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Molecular Imaging of Atherosclerotic Coronary Plaques by Fluorescent Angioscopy

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1. Introduction

It is generally believed that the coronary plaques with lipid-laden thin fibrous cap and a large lipid core beneath are vulnerable and imaging methods such as intravascular ultrasonography[1], optical coherence tomography[2], and angioscopy[3-5] are clinically employed to detect this type of plaques. However, the coronary plaques that have a thin cap composed of tight calcium layers are frequently observed during post-mortem examinations[3]. Moreover, plaques wherein the deposition of lipids and macrophages is confined to just the superficial layers and in which a lipid core is not present also exist[3,6]. These evidences indicate the necessity of detailed molecular characterization for the detection of vulnerable plaques.

Oxidized low-density lipoprotein (ox-LDL) plays an important role in the initiation, progression and destabilization of atherosclerotic plaques by inducing the proliferation and elongation of survival of macrophages[7,8].

Normal collagen fibers (CFs) that contain collagen-I in abundance protect the coronary plaques against mechanical stress. During plaque growth, collagen-I is replaced by collagen-III, -IV and/or -V[9-11], and CFs are degenerated, disrupted, and finally destroyed by matrix metalloproteinases released by macrophages[12]. During this process, macrophages accumulate lipids such as cholesteryl esters (CEs) and ox-LDL[13,14] and become foam cells while simultaneously producing ceramide within themselves[15]; their death results in formation of the lipid core. Therefore, demonstrating the lack of collagen-I which is mainly contained in normal CFs, deposition of lipids and existence of ox-LDL and the substances that comprising ox-LDL is an essential requisite for the detection of vulnerable plaques, but *in-vivo* clinical tools to visualize them in the coronary plaques are lacking.

Structurally, ox-LDL is composed of cortex and core. The cortex is composed of lysophosphatidylcholine (LPC), free cholesterol (C) and Shiff base derived by oxidation

from apolipoprotein B (apoB), and the core is composed of triglyceride (TG), cholesteryl esters (CEs), and proteins (Figure 1).

High-density lipoprotein (HDL) is composed of the same substances except Apo B, and plays a key role in reverse cholesterol transport but also stimulates prostacyclin release, enhances endothelial repair, inhibits monocyte recruitment into the arterial wall, and inhibits progression of and enhances regression of atherosclerosis[16, 17].

LPC itself is a pro-inflammatory substance, and plays a critical role in the atherogenic activity of ox-LDL. This substance is generated by lipoprotein-associated phospholipase A_2 [18]; induces vascular endothelial cell dysfunction[19]; causes endothelial cell apoptosis by DNA fragmentation[20]; stimulates adhesion and activation of lymphocytes and initiates chemotaxis of macrophages[21]; causes transformation of vascular smooth muscle cells that is characteristic of atherosclerosis.

Apolipoprotein B-100 (apo B-100) is a major protein of low density lipoprotein (LDL)[22]. Serum Apo B-100 is elevated in patients with type 2 diabetes mellitus[23] as well as in those with coronary artery disease[24]. Apo B-100 and apo B-100/apo A-1 ratio predict not only coronary artery disease[25] but also other cardiovascular disease[26], and metabolic syndrome[27]. Medical treatments have been directed to lower Apo B-100[28].

TG is also considered an important risk of cardiovascular disease, but lowering TG levels remains difficult to achieve[29, 30].

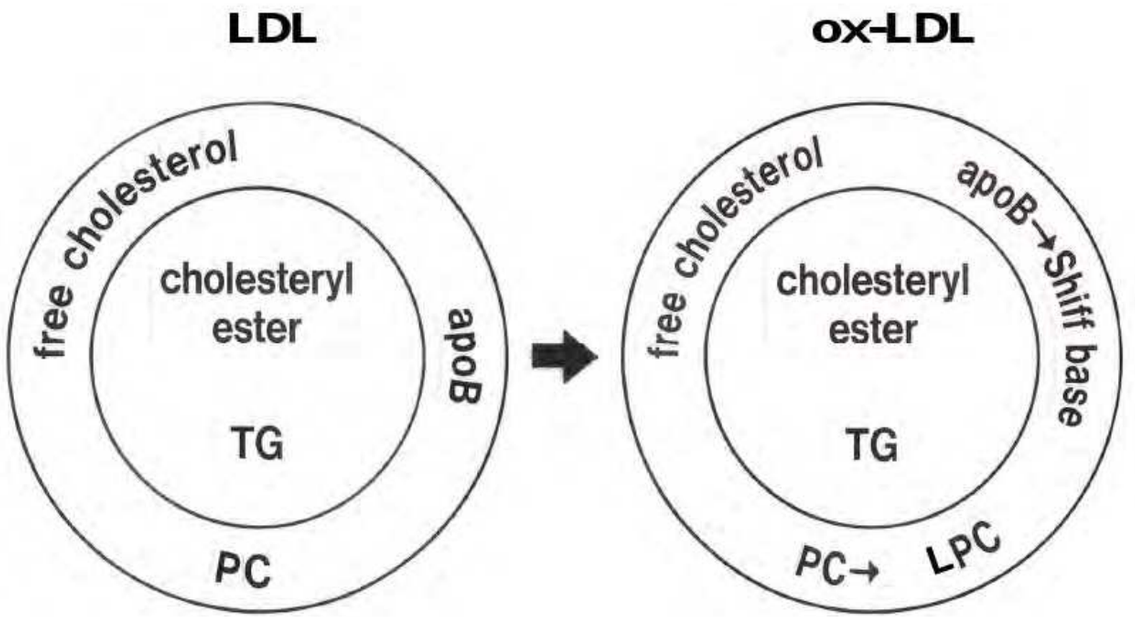
Although these substances in serum are measureable, measurement of these substances in the atherosclerotic plaques is difficult. If they become visible in patients *in-vivo*, initiation, progression and destabilization, and the effects of medical and interventional therapies on atherosclerotic plaques can be objectively evaluated.

Based upon the knowledge about the pathophysiology of atherosclerosis, *in-vitro* and *in-vivo* animal studies and clinical trials have been performed using different tracers for plaque imaging studies, including radioactive-labelled lipoproteins, components of the coagulation system, cytokines, mediators of the metalloproteinase system, cell adhesion receptors, and even whole cells, or antibodies of the substances composing atherosclerotic plaques. However the majority of them are still not applicable clinically.

Spectroscopy has been attempted intensively for molecular imaging of lipids within the atherosclerotic lesions *in-vitro*[31,32]. However, its clinical application started only recently[33]. Also, magnetic resonance imaging or computed tomography has been attempted for lipid imaging, however their clinical application is yet not established[34,35].

Although invasive, angioscopy is a clinically established high resolution technique, which enables direct, colored and three-dimensional imaging of the coronary arterial wall. Recently, fluorescent angioscopy, both color and near-infrared, was developed and is used for molecular imaging of the substances within the human coronary plaques not only *in-vitro*[36-39] but also in patients *in-vivo*[36,37].

The present article reviews recent advances in molecular imaging of the substances comprising atherosclerotic coronary plaques by fluorescent angioscopy.



TG: triglyceride. apo B: apolipoprotein B . PC: phosphatidylcholine. LPC: lysophosphatidylcholine.

Fig 1. Schematic Representation of Structure of Low-density Lipoprotein (LDL) and Oxidized Low-density Lipoprotein (Ox-LDL).

