Quantitative Evaluation of Lipid Volume Fraction in Atherosclerotic Plaque Phantoms by Near-infrared Multispectral Imaging at Wavelengths around 1200 nm

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Abstract Near-infrared multispectral imaging (NIR-MSI) is a potentially effective technique for quantitative evaluation of atherosclerotic plaque. NIR light shows high penetration for biological tissues and the NIR region includes the characteristic absorptions of lipid-rich vulnerable plaques, especially at wavelengths near 1200 nm. In this study, a quantitative method for assessing lipid volume fraction in plaques—one of the factors of plaque vulnerability—was developed using NIR-MSI at three wavelengths around 1200 nm. Atherosclerotic phantoms with lipid volume fractions of 100, 80, 60, 40, and 20 vol% were prepared and measured by NIR-MSI at three wavelengths: 1150, 1200, and 1300 nm. The acquired datasets were processed by the spectral angle mapper method. Consequently, lipid-enhanced multispectral images of the phantoms were created. In addition, the differences in lipid volume fraction were evaluated and the fractions were classified into six grades quantitatively. These results show the potential of NIR-MSI for the quantitative evaluation of vulnerable plaques.

Keywords: spectroscopy, multispectral imaging, near-infrared, atherosclerotic plaque.


1. Introduction

Atherosclerosis is a disease of the arterial wall occurring at susceptible sites in the major conduit arteries. It is initiated by lipid retention, oxidation, and modification, which provoke chronic inflammation, ultimately causing thrombosis or stenosis. Atherosclerotic lesions can cause stenosis with potentially lethal distal ischemia, or they can trigger thrombotic occlusion of major conduit arteries to the heart, brain, legs, and other organs. Identification of these lesions, known as “vulnerable plaque” or “unstable plaque,” is essential for the development of treatment modalities to stabilize such plaques [1–3].

Vulnerable plaque cannot be identified by evaluation of stenosis, but is instead characterized by the large necrotic lipid core with high cholesterol accumulation and the presence of a thin-cap fibroatheroma (TCFA) [4]. However, standard diagnostic modalities for atherosclerosis, such as angiography and computed tomography, mainly evaluate the stenosis, which is not related to plaque type. Therefore, a novel method that provides more detailed information allowing the identification of vulnerable plaque is required [5].

Some intravascular imaging modalities have been studied for early detection of plaque segments considered to be vulnerable with a risk of causing clinical events [6]. However, although angioscopy provides real-time spatial information concerning the arterial intima, this method requires clinicians to make subjective judgments concerning subtle color differences and is hence unreliable for identifying vulnerable plaque [7]. Methods such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) that provide cross-sectional images of arterial walls are used for detecting vulnerable plaque. However, the spatial resolution of IVUS (70–200 μm) is insufficient for detecting the thin fibrous cap of vulnerable plaque. Although OCT has a higher spatial resolution (<10 μm), its penetration depth is limited to approximately 2 mm [8–10]. In addition, active attempts have been made to diagnose plaque using intravascular photoacoustic imaging that provides relatively high spatial resolution and penetration depth [11]. However, although these methods provide important structural information, none allow complete characterization of plaque.

Recently, spectroscopic techniques have been applied to the characterization of plaque. A number of studies have demonstrated the applicability of spectroscopy to evaluate the chemical composition of lipids in plaque [12–15]. Especially, these spectroscopic techniques are integrated with tomographic imaging such as IVUS or photoacoustic imaging, and they show promise for identification of vulnerable plaque [16, 17].

Multispectral imaging (MSI) is also a promising spectroscopic imaging technique used in multimodality imaging with angiography. Furthermore, this approach uses multiple wavelengths and allows advanced compositional analysis. In addition, near-infrared (NIR) light is suitable for biomedical imaging because of the high optical penetration depth in biological tissues. In our previous work, we detected atherosclerotic plaque phantoms using a camera system of NIR-MSI at a dozen wavelengths around 1200 nm [18]. The phantoms were observed through saline or blood layers, using the NIR-MSI system at wavelengths around 1200, 1700, and 2300 nm. In saline and blood environments, wavelengths around 1200 nm were optimal for lipid detection [19]. However, quantitative evaluation of lipid volume fraction to characterize plaque types had not been performed.
The purpose of this paper is to describe a quantitative method for diagnosing vulnerable plaque by NIR-MSI. Lipid volume fraction is one of the important factors of plaque vulnerability. In this study, the lipid volume fraction in atherosclerotic phantom was evaluated quantitatively by a camera system at three wavelengths near 1200 nm. The potential of NIR-MSI for the diagnosis of atherosclerotic plaques is presented.

