each section. Next, they numbered every other square that was contained within the plaque boundary following a checkerboard pattern.

![Figure 1](image_url)

**Figure 1** ROIs selection for ex vivo accuracy assessment. Four investigators were engaged in the separate tasks of (A) drawing histology image outlines on transparent paper with a grid of $1/3 \times 1/3 \text{ mm}$ regions and highlighting alternating regions; (B) finding matching regions on corresponding VH IVUS images and interpreting the VH IVUS outcome in each ROI; and finally (C) interpreting the pathology outcome in all ROIs. The investigators were blinded to each other’s results.
This overhead (without the underlying histology image) was provided to the third investigator who translated the numbered squares to corresponding positions on the matched and scaled VH image printouts. This step is necessary since the paraffin embedding process in histology preparation results in warping of the tissue. A third investigator interpreted and recorded the dominant VH IVUS tissue type within each of these heterogeneous ROIs. This third investigator was blinded to the histology data. The traced overhead projection sheets were then provided to a fourth investigator who interpreted the dominant feature of the histology slide for each of the numbered ROIs and was blinded to the matched VH IVUS image. A total of 1060 regions were thus analysed (Figure 1).

In addition to this analysis, the third investigator also identified ROIs that were located behind densely calcified ROIs in the radial direction of the ultrasound backscatter. Calcium is known to attenuate or inhibit ultrasound and hence cast a ‘shadow’ on tissue located at deeper locations radially with respect to the IVUS transducer. This phenomenon is more common with the rotational IVUS modality where the IVUS catheter is constructed of a single unfocused ultrasound transducer. The singularity of the transducer lends it more prone to shadowed regions due to calcifications, because the single unfocused transducer responsible for imaging has backscatter blocked from the calcium in the path of the ultrasound. In contrast, the previous work with the 20-MHz solid-state IVUS modality was less prone to the calcium-induced shadowing due to the synthetic aperture image formation from a multi-element transducer array. Thus, in the present study, the accuracy results were determined by both including and excluding regions that were potentially shadowed by the heavily calcified areas.

In an independent manner, two interventional cardiologists (C.M.C. and H.M.G.) analysed 253 randomly selected heterogeneous ROIs (30 random lesions). The comparison was performed blinded to the histologic findings to assess the inter- and intra-observer reproducibility of

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![Figure 2](image_url)

**Figure 2** VH IVUS predictive accuracy for tissue types including all ROIs \(n = 1060\). (A) Agreement between VH IVUS and histology and truth table for the accuracy measurements. (B) VH IVUS predictive accuracy, sensitivity, and specificity by tissue types compared with histology. DC, dense calcium; FT, fibrous tissue; FF, fibro-fatty; NC, necrotic core.
VH IVUS image interpretation. At first, the VH IVUS tissue-type classification was performed in consensus between the two physicians to compare with the validation analysis done by the four investigators. Later, after a period of 1 month, the same 253 ROIs were re-analysed by the same two interventional cardiologists, to assess the intra-observer reproducibility.

Data analysis
The statistical computation that was applied is based on a test for a single state. Thus, for each computation, the results were interpreted as being in one of two states: chosen tissue type or not of the chosen tissue type. Since this manuscript addresses four plaque tissue types, one of the types is chosen (Type X) and the three remaining tissue types are combined as ‘not of type X’. Then the common definitions for sensitivity, specificity, and accuracy can be applied. Finally, the Kappa statistic was computed for determining the inter- and intra-observer reproducibility of the 45 MHz VH IVUS images.

Results

45 MHz VH IVUS algorithm ex vivo accuracy assessment
VH IVUS tissue maps were constructed from 45-MHz IVUS data collected from a total of 120 lesions from 60 coronary artery specimens (51 hearts, 42 male, 9 female; 14 black, 37 white; average age 57.0 ± 12.7 years). The ex vivo accuracy was determined for all 1060 ROIs and again after excluding the 290 ROIs shadowed by calcium. Figure 2 describes the truth table for inclusion of all ROIs and the corresponding accuracy statistics of sensitivity, specificity, and predictive

![Figure 3](image-url)  
**Figure 3** VH IVUS predictive accuracy for tissue types excluding all calcium-shadowed ROIs (n = 770). (A) Agreement between VH IVUS and histology and truth table for the accuracy measurements. (B) VH IVUS predictive accuracy, sensitivity, and specificity by tissue types compared with histology. DC, dense calcium; FT, fibrous tissue; FF, fibro-fatty; NC, necrotic core.
accuracy. When including all ROIs, the predictive accuracy ranged from a low of 85.8% for fibro-fatty to a high of 90.8% for fibrous tissue type, with an overall accuracy of 76.7% for all tissue types combined. The sensitivities were all >81% except for dense calcium which is relatively low at 55.8%. The lowest specificity was for fibro-fatty at 84.9% with the other three tissue types at 90% or higher.