2. Fluorescent angioscopy system

a. Color fluorescent angioscopy

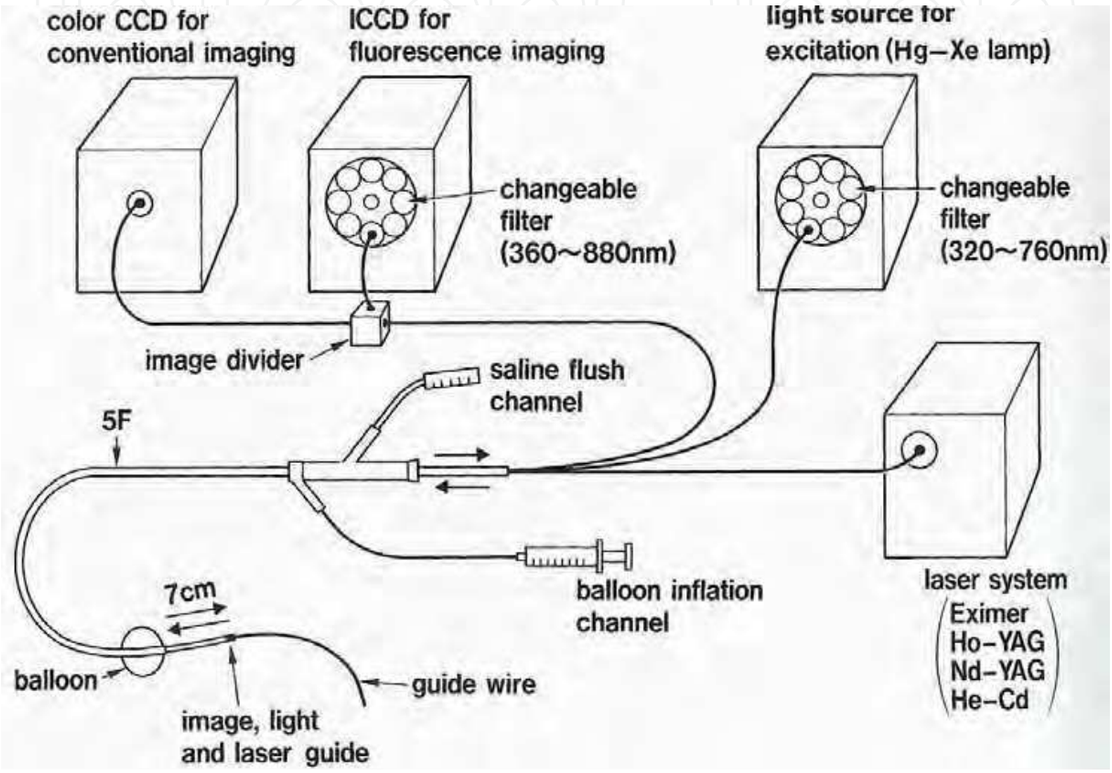
The color fluorescent angioscopy (CFA) system is composed of a fluorescence-excitation unit, an angioscope, a fluorescence-emission unit and a camera. The fluorescence-excitation unit is composed of a mercury-xenon lamp and seven sets of band-pass filter (BPF) discs, exchangeable by rotation for selection of the desired wave length of light ranging from 320- to 760-nm. The remaining single disc, which did not have any filter, was used to irradiate white light for carrying out conventional angioscopy. The angioscope (modified VecMover, Clinical Supply Co, Gifu, Japan) was composed of a 2.5-F fiberscope which contained 6000 quartz fibers for the image guide and 300 quartz fibers for the light guide. This fiberscope was incorporated in a 5-F guiding balloon catheter and was steerable along a 0.014-inch guide wire; it also enabled observation of a coronary segment up to 7 cm in length by a single saline flush. This angioscope was approved for clinical use by the Japanese Ministry of Health and Labor and Welfare, and is widely used clinically in Japan.

The fluorescence-emission unit is composed of seven sets of dichroic membrane (DM) and 7 sets of band-absorption filter (BAF) discs which receive light ranging from 360- to 880-nm and is connected to a 3CCD digital camera (C7780, Hamamatsu Photonics, Hamamatsu). The obtained images are displayed on a computer screen through a camera controller (C7780, Hamamatsu Photonics, Hamamatsu).

To observe the vascular lumen, the light and image guides are connected to the excitation and emission units, respectively. After selecting the desired BPF and BAF, the light is irradiated through the BPF and the light guide toward the target. A pair of BPF of 345±15

nm and BAF of 420 nm ("A" imaging), and another pair of 470±20 nm BPF and BAF of 515 nm ("B" imaging) are usually used for imaging The evoked fluorescence is received by the digital camera through the DM and BAF for successive three-dimensional imaging at an adequate time-interval from 0.01 to 1 s. The details of this CFA system are described elsewhere.[41]

The intensity of the fluorescence images is arbitrarily defined as strong, weak and absent when the exposure-time required for imaging is within 0.5, more than 0.5 and within 1 and more than 1 s, respectively (Figure 2).



Cited from Ref. 41, with permission.

Fig. 2. Schematic Representation of A Fluorescent Angioscopy System

b. Near-infrared fluorescent angioscopy (NIRFA)

For NIRFA, the BPF and BAF of the angioscopy system used for color fluorescent angioscopy system are replaced by a 710±25 nm BPF disc and 780-nm BAF disc by rotation, respectively, and color CCD camera is replaced by an intensified CCD camera (C3505, Hamamatsu Photonics)[37].

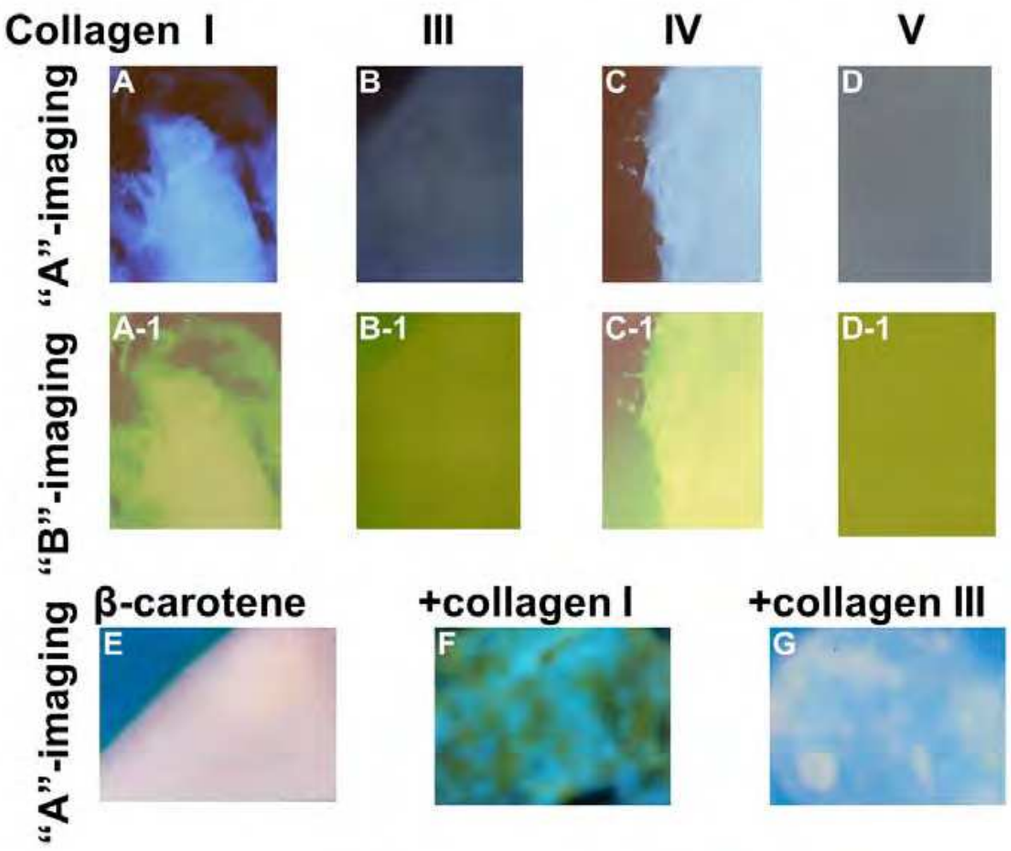
3. Color fluorescence of major substances that comprising atherosclerotic plaques examined by fluorescent microscopy

a. Autofluorescence at "A"-imaging

Among the major substances that constitute atherosclerotic plaques, collagen-I and -IV exhibit blue and light blue autofluorescence, respectively whereas collagen-III and -V did

not. Blue or light blue auto-fluorescence was not exhibited by other substances. Calcium phosphate, ceramide, and β-carotene which co-exists with lipid in the vascular wall, exhibited white, purple and orange auto-fluorescence, respectively (Figure. 3; Table 1)

In the presence of β-carotene, collagen-I and -IV exhibited green fluorescence, collagen-III and -V showed white fluorescence, cholesterol (C) exhibited yellow fluorescence and cholesteryl esters (CEs) showed orange fluorescence (Figure 3; Table 1).



From A to D: "A"-imaging of and E to H: "B"-imaging of collagens-I, -III, -IV and -V, respectively. By "A"-imaging, blue fluorescence was seen in collagen-I, no fluorescence in collagen-III, light blue fluorescence in collagen-IV and no fluorescence in collagen-V. I: β-carotene (10^{-5} M) showing orange in fluorescence color by "A"-imaging. J: mixture of collagen-I and β-carotene showing green in fluorescent color by "A"-imaging. K: mixture of collagen-III and β-carotene showing white-to-light blue in fluorescence by "A"-imaging. Horizontal bar of each panel: 100 μm. cited from Ref. 36, with permission.