2. Materials and Methods

2.1 Samples

Atherosclerotic phantoms were prepared using a biological tissue model and bovine fat. The biological tissue model was a normal tissue simulation model consisting of 0.5 mg/mL hemoglobin (H7379-10G; Sigma-Aldrich, St. Louis, USA), 0.1 g/mL gelatin (G2500-500G; Sigma-Aldrich, St. Louis, USA), and 2.0% intralipid (FB-011L20; Terumo, Tokyo, Japan) [20]. The bovine fat was used to mimic the optical absorption characteristics of lipid in vulnerable plaque. Figure 1 shows the absorption spectra of bovine fat and atherosclerotic plaque of rabbit artery measured by Fourier transform NIR spectroscopy (FT-NIR). In these two spectra, similar absorption peaks were found at the wavelength band around 1200 or 1700 nm. Figure 2(a) shows a schematic illustration of the atherosclerotic phantom, which has a disc shape with a radius of 18 mm and a thickness of 1 mm. The simulated plaque area is set at the center with a diameter of 2–3 mm, and is filled with the mixture of bovine fat and biological tissue model. The lipid volume fraction was 100, 80, 60, 40, or 20 vol%.

2.2 Optical setup

Figure 2(b) shows a schematic diagram of the NIR-MSI system used in the present study. The device employed a 150 W halogen lamp (LS-H150IR-FBC; Sumita Optical Glass, Inc., Saitama, Japan) and a specific wavelength was selected by band-pass filter. The phantom was irradiated by narrow band NIR light uniformly using a ring right guide (GF10-1-LR; Sumita Optical Glass, Inc., Saitama, Japan) that produced broadband NIR illumination at wavelengths from 800 to 2200 nm. The NIR light was guided by a straight light guide (GF9.5-1-LR-R50; Sumita Optical Glass, Inc., Saitama, Japan) and a specific wavelength was selected by band-pass filter.

2.3 Image processing

The reflectance \( R_\lambda (\lambda) \) at wavelength \( \lambda \) at a point with spatial coordinates \((i,j)\) was calculated using Eq. (1).

\[
R_\lambda (\lambda) = \frac{I_\lambda(\lambda) - D_\lambda(\lambda)}{W_\lambda(\lambda) - D_\lambda(\lambda)}
\]

Here, \( I_\lambda(\lambda) \) is the intensity of the diffuse reflection light from the sample measured by the MCT camera, \( D_\lambda(\lambda) \) is the dark intensity measured in the absence of illumination to correct for dark current, and \( W_\lambda(\lambda) \) is the diffuse light intensity from a diffuse reflection standard (WS-1; Ocean Optics, FL, USA). The apparent absorbance at each pixel was calculated using the modified Beer–Lambert law [18] given in Eq. (2).

\[
A_\lambda (\lambda) = -\log_{10}[R_\lambda (\lambda)]
\]

The spectral angle mapper (SAM) algorithm was used for data analysis. SAM is a classification method that permits rapid mapping by calculating the pixel-by-pixel similarity between the calculated reflection spectrum of an image and a reference reflection spectrum [22]. The reference reflection spectrum used was for bovine fat and was measured using FT-NIR, as shown in Figure 1. Both the measured and reference spectra were treated as vectors in \( n \)-dimensional space. Here \( n \) is the number of wavelength. The spectral similarity costh was determined using Eq. (3).

\[
\cos \theta = \frac{A \cdot B}{|A||B|}
\]

Here \( A \) is the vector at each pixel in the sample image, and \( B \) is the reference vector.