The truth table resulting from the removal of the ROIs positioned behind calcium with the corresponding accuracy statistics is presented in Figure 3. The predictive accuracy ranged from a low of 93.0% for fibrous to a high of 96.8% for fibro-fatty tissue, with an overall accuracy of 88.6% for all tissue types combined. The sensitivities fall in the 82–93% range, while the specificity values remain high with all values >90%.

### Observer reproducibility for VH IVUS image interpretation

Table 1 describes the inter-observer reproducibility analysis of VH IVUS image interpretations, without exclusion of shadowed ROIs (n = 253). There was substantial agreement \( k = 0.66 \) between the assessment performed by the four investigators and the assessment performed by the independent interventional cardiologists. Table 2 summarizes the intra-observer reproducibility analysis without exclusion of shadowed ROI, where there was higher agreement \( k = 0.88 \).

### Table 1  Inter-observer reproducibility of VH IVUS interpretation without exclusion of shadowed ROIs (n = 253)

<table>
<thead>
<tr>
<th>Developer</th>
<th>IC</th>
<th>DC</th>
<th>FF</th>
<th>FT</th>
<th>NC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>17</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td></td>
<td>25 (9.9%)</td>
</tr>
<tr>
<td>FF</td>
<td>1</td>
<td>50</td>
<td>9</td>
<td>3</td>
<td></td>
<td>63 (24.9%)</td>
</tr>
<tr>
<td>FT</td>
<td>0</td>
<td>11</td>
<td>93</td>
<td>8</td>
<td></td>
<td>112 (44.3%)</td>
</tr>
<tr>
<td>NC</td>
<td>1</td>
<td>2</td>
<td>15</td>
<td>35</td>
<td></td>
<td>53 (20.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (7.5%)</td>
<td>63 (24.9%)</td>
<td>118 (46.6%)</td>
<td>53 (20.9%)</td>
<td>253</td>
<td></td>
</tr>
</tbody>
</table>

IC, interventional cardiologist.

### Table 2  Intra-observer reproducibility of VH IVUS interpretation without exclusion of shadowed ROIs (n = 253)

<table>
<thead>
<tr>
<th>IC 2nd analysis</th>
<th>IC 1st analysis</th>
<th>DC</th>
<th>FF</th>
<th>FT</th>
<th>NC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
<td>19 (7.5%)</td>
</tr>
<tr>
<td>FF</td>
<td>0</td>
<td>61</td>
<td>3</td>
<td>0</td>
<td></td>
<td>64 (25.3%)</td>
</tr>
<tr>
<td>FT</td>
<td>0</td>
<td>2</td>
<td>112</td>
<td>9</td>
<td></td>
<td>123 (48.6%)</td>
</tr>
<tr>
<td>NC</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>42</td>
<td></td>
<td>47 (18.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (7.5%)</td>
<td>63 (24.9%)</td>
<td>118 (46.6%)</td>
<td>53 (20.9%)</td>
<td>253</td>
<td></td>
</tr>
</tbody>
</table>

IC, interventional cardiologist.

### Discussion

The main findings of the present manuscript can be summarized as follows: first, a statistically robust algorithm was designed to yield analysis of 45-MHz IVUS backscatter, resulting in colour-coded tissue maps of the data; second, the VH IVUS algorithm with the 45-MHz catheter had predictive accuracy >85.8% for each tissue type compared with histology; third, the predictive accuracy for each tissue type improved to >91.9% when calcium-shadowed areas were not taken into consideration; forth, tissue types classification and resulting interpretation of VH IVUS images showed high intra- and inter-observer reproducibility.