Fig. 3. Fluorescence of Collagen Subtypes Visualized by "A"- and "B"-Imagings of Color Fluorescent Microscopy (CFM) Before and After Mixing with β-Carotene.

	Color fluorescent microscopy Fluorescence evoked by dyes									
	AF		EB		TB		NB		Ho+TB	
	"A"	"B"	"A"	"B"	"A"	"B"	"A"	"B"	"A"	"B"
LDL	-	-	-	-	V	O	-	**Br**	R	**Go**
Ox-LDL	-	-	**V**	**RBr**	V	O	-	-	V	**Go**
VLDL	-	-	-	R	V	O	-	-	P	O
HDL	-	-	R	R	P	R	SB	G	-	P
LPC	-	-	R	R	**R**	**R**	P	**R**	V	**R**
PC	-	-	-	O	P	O	-	-	P	O
TG	-	-	-	-	-	-	P	Br	-	-
Apo B-100	-	-	-	R	P	O	-	**Go**	P	**R**
Apo A-1	-	-	-	-	-	-	-	-	-	-
Apo E-2	-	-	-	-	-	-	-	-	-	-
MMP-9	-	-	-	-	-	-	-	-	-	-
Cholesterol	W	Y	-	O	-	-	-	-	W	G
Chole oleate	-	-	-	-	-	-	-	-	-	-
Chole linoleate	-	-	-	-	-	-	-	-	-	-
7-Keto chol	-	-	-	-	-	-	-	-	-	-
5-Cholesten-3 β -ol	-	-	-	-	-	-	V	Br	-	-
Oleic acid	-	-	-	-	-	-	-	-	-	-
Linoelic acid	-	-	-	-	-	-	-	-	-	-
Collagen I	**B**	**G**	-	-	-	-	B	G	-	-
Collagen IV	**LB**	**G**	-	-	-	-	LB	G	-	-
Collagen III, V	-	-	-	-	-	-	-	-	-	-
Heparan sulfate	-	-	-	-	-	-	-	-	-	-
Hyaluronic acid	-	-	-	-	-	-	-	-	-	-
Albumin	-	-	-	-	-	-	-	-	-	-
Globulins	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-
Ceramide	P	G	-	-	-	-	**R**	**R**	-	-
Calcium phosphate	W	Y	B	Y	-	-	-	-	-	-
β -Carotene	**O**	**O**	O	O	-	-	-	-	P	O

AF: autofluorescence. EB: Evans blue. TB: Trypan blue. NB: Nile blue. Ho: Homidium chloride.
"A" : "A"-imaging.
"B" : "B"-imaging.
LDL: low-density lipoprotein. Ox-LDL: oxidized low-density lipoprotein. VLDL: very low-density

lipoprotein. HDL: high-density lipoprotein. LPC: lysophosphatidylcholine. PC: phosphatidylcholine. TG: triglyceride. Apo: apolipoprotein. MMP: matrix metalloproteinase. Chole: cholesteryl. 7-Keto chol: 7-ketocholesterol.
B: blue. **Br**: brown. **G**: green. **Go**: gold. **LB**: light blue. **LY**: light yellow. **O**: orange. **P**: purple. **R**: red. **RBr**: reddish brown. **SB**: sky blue. **V**: violet. **W**: white. **Y**: yellow. - : no significant fluorescence.
Underlined colors are characteristic for the corresponding substances.

Table 1. Fluorescence Color of the Major Substances That Comprising Atherosclerotic Plaques

b. Autofluorescence at "B"-imaging

Collagen I and IV exhibit green autofluorescence whereas collagen III and V not. Calcium phosphate, ceramide and β-carotene exhibit yellow, yellow and orange autofluorescence, respectively. Other major subtances comprising atherosclerotic plaques do not exhibit auto-fluorescence (Table 1).

c. Color fluorescence of major substances that comprising atherosclerotic plaques evoked by markers

Evans blue dye (EB) has been clinically used for intravascular imaging[41-43], and its beneficial effects proved[44].

Oxidized low-density lipoprotein (ox-LDL) does not show autofluorescence, but presents a violet and a reddish brown fluorescence in the presence of EB by "A"- and "B"-imaging, respectively (Figure 4). This combination of fluorescent colors is not exhibited by any other major substances in the atherosclerotic plaques, indicating that this combination of fluorescent colors is due to ox-LDL (Table 1).

TB has been clinically applied for treatment of Tripanosoma parasitemia many years ago[45, 46]. Although LPC and TB do not exhibit autofluorescence independently, a red fluorescence is evoked at both "A"- and "B"-imaging when they are mixed together. PC exhibits a pink and an orange fluorescence by "A"- and "B"-imaging, respectively[38] (Figure 4).

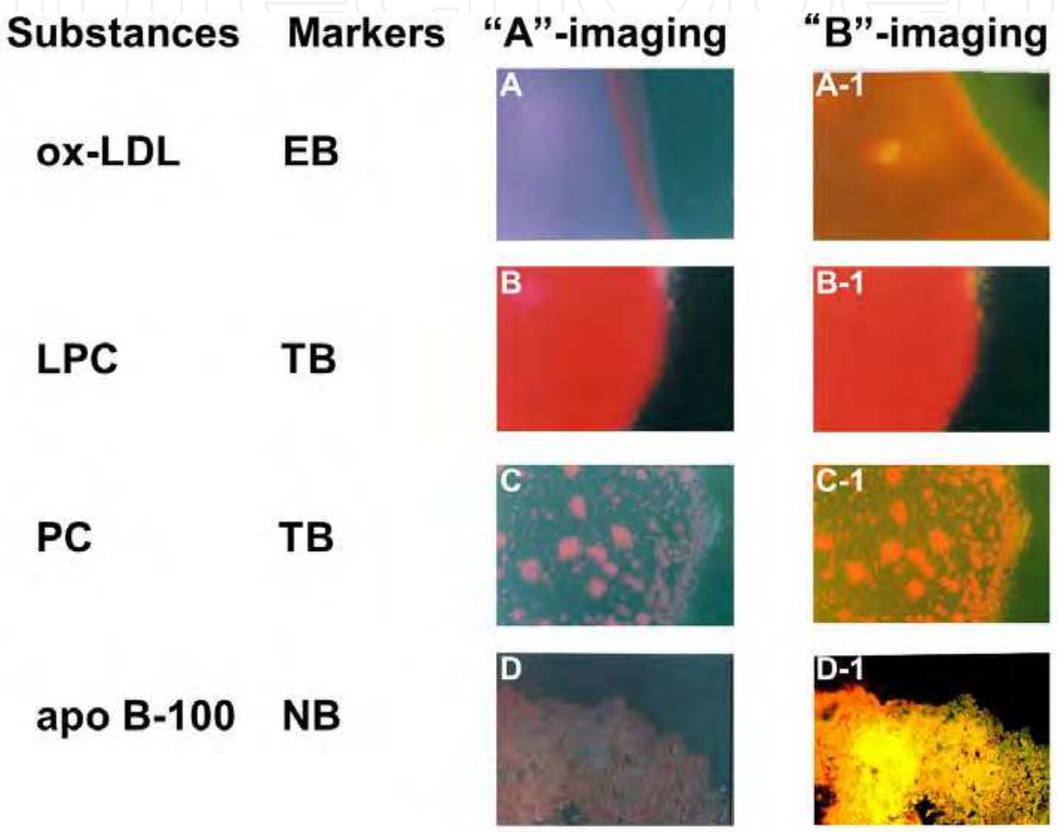
Nile blue dye (NB), which has been used as a electromechanical biosensor of DNA[47]. Apolipoprotein B-100 (apo B-100) exhibits no fluorescence at "A"-imaging but exhibits a golden fluorescence in the presence of NB at "B"-imaging (Figure 4, Table 1).

In the presence of a mixture of homidium chloride (Ho) and TB, ox-LDL and LDL exhibit a golden fluorescence whereas LPC and apo B-100 a red fluorescence at "B"-imaging (Table 1).

Since not exhibited by other major substances comprising atherosclerotic plaques, these fluorescent colors can be used for identification of the substances mentioned above.