In this paper, two types of multispectral images were created using the spectral similarity costh. First, lipid-enhanced multi-

![Fig. 1 Absorption spectra of bovine fat and atherosclerotic plaque of rabbit artery in the near-infrared range.](image)

![Fig. 2 (a) Schematic representation of atherosclerotic phantom with 100, 80, 60, 40 or 20 vol% lipid volume fraction. (b) Schematic representation of the NIR-MSI system.](image)
spectral images were processed. The pixels with high spectral similarities were visibly enhanced in the 256-level gradation process and salt-and-pepper noises were removed using a median filter. Second, lipid classification images were created quantitatively. The pixels were encoded by six colors using the correlation between the lipid volume fraction and $\cos \theta$ in multispectral images.

3. Results

3.1 Lipid-enhanced multispectral images of phantoms

Figure 3 shows the lipid-enhanced multispectral images of atherosclerotic phantoms with lipid volume fractions of 100, 80, 60, 40, and 20 vol%, and the visible light image of a phantom with 100 vol%. In the visible light image, the simulated plaque area was not visible. Conversely, the simulated plaque areas were enhanced in all multispectral images. High contrast was clearly observed between the simulated plaque area and the biological tissue area, especially in high lipid volume fraction phantoms, but the contrast was lower in low lipid volume fraction phantoms (20 and 40 vol%). In addition, the differences in lipid volume fraction among various lipid volume fractions were hardly distinguishable in lipid-enhanced multispectral images.

3.2 Investigation of the relationship between lipid volume fraction and $\cos \theta$

The relationship between the lipid volume fractions in the phantoms and $\cos \theta$ was evaluated for quantitative analysis of the phantoms (Figure 4). The $\cos \theta$ was extracted from the simulated plaque area with 900 pixels in each multispectral image, and the data for 0 vol% was extracted from the biological tissue area in all the phantoms. There was a good correlation between the lipid volume fraction and $\cos \theta$. These data plots were fitted by exponential function and the threshold of lipid volume fraction was defined for quantitative analysis.

3.3 Classification images of lipid volume fraction

Since the former experiment (Figure 3) established that lipid volume fraction in the phantoms is not distinguishable in lipid-enhanced multispectral images, we created quantitative classification images using the threshold of $\cos \theta$. Figure 5 shows lipid

![Fig. 3 Lipid-enhanced multispectral images of atherosclerotic phantoms with lipid volume fractions of 100, 80, 60, 40, 20 vol%, and visible light image of a phantom with 100 vol%. The simulated plaque areas were enhanced in all lipid-enhanced multispectral images.](image)

![Fig. 4 Relationship between lipid volume fraction and spectral similarity $\cos \theta$.](image)

![Fig. 5 Lipid classification images of atherosclerotic phantoms with 100, 80, 60, 40, 20 vol%. The lipid volume fractions were classified into six grades of vol%: 100–90, 90–70, 70–50, 50–30, 30–10, and 10–0.](image)
classification images of the atherosclerotic phantoms. In this investigation, five thresholds were chosen and the lipid volume fractions were classified into six grades: 100–90, 90–70, 70–60, 60–40, 40–20, and 20–0 vol%. The simulated plaque areas in all phantoms were graded clearly in lipid classification images, even though low lipid volume fraction phantoms had poor visibility in lipid-enhanced multispectral images. However, false detections were observed around the simulated plaque area in high lipid volume fraction phantoms of 100 or 80 vol%, due to the diffusion of light in the phantoms.

4. Discussion

Atherosclerotic plaques have absorption bands around 1200, 1450, and 1700 nm as shown in Figure 1. The bands around 1200 and 1700 nm correspond to the second and first overtones of the CH stretching vibration mode, respectively. The band around 1450 nm corresponds to the first overtone of the OH stretching vibration mode.

