Detection of Fibrous Cap in Atherosclerotic Plaque by Intravascular Ultrasound by Use of Color Mapping of Angle-Dependent Echo-Intensity Variation

Takafumi Hiro, MD, PhD; Takashi Fujii, MD, PhD; Kyounori Yasumoto, MD; Takashige Murata, MD; Akihiro Murashige, MD; Masunori Matsuzaki, MD, PhD

**Background**—The thickness of the fibrous cap is a major determinant in the vulnerability of atherosclerotic plaque to rupture. It has been demonstrated that intravascular ultrasound (IVUS) backscatter from fibrous tissue is strongly dependent on the ultrasound beam angle of incidence. This study investigated the feasibility of using a new IVUS color mapping technique representing the angle-dependent echo-intensity variation to determine the thickness of the fibrous cap in atherosclerotic plaque.

**Methods and Results**—Nineteen formalin-fixed noncalcified human atherosclerotic plaques from necropsy were imaged in vitro with a 30-MHz IVUS catheter. The IVUS catheter was moved coaxially relative to the plaque. The images showing maximum and minimum echo intensity of the plaque surface were selected to calculate the angle-dependent echo-intensity variation. A colorized representation of the echo-intensity variation in the plaque was obtained from the 2 IVUS images. A clearly bordered area with large variation in echo intensity was revealed for each plaque surface in the colorized IVUS image. The thickness \( (x, \text{mm}) \) of this area correlated significantly with that of fibrous cap \( (y, \text{mm}) \) measured from histologically prepared sections as \( y=1.05x-0.01 \) \( (r=0.81, P<0.0001) \). Bland-Altman analysis also supported the reliability of this method (mean difference, 0.00±0.10 mm).

**Conclusions**—This novel technique for color mapping the echo-intensity variation in IVUS provided an accurate representation of the thickness of the fibrous cap in atherosclerotic plaque. This method may be useful in assessing plaque vulnerability to rupture in atherosclerosis. (*Circulation. 2001;103:1206-1211.*)

**Key Words:** atherosclerosis ■ echocardiography ■ imaging ■ plaque ■ ultrasonics

Intravascular ultrasound (IVUS) imaging provides a detailed arterial cross section with accurate morphometric representation of atherosclerotic plaque dimensions in vivo.1–9 It was originally expected that IVUS imaging would allow characterization of the tissue components of atherosclerosis on the basis of their characteristic intensity pattern.2,3,10–13 Subsequent studies, however, have demonstrated significant limitations in tissue characterization by IVUS intensity patterns alone, especially in discriminating fibrous and fatty tissues.14–17

IVUS images of atherosclerotic plaque vary in intensity with changes in angle or position of the transducer relative to the plaque.18,19 It has been demonstrated that the ultrasound backscatter of fibrous tissue is strongly angle-dependent, whereas the backscatter from fatty tissue is influenced less by the ultrasound beam angle.18 Using a currently available IVUS system, we also documented previously that the angle dependence of fibrous/acellular tissue (fibrous cap) was significantly greater than that of the other tissue components except for calcified tissue.20 Identification of the fibrous cap is important, because the thickness of the fibrous cap is a major determinant of plaque vulnerability in atherosclerotic lesion. Therefore, the present study investigated the feasibility of using an IVUS color mapping technique developed in our laboratory that represents the angle-dependent echo-intensity variation to identify the fibrous cap within atherosclerotic plaque.

**Methods**

**IVUS Imaging**

Nineteen formalin-fixed noncalcified human femoral arteries obtained from necropsy were imaged by use of a 3.2F, 30-MHz IVUS catheter (CVIS/Boston Scientific) in saline at room temperature. The arteries imaged had <50% luminal cross-sectional narrowing.