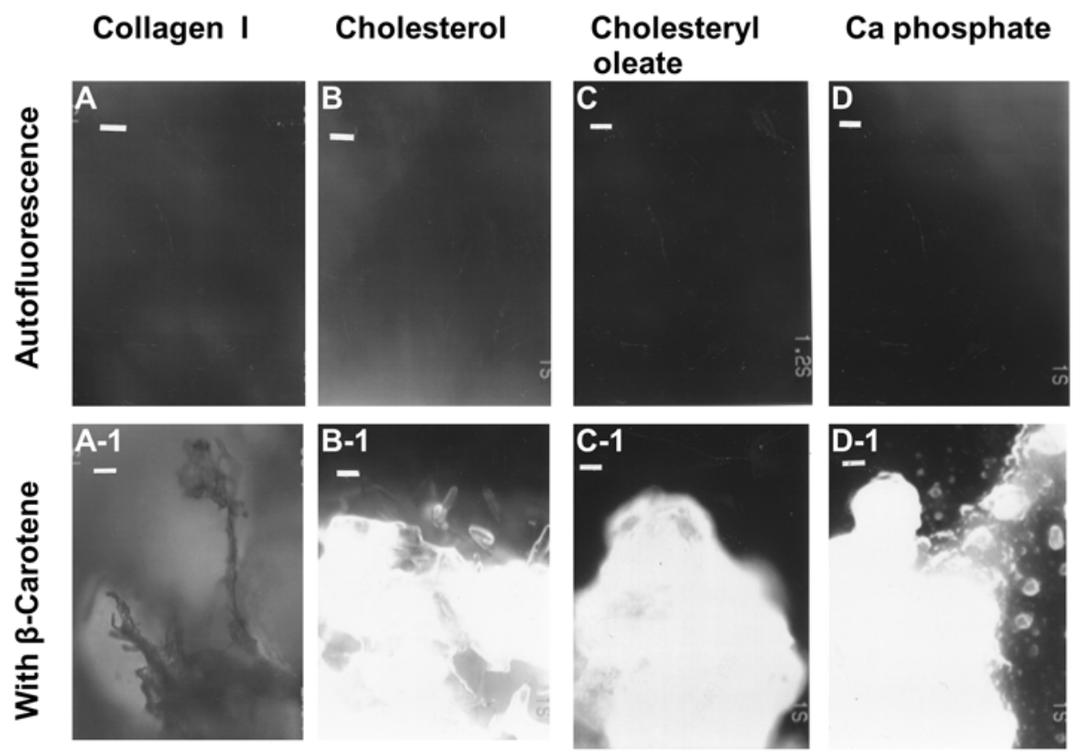
4. Near-infrared fluorescence (NIRF) of major substances that comprising atherosclerotic plaques

Cholesterol, cholesteryl esters and calcium phosphate (Ca) individually do not exhibit NIRF but exhibit NIRF in the presence of β-carotene which is known to coexists with lipids in the vascular wall. The other substances that are contained in atherosclerotic plaques did not[37] (Figure 5; Table 2).



EB: Evans blue. TB: Trypan blue. NB: Nile blue. Ho+TB: Homidium chloride and Trypan blue.

Fig. 4. Fluorescent Colors of Major Lipid Components Evoked by Biocompartible Markers Examined by Microscopy



Images before (upper panels) and after mixing with β-carotene (lower panels); From A to D: collagen I, free cholesterol, cholesteryl oleate, calcium phosphate, respectively; From A-1 to D-1: corresponding NIRFM images evoked after mixing with β-carotene (10^{-5}M). Horizontal bar in each panel: 100μm. Cited from Ref. 37, with permission.

Fig. 5. Near-infrared Fluorescent Microscopy (NIRFM) Images of Collagen I, Cholesterol, Choresteryl Oleate and Calcium Phosphate.

Substances		NIRF	
		Autofluorescence	with β-Carotene
Cholesterol	(c)	-	+
Chole oleate	(c)	-	+
Chole linoleate	(c)	-	+
5-Cholesten-3β-ol	(l)	-	+
7-Ketochol	(c)	-	-
Oleic acid	(c)	-	-
Linoleic acid	(c)	-	-
Ox-LDL	(l)	-	-
LDL	(l)	-	-
VLDL	(l)	-	-
HDL	(l)	-	-
Apo B-100	(c)	-	-
Apo A-1	(c)	-	-
Apo E-2	(c)	-	-
Matrix metalloproteinase-9	(c)	-	-
PC	(c)	-	-
LPC	(c)	-	-
TG	(l)	-	-
Collagens I, III, IV, V	(c)	-	-
Hyaluronic acid	(c)	-	-
Ceramide	(c)	-	±
Heparan sulfate	(c)	-	-
Albumin	(c)	-	-
Globulins	(c)	-	-
Calcium phosphate	(c)	-	+
β-Carotene	(l)	-	

(c): crystal. (l): liquid. - : NIRF absent. ±= NIRF weak. += NIRF strong.

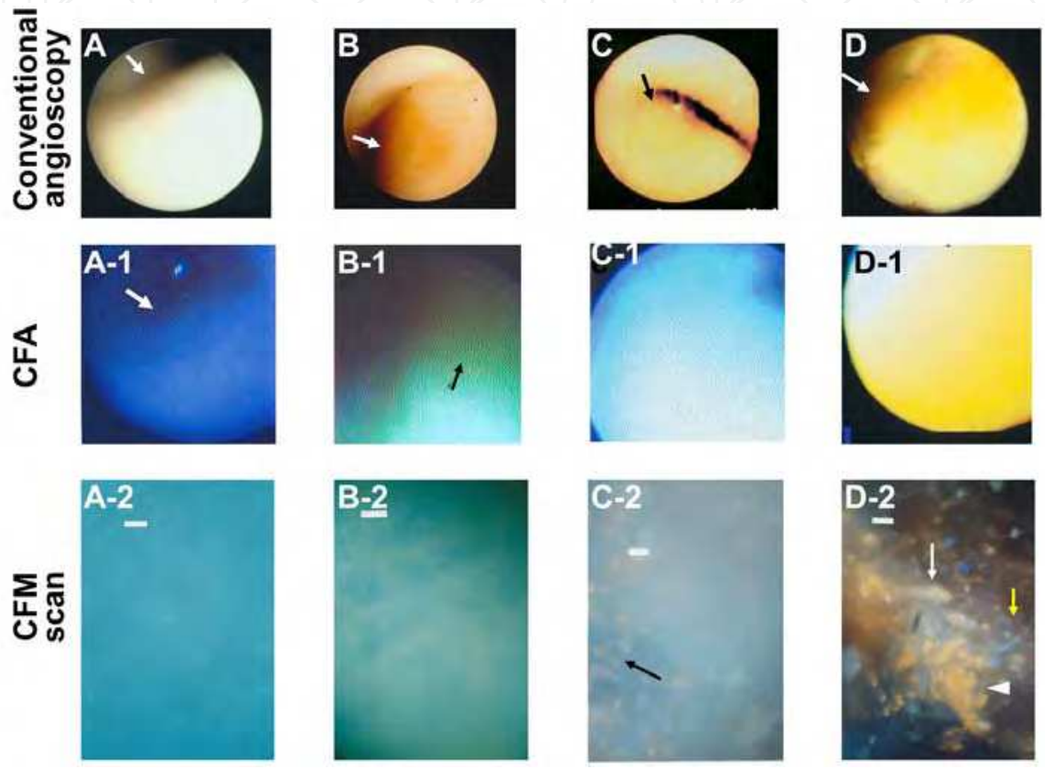
Table 2. Near-infrared Fluorescence (NIRF) of the Major Substances That Comprising Atherosclerotic Plaques