Vulnerable plaque is typically characterized by the presence of a TCFA and a lipid-rich necrotic core. Previously, many assessments of TCFA were performed in vulnerable plaques using intravascular tomographic imaging such as OCT and IVUS. In this study, the atherosclerotic phantoms were successfully imaged by OCT and IVUS. The spectral band around 1200 nm has good optical penetration in biological tissues. In our previous study, lipid-rich cores were also assessed by virtual histology IVUS, angioscopy and other modalities [25, 26]. In addition, multimodalities of IVUS and NIR spectroscopy have been proposed as novel methods for quantitative diagnosis of the lipid-rich necrotic core or lipid distribution with chemical composition [16, 17]. On the other hand, NIR-MSI is expected to be useful for multimodality imaging with angioscopy because it may allow spatial acquisition of spectral data and visualization of the interior of blood vessels at the same time. NIR-MSI would provide new diagnostic criteria for plaques based on the chemical composition.

In this study, the atherosclerotic phantoms were successfully classified in lipid classification images when the lipid volume fraction was 20 vol% or higher. Typical disrupted vulnerable plaques have lipid volume fractions in excess of 40 vol%, although the 40 vol% varies depending on the form and location of the plaque [27, 28]. Therefore, NIR-MSI with three wavelengths around 1200 nm has the potential to evaluate vulnerable plaques. However, higher sensitivity may be required for the detection of stable plaques that typically have lipid volume fractions lower than 20 vol%.

Additionally, the thickness of the fibrous cap in plaques should be considered when using NIR-MSI for quantitative evaluation of plaques. The depths of the lipid-rich necrotic cores vary greatly among plaques, although we evaluated atherosclerotic phantoms with exposed lipid areas in this study. However, the spectral band at 1200 nm has relatively high optical penetration depth in biological tissues. In our previous study, the lipid cores were detected up to a depth of at least 1 mm [18]. We evaluated the effect of lipid location by examining the relationship between the depth of simulated plaque area and spectral similarity (Figure 6). In this experiment, layers of biological tissue model with thicknesses ranging from 0 to 1 mm were overlaid on the atherosclerotic phantoms. There was a good correlation between the depth and the spectral similarity. This result indicates that plaque location can be estimated. However, simultaneous evaluation of the depth and lipid volume fraction is difficult. Using multiple wavelength bands may allow the simultaneous evaluation. The spectral signal from deep plaques will be attenuated by scattering and absorption of overlaid tissues, and may well interfere with accurate measurement of spectral similarity. Moreover, spectral similarity is affected by the thickness of plaque. Thus, the lipid volume fraction and the depth of plaques should be estimated comprehensively beforehand in clinical situations.

To apply MSI to angioscopy, it is necessary to consider the effects of blood and the saline flush used in the intravascular environment. The spectral band around 1200 nm has good optical penetration for water and hemoglobin, and the atherosclerotic phantoms could be observed under saline at a depth of 10 mm or under arterial blood at a depth of 1 mm in our previous research [19]. NIR-MSI at 1200 nm is potentially capable of evaluating plaques through saline when used with an angioscope, because the focus of the angioscope is around 2 mm.

The wavelength band around 1700 nm is also promising for the diagnosis of vulnerable plaques using NIR-MSI. Although the wavelength band at 1700 nm has high absorbance for water and biological tissues compared to 1200 nm, the absorption peak for lipid is much stronger at 1700 nm [21, 29]. Besides, less scattering of light by blood cells is expected at longer wavelengths [30]. This wavelength band may be suitable for the detection of superficial lipid-rich necrotic core through arterial blood. In future studies, NIR-MSI at these spectral ranges will be assessed with an angioscopic system.

5. Conclusion

A quantitative method for the evaluation of lipid volume fraction in plaques using NIR-MSI at 1200 nm was developed. This method utilizes the correlation between the lipid volume fraction and costh, and is analyzed by SAM. As a result, atherosclerotic phantoms were detected and classified quantitatively when the lipid volume fraction was 20 vol% or higher. This study shows the potential of NIR-MSI for diagnosing vulnerable plaques and suggests that multimodality imaging using NIR-MSI and angioscopy may provide new diagnostic criteria of plaques based on the chemical composition.
Conflict of Interest

We have no conflicts of interest relationship with any companies or commercial organizations based on the definition of Japanese Society of Medical and Biological Engineering.

References

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Ryo Nagao received his master’s degree in engineering from Graduate School of Engineering, Osaka University, Japan, in March 2015. His study covered the field of spectroscopic imaging for medical diagnosis.

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