In this study, calcified plaques were not studied, because the echo intensity of calcium usually saturates the gray-scale value, and the angle dependence of the calcium echo intensity is difficult to assess.
Calcified tissue is readily identified by visual inspection with a high sensitivity and specificity. The current concern for tissue characterization of plaque is how to discriminate between fibrous and fatty tissue.

An acoustic reference point was established by suturing a surgical needle into the wall of the artery perpendicular to the long axis. This technique ensured that the same cross section was imaged for all studies and that the ultrasound images corresponded exactly to the cross section chosen for histological analysis. The IVUS catheter was inserted into the artery until the surgical needle echo was visualized. The catheter was kept parallel to the long axis of the artery but was moved coaxially at the same cross section as confirmed by identification of the needle echo. The coaxial angular span was determined by the capability of the catheter to move inside the arterial lumen.

The images were optimized under visual inspection by manipulating the system settings. The gain settings were determined with the intent of maximizing image morphology without excessive dropout, not saturating adventitial intensity, and minimizing noise. After the first image was optimized, the gain was then fixed for all images within the artery. These settings were used for all other arterial segments studied. The ultrasound images were recorded on super-VHS tape. The ramp setting options were not used in the IVUS system.

Data Acquisition
The arterial images with the needle acoustic reference were digitized on a Macintosh computer. An on-screen image analysis application (NIH Image, public domain software) was used to measure gray-scale video intensity for each plaque portion. Because the absolute energy of the reflected ultrasound intensity cannot be obtained with regular IVUS machines, the intensity of each segment was measured by use of a relative intensity (RI) index calculated as $RI = (I_{ROI} - I_{bkg}) / (I_{ref} - I_{bkg})$, where $I_{ROI}$ represents the echo intensity of the subject region of interest (ROI), $I_{ref}$ the echo intensity of a standard reflector with the same gain settings, and $I_{bkg}$ the intensity of background of the equivalent ROI. The background area was chosen from an IVUS image in the saline bath before the catheter was inserted into the artery. Each ultrasound image had a gray scale of 256 levels on a 640×480-pixel display. A piece of tissue paper was placed in the saline bath and imaged as the standard reflector. As previously reported, this index has proved to be reproducible, varying by only 3.0% with changes in the overall gain setting, provided that the videointensity of any area does not become saturated.

IVUS Color Mapping of Angle-Dependent Echo-Intensity Variation
Representative examples of the angle dependence of IVUS backscatter from an arterial plaque are shown in Figure 1. Our previous study demonstrated that the part of the plaque for which backscatter was most sensitive to changes in angle corresponded to fibrous/acellular tissue. Therefore, a 0.5×0.5-mm box, the ROI, was placed around the plaque surface (Figure 2). The intensity of the ROI was then measured for all video frames to select the images with the maximum and minimum echo intensity. It was documented that the distance between the transducer and the tissue did not reveal any significant effect on the echo intensity in this setting.

When the IVUS catheter was moved coaxially inside the lumen, the arterial image moved on the video screen. Therefore, 2 tracking markers were visually determined at the edge of the plaque shoulder to match the 2 IVUS images on a pixel-by-pixel basis. Digital subtraction between 2 images was performed. Absolute value of difference in intensity for each pixel was then colorized.

**Figure 1.** Representative examples of angle dependence of IVUS backscatter from same arterial plaque. Each image was created by moving catheter within lumen to alter angle of incidence. *IVUS catheter; n indicates echo from surgical needle used as acoustic reference to ensure that same cross section was imaged.*

**Figure 2.** IVUS image acquisition. a, Echo intensity of ROI around plaque surface was measured for all video frames. b, Images with maximum and minimum echo intensity in ROI were then selected.
2 IVUS plaque images. In such cases, the 2 IVUS images were selected from the nondistorted images. After the matching, digital subtraction between the 2 images was performed with the relative intensity values. The absolute value of the difference in intensity for each pixel between the 2 images was then colorized.