5. Color fluorescent angioscopy of excised human coronary plaques

a. Detection of vulnerable coronary plaques based on collagen subtype imaging

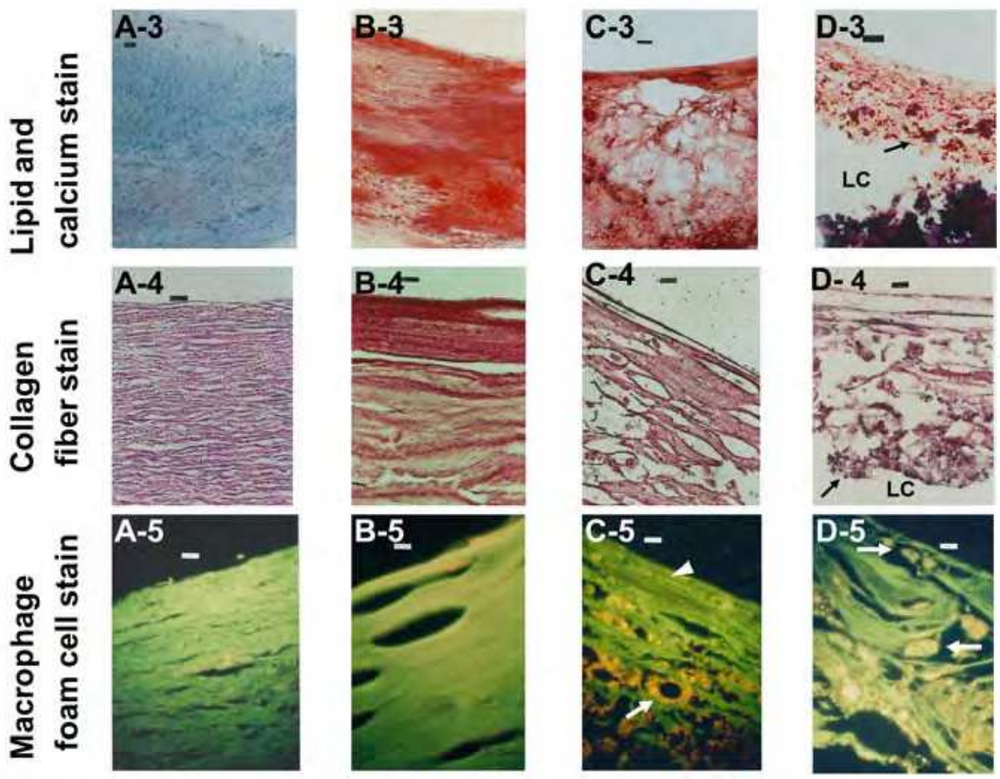
Excised human coronary plaques exhibit blue, green, white-to-light blue, or yellow-to-orange fluorescence. Fluorescent microscopic studies revealed that collagen subtypes, cholesterol, cholesteryl esters, calcium and β-carotene determine the fluorescent color of the plaques[36]. Histological examinations revealed abundant CFs without lipids in blue plaques;

CFs and lipids in green plaques; meager CFs and abundant lipids in white-to-light blue plaques; and the absence of CFs and deposition of lipids, calcium and macrophage foam cells in the thin fibrous cap in yellow-to-orange plaques, indicating that the yellow-to-orange plaques were most vulnerable[36] (Figures 6, 7).



From A to D: conventional angioscopic images of coronary plaques. From A-1 to D-1: corresponding CFA images using "A" imaging. From A-2 to D-2: corresponding CFM scanned images using "A" imaging. Horizontal bar:100 μ m. A: a white plaque observed during conventional angioscopy (arrow) exhibited blue fluorescence by CFA (arrow in A-1) and CFM scan (A-2). B: a yellow plaque observed during conventional angioscopy (arrow) exhibited green fluorescence seen during CFA (arrow in B-1) and CFM scan (arrow in b-2). C: a yellow plaque observed during conventional angioscopy (arrow) exhibited white-to-light blue fluorescence seen during CFA (C-1) and deposition of yellow substances in the white-to-light blue area (arrow in C-2). D: a yellow plaque observed during conventional angioscopy (arrow) exhibited yellow fluorescence observed during CFA (D-2) and deposition of orange (white arrowhead), white (white arrow) and blue (yellow arrow) substances in the area of no fluorescence by CFM scanning. Cited from Ref. 36, with permission.

Fig. 6. Relationships between Images of Coronary Plaques Produced by Conventional Angioscopy, Color Fluorescent Angioscopy (CFA) and Color Fluorescent Microscopy (CFM) Scanning

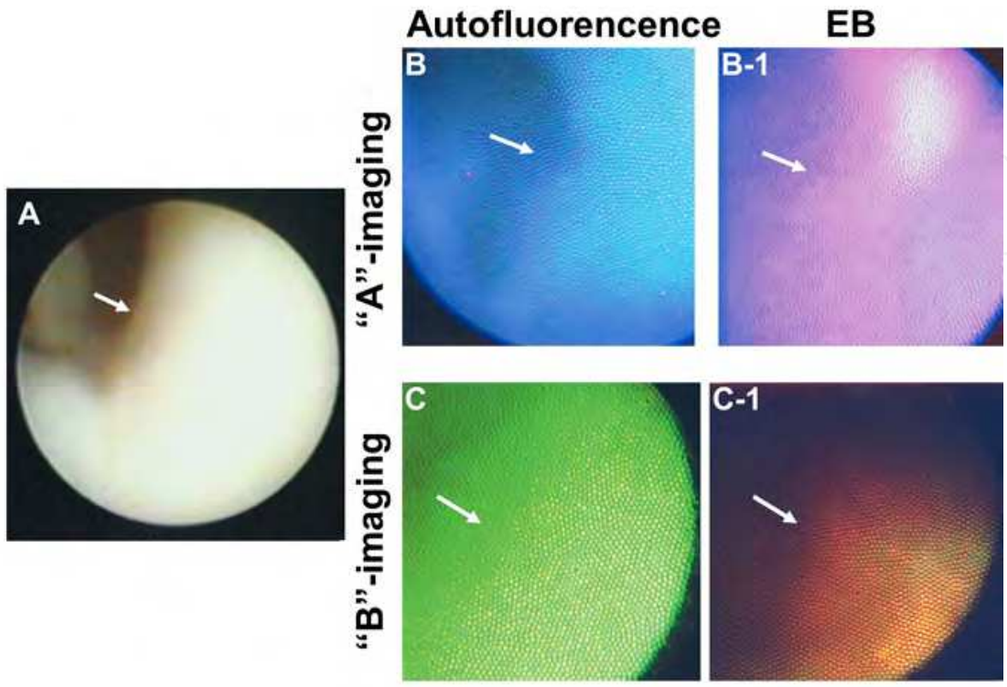


From A-3 to D-3: microscopic images after Oil-red O and methylene blue staining obtained from the same plaques in A, B, C and D as shown in Figure. 6, respectively. Red: lipids. Black: calcium. LC: lipid core. Horizontal bar: 100 μ m. From A-4 to D-4: CFs stained by silver staining. No normal CFs in D-4. Arrow in D-4: plaque debris. Horizontal bar: 20 μ m. From A-5 to D-5: images of ceramide in macrophage foam cells obtained by "B" imaging of CFM after Ziel-Neelsen staining. Orange fluorescence (arrows): ceramide. Arrowhead in C-5: residual CFs. Horizontal bar: 20 μ m. A-3: a plaque without lipids, with abundant normal CFs (A-4) and without macrophage foam cells (A-5). B-3: a plaque with lipids, with thick CFs (B-4) but without macrophage foam cells (b-5). C-3: a plaque with lipid deposition and cavity formation, meager CFs (C-4) and disseminated macrophage foam cells (arrow inC-5). D-3: a plaque with a thin fibrous cap with lipids and calcium (arrow in D-3), without CFs (D-4) and with multiple macrophage foam cells (arrows inD-5). Cited from Ref. 36, with permission.

Fig. 7. Lipids, Calcium Compounds, Collagen Fibers (CFs), and Macrophage Foam Cells in the Same Plaques as Those Shown in Figure. 6.

b. Oxidized low-density lipoprotein (Ox-LDL) imaging

After the administration of EB in an *ex-vivo* study, not only the yellow plaques but also the white plaques studied by conventional angioscopy frequently presented a violet fluorescence by "A"-imaging and a reddish brown fluorescence by "B" imaging, indicating the existence of ox-LDL (Figure 8). The distribution of this fluorescence appeared in a patchy or diffuse manner. There was a tendency for this fluorescent color to appear more frequently in yellow plaques rather than the white plaques classified by conventional angioscopy[36]. By scanning microscopy, a violet and a reddish brown fluorescence distributed diffusely or in web-like configuration, indicating plaque to plaque differences in deposition pattern of ox-LDL[36].