The present work is the first histologic validation of VH IVUS using a 45-MHz rotational IVUS catheter. Adapting the VH IVUS algorithm to the 45-MHz catheter is non-trivial since the physical properties of the target plaque tissue types are quite distinct from the same tissue at the lower 20 MHz bandwidth, that was previously reported. One key difference is that the attenuation at 45 MHz is considerably greater than at 20 MHz for both blood and tissue. Another consideration is the fact that the 20-MHz catheter has a multi-element transducer array with synthetic aperture image formation, while the 45-MHz catheter has a single mechanically rotated unfocused transducer. It has been shown that these factors influence the ultrasound’s depth of field and characteristic resolution. Figure 4 illustrates an example of IVUS greyscale images obtained at 20 and 45 MHz from the same location in an ex vivo coronary artery sample, with the corresponding VH IVUS images and
histologic findings. It is evident that the 20 MHz image has greater IVUS depth of penetration and the 45 MHz image has greater axial resolution (i.e. in the direction of the ultrasound beam) while it lacks in the depth of penetration due to higher attenuation (Figure 4). In this 45-MHz IVUS study, the greater effect of calcium-induced shadowing and loss of signal with depth can be observed in the increased amount of fibro-fatty tissue appearing behind dense calcifications as demonstrated in the 45 MHz VH IVUS image in Figure 4. This is due to the fact that spectral properties of backscatter from atherosclerotic fibro-fatty tissue can overlap with properties of backscatter from attenuated ultrasound, resulting in an incorrect classification of tissue type. This phenomenon also explains the low sensitivity observed for dense calcium and the low specificity observed for fibro-fatty tissue in the ROI-cohort without exclusion of calcium-shadowed ROIs. Hence, for clinical studies utilizing the 45 MHz VH IVUS algorithm, regions with dominant shadowing can be excluded from analysis. This greatly increases the sensitivity of dense calcium and to a lesser extent the sensitivity of necrotic core tissue type while increasing the specificity of the fibro-fatty tissue type.

The results lend themselves to direct comparison with previously reported measurements for the 20 MHz VH IVUS algorithm. The previous manuscript reported predictive accuracy in the range of 93.5–96.7% which is comparable to the 45-MHz VH IVUS results when excluding ROIs located behind heavy calcifications (93–95.5%) and ranged from ~85–90% when including all ROIs.

It may be argued that, with similar catheter accuracy for tissue types, the change for the 45-MHz IVUS catheter is inconsequential. However, the similar accuracy between the 20 and the 45 MHz is in terms of correct colour code interpretation related to histology and not to any dimensional assessment. The higher axial resolution of the 45 MHz IVUS has potential to improve the coronary atherosclerosis research and raises several questions to be answered in the future. The higher resolution may improve the discernment of the in vivo plaque phenotype, identifying with more precision, for instance, if a necrotic core is in contact with the lumen or not. In addition, the better resolution may enhance the ability to quantify tissue-type changes with application in natural history of atherosclerosis trials, bioresorbable coronary scaffold degradation, pharma interventions, etc.

The reported inter-observer reproducibility analysis showed consistent agreement between the four investigators and the two independent interventional cardiologists and may translate to clinical utility for this tool. Additionally, the intra-observer reproducibility demonstrated stable response in a relatively large sample of ROIs.

**Limitations**
Although the 45-MHz catheter has improved axial resolution, the detection of a thin fibrous cap, which defines plaque vulnerability (<65 μm in thickness), is still below the axial resolution of current IVUS, and this may lead to false-positive identification of

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**Figure 4** Examples of (A) 20 MHz and (B) 45 MHz IVUS images with corresponding VH IVUS images, collected ex vivo in a human coronary lesion (C). The 20 MHz IVUS image has lower axial resolution, but lesser attenuation in signal behind an area of dense calcification, and is displayed with a 10 mm field of view. Whereas, the 45 MHz IVUS image has higher axial resolution, but higher attenuation of signal behind the dense calcification (evident from the increased fibro-fatty tissue in the VH IVUS image), and is displayed with an 8 mm field of view. This plaque phenotype is classified as a calcified fibroatheroma as it has a visible fibrous cap covering >10% confluent dense calcium and necrotic core.
some vulnerable atheromas. However, with similar accuracy and yet lower resolution, the spectral analysis approach of ultrasound backscatter used with the 20-MHz IVUS catheter has shown prognostic relevance.9 –11

Conclusion

Tissue classification by 45 MHz VH IVUS technology, when not influenced by significant calcium-induced acoustic attenuation, provided combined tissue accuracy >88% to identify tissue types compared with the gold standard histologic assessment, with high inter- and intra-observer reproducibility.

Conflict of interest: A.N. is an employee of Volcano Corporation. R.J.F., D.G.V., and M.P.M. were employees of Volcano Corporation at the time the 45 MHz VH IVUS algorithm was developed. The other authors did not receive grants or financial support from industry or from any other source.

References