**Histological Study**

After the arteries were imaged by IVUS, the needle was removed and the needle site marked with India ink. The specimens were processed for histology and stained with Masson’s trichrome stain. Histological views of each sample were also digitized on computer to measure the thickness of the fibrous/acellular area in the plaque samples.

**Statistics**

Values are expressed as mean±SD. The accuracy of ultrasound measurements compared with histological measurements was assessed with 2 different analyses: (1) linear regression and (2) Bland-Altman analysis of agreement. The latter analysis was used to compare the mean difference and SD between the values from ultrasound and histology. The mean difference in measurements represents the bias of ultrasound relative to histology; the SD indicates a measure of precision of value from IVUS compared with that from histology. In these analyses, a value of $P<0.05$ was considered significant.

**Results**

**IVUS Color Mapping of Plaque in Vitro**

A representative example of color mapping of angle-dependent echo-intensity variation of IVUS plaque image compared with histology is shown in Figure 4. This mapping was obtained from the same plaque cross section as shown in Figure 1. Colorization was obtained with a public domain software program (NIH image), which provided white to yellow for higher values, blue for middle values, and purple to black for lower values for each pixel. A clearly bordered area with large echo-intensity variation was revealed at the surface of plaque. This area seemed to correspond to the fibrous/acellular area at the surface of the plaque. Figure 5 shows 3 representative examples of plaque, which have different thickness of the area at the plaque surface.

**Validation Study**

The thickness of the demarcated area at the plaque surface in the color IVUS image and the thickness of the fibrous/acellular area in histology were measured for the same segment at the same distance from the edges of the plaque shoulder. There was significant correlation ($r=0.81$, $P<0.0001$) in thickness between the area in the IVUS image and that in histology (Figure 6). Bland-Altman analysis revealed that the bias of the color IVUS measurement of the thickness of the fibrous/acellular area was $0.00±0.10$ mm compared with the histological measurement (Figure 7).

**Discussion**

The present study introduces a novel technique, developed in our laboratory, for the color mapping of the angle-dependent echo-intensity variation in IVUS. The major finding of this
study was that this technique provides an accurate representation of the thickness of the fibrous cap in atherosclerotic plaque. This method may have clinical relevance because the fibrous cap is composed primarily of fibrous/acellular tissue, and the thickness of this capsule covering the lipid core is related to plaque vulnerability.22,23

Accuracy of the Present Color Mapping Technique
The accuracy of this method for measuring the thickness of the fibrous cap was tested against histological measurements and was found to be acceptable according to both the correlation study and Bland-Altman analysis. The Bland-Altman analysis showed that the bias of the color IVUS measurement of the thickness of fibrous cap was 0.00±0.10 mm compared with the histological measurement. Considering the resolution of IVUS, which was ≈0.05 mm,24 this SD of 0.10 mm was quite acceptable. It has been documented that plaque is prone to rupture when the thickness of fibrous cap becomes <0.2 mm.25 Therefore, our method may prove useful in discriminating vulnerable plaque in atherosclerotic lesion.

A structure that is bigger than the ultrasound wavelength generates directional backscatter.26,27 The direction of the backscatter is determined by the ultrasound beam angle of incidence as well as the shape of the structure. If the shape is a flat surface, the backscatter directed to the transducer reaches a maximum when the ultrasound incidence is perpendicular to the surface. The collagen fibers at the surface of the fibrous cap are arranged parallel along the plaque surface, providing a flat acoustic interface. Furthermore, the border between the fibrous cap and the lipid-rich area is usually distinct, displaying a significant difference in acoustic impedance. This distinct border is probably responsible for generation of the backscatter directivity, giving a clearly marked echo pattern at the plaque surface.

Comparison With Other Methods of Tissue Characterization
It was originally expected that tissue components within plaque could be identified from the videointensity pattern of

Figure 5. Plaque examples with a clearly bordered demarcated area at surface represented by IVUS color mapping of angle-dependent echo-intensity variation, which corresponded to fibrous cap. Three different plaques with such areas of different thickness are shown (A through C).