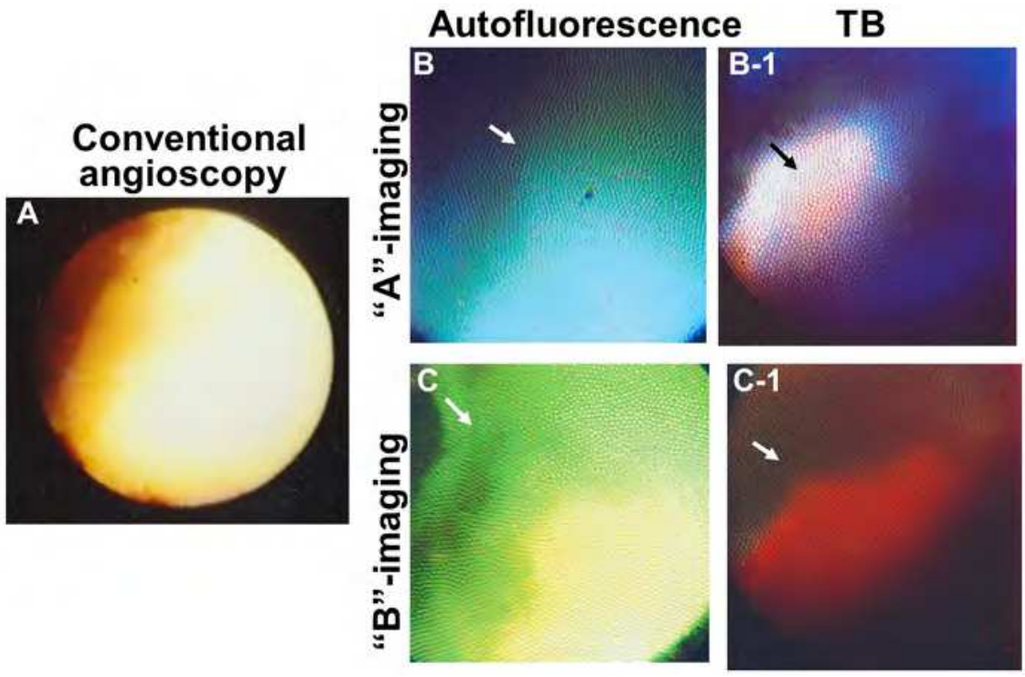


A: a white plaque imaged by conventional angioscopy. Arrow: the portion observed by CFA. B: "A"-image of the same plaque before administration of EB. The plaque showed green autofluorescence, indicating existence of collagens-I and/or -IV (Table 1). B-1: CFA image after administration of EB. The plaque showed diffuse and violet fluorescence. C: "B"-image of the same plaque, showing green autofluorescence. C-1: "B"-image after administration of EB, showing reddish brown fluorescence. This combination of EB-evoked fluorescent color indicates the existence of ox-LDL. Cited from Ref. 36, with permission.
Blue fluorescence (arrowhead in A-1) and green fluorescence(arrowhead in B-1) indicate collagen I and/or IV.

Fig. 8. Ox-LDL in An Excised Coronary Plaque Imaged by Color Fluorescent Angioscopy (CFA)

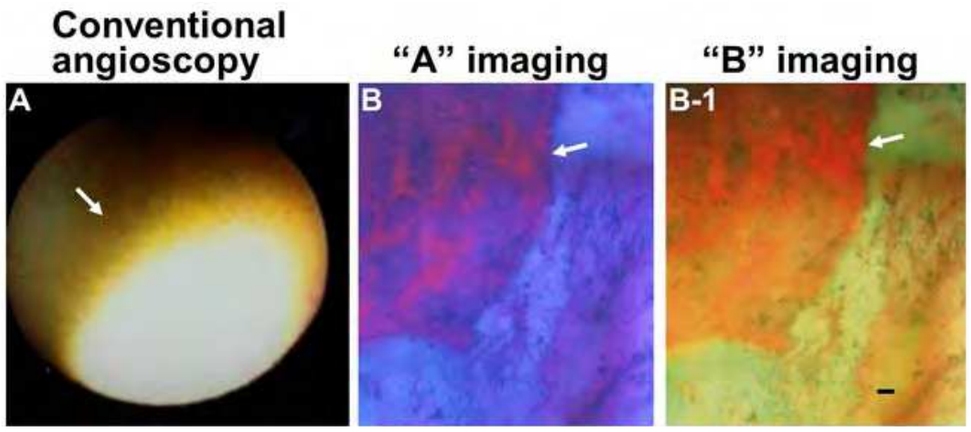
c. Lysophosphatidylcholine (LPC) imaging

The red fluorescence of LPC was investigated by color fluorescent angioscopy in the excised human coronary plaques. This fluorescent color both at "A"-and "B"-imaging was detected frequently by color fluorescent angioscopy in both white and yellow plaques that were classified by conventional angioscopy using white light (Figure 9). As in case of ox-LDL, this fluorescent color was found to be distributed in web-like or diffuse configuration by color fluorescent microscopic scanning[38] (Figure 10).



A: a yellow plaque imaged by conventional angioscopy. Arrow: the portion observed by CFA. B and C: CFA images of the same plaque before administration of TB. The plaque showed green and blue fluorescence in mosaic pattern by "A" imaging, and green and yellow fluorescence in mosaic pattern by "B" imaging, indicating the co-existence of collagen I and/or IV and lipids. B-1 and C-1: CFA images after administration of TB. The plaque showed red fluorescence by both" A"-and "B"-imaging, indicating the existence of LPC. Cited from Ref. 38, with permission.

Fig. 9. Lysophosphatidylcholine (LPC) in A Coronary Plaque Imaged by Color Fluorescent Angioscopy (CFA)



A: yellow plaque imaged by conventional angioscopy. B and B-1: CFM scanned luminal surface of the same plaque after the application of TB, showing wed-like distribution of red fluorescence and therefore web-like deposition of LPC (arrows). Horizontal bar in B-1: 100 μm. This bar is also applicable to B. Cited from Ref. 38, with permission.

Fig. 10. Distribution Patterns of Lysophosphatidylcholine (LPC) in A Coronary Plaque by Color Fluorescent Microscopy (CFM) Scanning

d. Apo B-100 imaging

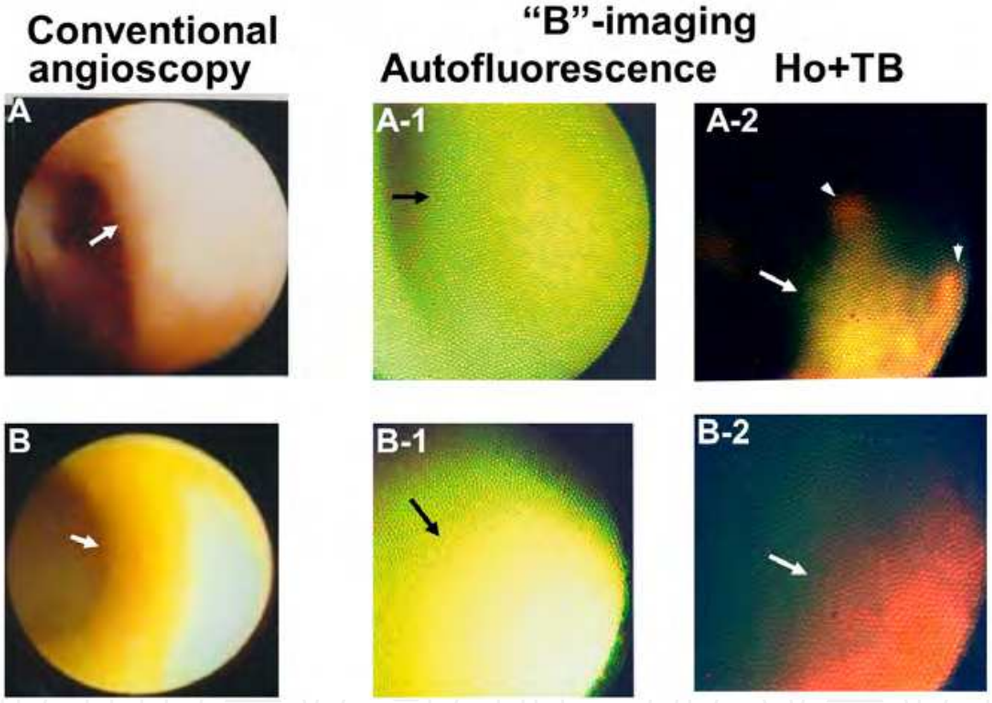
In the presence of NB, golden fluorescence, diffuse, spotty or web-like configuration, was observed in excised human coronary arteries by "B"-imaging, indicating deposition of apo B-100. However, the relationship between deposition patterns of this substance and plaque structure by histology is not clarified.

e. Triglyceride (TG) imaging

Imaging of TG, a major component of the core of ox-LDL, by CFA is yet not successful because of a lack in appropriate biocompartible markers.

f. HDL and LDL imaging

Selective imaging of HDL and LDL by fluorescent angioscopy is yet not successful as in case of TG.