Figure 6. Correlation between thickness of demarcated area with highly directive echo intensity at plaque surface in color IVUS and that of fibrous cap in histology.

Figure 7. Bland-Altman analysis for color IVUS image vs histology in measurement of thickness of fibrous cap.
IVUS images. Subsequent studies, however, demonstrated significant limitations of tissue characterization by IVUS intensity patterns alone, especially in discriminating fibrous and fatty tissues or in assessing plaque vulnerability.

To overcome the limitations, several methods of quantitative tissue characterization have been proposed to discriminate fibrous and fatty plaque. These included radiofrequency signal analysis, such as integrated backscatter analysis, and attenuation slope mapping. These methods, however, required the averaging of vectors in some ROIs or subwindows, causing difficulty in detecting small tissue heterogeneities. These methods also required special equipment that is not supplied with currently available IVUS machines. Furthermore, these methods have potential limitations in that even the radiofrequency signal is strongly influenced by the ultrasound beam angle.

Two major advantages of our technique are that it can be performed with commercially available IVUS machines and that it provides an easily recognizable visualization of the fibrous cap. Visualization of the fibrous cap may also be available with a gray-scale coding of angle-dependent echo-intensity variation in our method. The greatest advantage of using colorization, however, may be that the colorized image can be superimposed on a regular IVUS image in routine diagnostic IVUS procedures.

Study Limitations
When the present method is applied in vivo, the coaxial movement of the catheter tip within the coronary artery lumen might be seen as a problem. A new pioneering method that provides a catheter-tip manipulation system with micromachine-based multiple joints, which is already available as an intermediary pilot device in angioscopy, could well overcome this difficulty.

There were some limitations in measurement in this study. The arteries imaged in the present study were placed in a saline bath at room temperature of 20°C. The IVUS system assumes a constant velocity of sound of 1540 m/s, which is the velocity of sound in water at a body temperature of 37.0°C. The measurement of a tissue size is influenced by sound speed inside the tissue. It has been reported that the velocities of sound inside fibrous tissue and fatty tissue within plaque at 20.0°C are 1514 m/s and 1532 m/s, respectively. Therefore, the influence of the imaging condition we used was negligible in the measurement of fibrous capsule thickness.

Another limitation was that the arteries were imaged after they were fixed by formalin. It has been reported that in vessels with <50% luminal cross-sectional narrowing, histological fixation and processing changed the size of luminal cross-sectional area but did not change absolute wall area. The arteries imaged in the present study had <50% luminal cross-sectional narrowing. Therefore, the effect of histological fixation and processing was not so significant in the measurement of thickness of fibrous cap. It is known that formalin fixation can enhance echogenicity of tissue. Several reports, however, have documented that formalin fixation does not significantly affect the morphology and qualitative echo patterns of plaque tissue. Therefore, it can be reasonably extrapolated that the distinct acoustic border between the fibrous cap and the lipid-rich area exists in arterial plaques in vivo as well.

Conclusions
The present study introduces a novel technique, developed in our laboratory, for color mapping the angle-dependent echo-intensity variation in IVUS. This novel method of tissue characterization provides an accurate representation of the thickness of fibrous cap in atherosclerotic plaque within a bias of 0.00±0.10 mm. This method may prove useful in assessing plaque vulnerability in atherosclerosis.

Acknowledgment
This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Japan (grant No. 12670672).

References
Detection of Fibrous Cap in Atherosclerotic Plaque by Intravascular Ultrasound by Use of Color Mapping of Angle-Dependent Echo-Intensity Variation
Takafumi Hiro, Takashi Fujii, Kyounori Yasumoto, Takashige Murata, Akihiro Murashige and Masunori Matsuzaki

_Circulation_. 2001;103:1206-1211
doi: 10.1161/01.CIR.103.9.1206

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/103/9/1206

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/