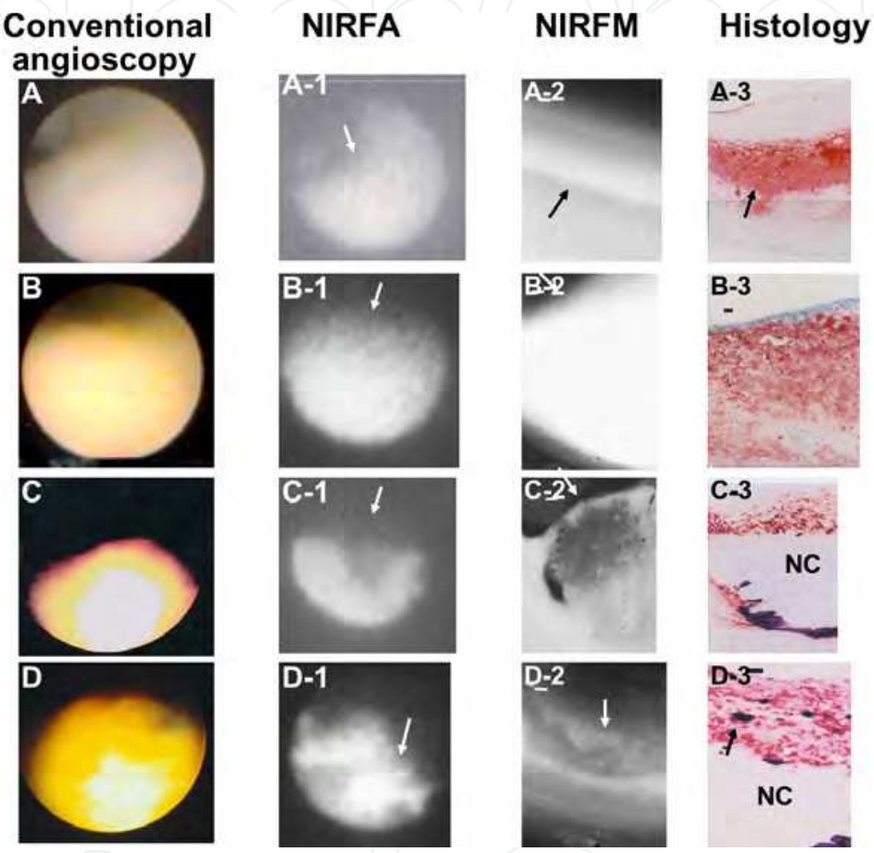


A: Yellow plaque imaged by conventional angioscopy. Arrow: the portion observed by CFA A-1 and A-2: CFA images of the same plaque before and after administration of Ho and TB solution (Ho+TB), respectively. The plaque showed greenish yellow autofluorescence before (arrow in A-1) and red (arrowheads in A-2) and golden fluorescence (arrow in A-2) in a mosaic pattern after the administration of Ho and TB, indicating co-existence of ox-LDL and/or LDL, and LPC and /or apo B-100. B: Yellow plaque by conventional angioscopy (arrow). B-1 and B-2: CFA images before and after administration of Ho and TB solution (Ho+TB), respectively. The plaque showed yellow autofluorescence (arrow in B-1) before and red fluorescence (arrow in B-2) after the administration of Ho and TB solution, indicating deposition of LPC and/or apo B-100.
Cited from Ref. 39, with permission.

Fig. 11. Oxidized Low-density Lipoprotein (Ox-LDL), LDL, Lysophosphatidylcholine (LPC), and Apolipoprotein (ApoB) in Coronary Plaques Imaged by Color Fluorescent Angioscopy (CFA)

g. Simultaneous imaging of oxidized low-density lipoprotein (Ox-LDL), low-density lipoprotein (LDL), lysophosphatidylcholine (LPC) and apolipoprotein B-100 (apo B-100)

By "B"-imaging, ox-LDL and LDL (group A) exhibit golden fluorescence, while LPC and apo B-100 (group B) exhibit red fluorescence. By color fluorescent angioscopy, coronary plaques frequently exhibited red and golden fluorescence in a mosaic pattern, indicating co-deposition of A and B. Red fluorescence was also observed in a small number of plaques, indicating solitary deposition of B[39] (Figure 11).



From A to D: the images of coronary plaques by conventional angioscopy.
From A-1 to D-1: corresponding NIRFA images of the same plaques. From A-2 to D-2 : corresponding NIRFM scanned images of the cut wall surface of the same specimens . From A-3 to D-3: corresponding histological images after staining with Oil Red-O and methylene blue. Red and black portions indicate lipids and calcium, respectively. Horizontal bar at the left upper corner of each panel: 100 μm.
A: a white plaque. From B to D: yellow plaques. A-1 and B-1: homogenous type. Arrows: homogenous NIRF; C-1: doughnut-shaped type. Arrow: NIRF absent portion surrounded by strong NIRF region; D-1: spotty type. Arrow: spots. A-2 and B-2: homogenous NIRF (arrows); C-2 : necrotic core lacking NIRF (arrow) surrounded by strong NIRF region; D-2: fibrous cap with strong NIRF spots (arrow). A-3: homogenous deposition of lipids deep in the plaque (arrow); B-3: lipid deposition in entire plaque. C-3: lipid-deposited fibrous cap with a necrotic core (NC) belowD-3: calcium particles distributed within a lipid-laden fibrous cap. Red: lipids. Black: calcium compounds (arrow).
Horizontal bar: 100μm. Cited from Ref. 37, with permission.

Fig. 12. Relationships Among Conventional Angioscopic, Near-infrared Fluorescent Angioscopic (NIRFA), and Near-infrared Fluorescent Microscopic (NIRFM) Scanned Images and Histological Changes in Excised Human Coronary Plaques.

6. Near-infrared fluorescent angioscopy of excised human coronary plaques

a. Cholesterol (C) and cholesteryl ester (CE) imaging

In an ***ex-vivo*** study, excised human coronary plaques were classified as those with NIRF and those without. The former plaques were classified into homogenous, doughnut-shaped and spotty types[37].

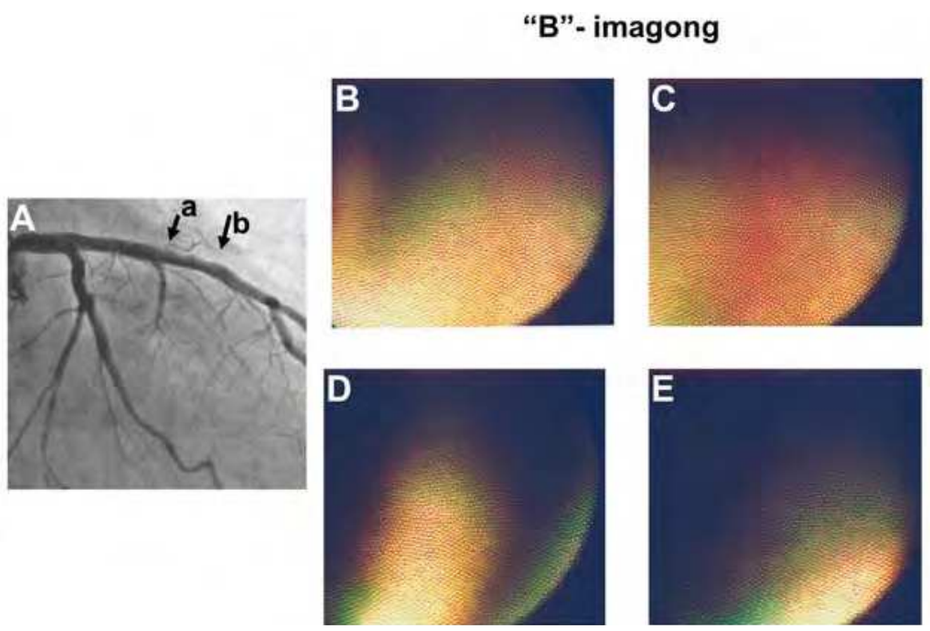
Histological examinations revealed that these image patterns were determined by the differences in the locations of cholesterol, cholesteryl esters and Ca, and that those deposited within 700 μ m in depth from plaque surface were imaged by NIRFA[37] (Figure 12).

7. Clinical application of fluorescent angioscopy

a. Oxidized low-density lipoprotein (ox-LDL)

In our catheterization laboratory, CFA was performed during routine coronary angioscopy in patients with coronary artery disease.

After selective injection of the EB solution into the coronary artery, not only the plaques but also the apparently normal coronary segments frequently exhibited a reddish brown fluorescence by "B"-imaging, indicating the existence of ox-LDL[36] (Figure 13).

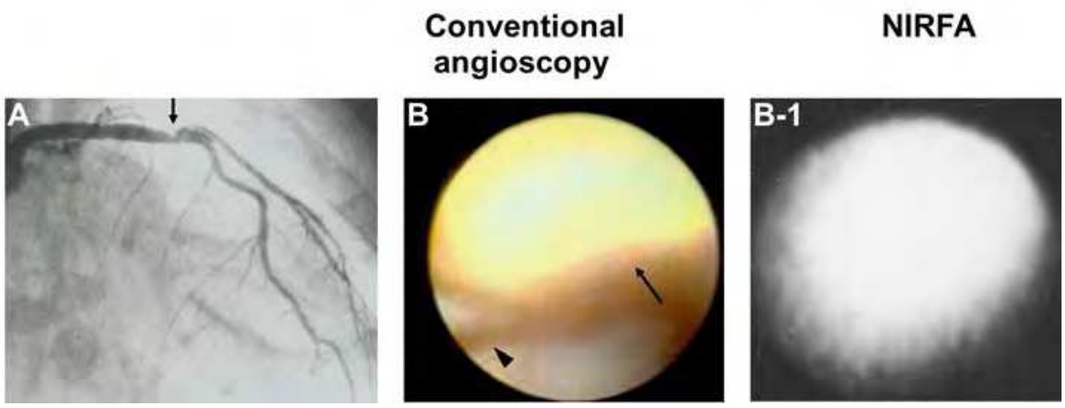


Reddish brown fluorescence observed in a non-stenotic proximal segment of the left anterior descending coronary artery after the intracoronary injection of EB in a patient with stable angina pectoris.
A: an angiogram of the left coronary artery. Arrows a to b: the proximal segment observed by CFA. The wall of the segment was uneven but significant stenosis was not found.
From .B to E: CFA images of the same segment obtained after injecting EB, by advancing the angioscope distally from a to b of panel A. Reddish brown portions indicate ox-LDL. The luminal surface was uneven, indicating early stage of atherosclerosis. Cited from Ref. 36, with permission.

Fig. 13. Ox-LDL Imaged by "B"-imaging of Color Fluorescent Angioscopy (CFA) in the Coronary Artery in A Patient with Angina Pectoris

b) Cholesterol and cholesteryl esters

The present authors examined coronary plaques by NIRFA during coronary angiography in 25 patients with coronary artery disease. Figure 14 shows a yellow plaque by conventional angioscopy. This plaque exhibited homogenous NIRF, indicating homogenous deposition of cholesterol and/ or choresteryl esters. Homogenous, doughnut-shaped or spotty NIRFA images were also observed in patients[37].



From A to C: angiogram, conventional angioscopic image and NIRFA image of a plaque in the proximal segment of the left anterior descending coronary artery (arrow in A). The yellow plaque (arrow in B) presented a homogenous type NIRF image (B-1). Arrowhead: guide wire.
Cited from Ref. 37, with permission.

Fig. 14. Near-infrared Fluorescent Angioscopy (NIRFA) Study in a 61-year-old Male with Stable Angina.

8. Discussion

The use of an antibody of individual components that comprising vessel wall is a more specific indicator for their imaging *in-vivo*. However, there are many limitations to its clinical application. Therefore, the use of a low molecular weight substance that selectively binds to individual components and presents specific fluorescence color is another option that can be used for the imaging of individual lipid components.

In the present study, low molecular weight dyes with, EB, TB, NB, Ho+TB were found to evoke fluorescence specific to ox-LDL and its components except TG and Schiff base. Thus, visualization of ox-LDL and its major components in a given plaque was achieved, enabling analysis of the differences in their deposition patterns in human coronary plaques. The mechanisms by which these dyes evoked fluorescence are not known. One possibility is that dyes became conjugated to the target substances to form an adduct to provoke a fluorescent color specific to individual substances.

Ox-LDL, which plays an important role in the initiation, progression and destabilization of atherosclerotic plaques, became visible for the first time by CFA in patients. Furthermore, the substances that comprising ox-LDL became visible by CFA or NIRFA in excised human coronary arteries.

However, CFA and NIRFA have been limited to collagen subtypes, ox-LDL, cholesterol and cholesteryl esters in clinical situations. Imaging of other substances by CFA or NIRFA in patients is yet not performed because clinical applicability of biocompartible markers other than EB is not established.

Fluorescent angioscopy faced some shortcomings; (1) The visualization by CFA is limited only to the substances deposited within 200μm from the vascular luminal surface and by NIRFA within 700 μm (Table 3). Therefore deposits in the deeper layers can not be analyzed by this system of CFA and NIRFA. (2) Differing in function to antibodies, biomarkers employed in CFA do not selectively bind to the target substance, and therefore, there may be other unknown substances which exhibit the same fluorescent color in the presence of the markers used. (3) Because a lens is used, the pictures obtained by CFA and NIRFA are fish-eye images and therefore quantitative assessment of the target substance is difficult (Table 3).

	Conventional angioscopy	CFA	NIRFA
1. Simultaneous visualization of two or more substances[39]	yes	yes	no
2. Determination of substance	no	yes	yes
3. Quantitative measurement of substances[41]			
Size	no	no	no
Density	no	no	no
Distance from luminal surface	no	no	no
4. Depth from luminal surface to the substances which can be visualized[37]	100-200 μm	200 μm	700 μm
5. Two-dimensional imaging	yes	yes	yes

CFA: color fluorescent angioscopy. NIRFA: near-infrared fluorescent angioscopy.

Table 3. Advantages and Disadvantages of Angioscopy

At present, imaging by fluorescent angioscopy is limited to several substances that comprise coronary plaques. By selecting adequate biomarkers, the substances other than those mentioned above may become visible.

Fluorescent angioscopy was established only recently and therefore its clinical application is limited to a few laboratories. As in spectroscopy, this technique will become prevalent when clinically applicable biomarkers for the substances comprising atherosclerotic lesions become complete.

9. Conclusion

LDL has cortex and core. The cortex is composed of PC, free cholesterol and apo B-100, and the core is composed of TG and cholesteryl esters. Ox-LDL is derived from LDL by oxidation of its component PC into LPC and apo B-100 into Shiff base, and plays an important role in the initiation, progression and destabilization of atherosclerotic plaques by inducing the proliferation and elongation of survival of macrophages.

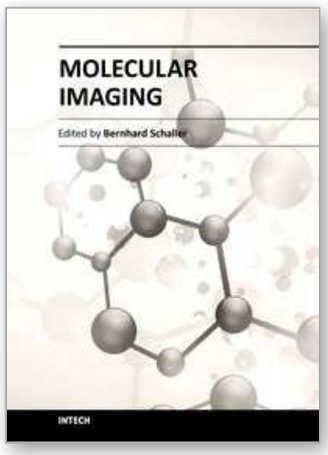
We succeeded in molecular imaging of ox-LDL not only in the excised human coronary plaques but also in coronary arteries in patients ***in-vivo*** by CFA using EB as a biomarker. Also, LPC, apo B-100, free cholesterol, and cholesteryl esters in human coronary plaques became visible by either CFA or NIRFA. These imaging techniques will give us much information which is otherwise not obtainable on the mechanisms of atherosclerosis in clinical situation.

10. References

[1] Ge J, Baumgart D, Hauda M, et al. Role of intravascular ultrasound imaging in identifying vulnerable plaques. Herz. 1999; 24: 32-41.
[2] Jang IK, Tearney GJ, MacNeil B, et al. In vivo characterization of coronary atherosclerotic plaque by use of optical coherence tomography. Circulation. 2005; 111: 1551-1555.
[3] Uchida Y. Coronary Angioscopy. Futura Publishing Co, NY, 2001: 11-81
[4] Uchida Y, Prediction of acute coronary syndromes by percutaneous angioscopy in patients with stable angina. Am Heart J 1995, 130: 195-203.
[5] Thieme T, Wernecke KD, Meyer R, et al. Angioscopic evaluation of atherosclerotic plaques: validation by histomorphologic analysis and association with stable and unstable coronary syndromes. J Am Coll Cardiol 1996; 28: 1-6.
[6] Uchida Y, Uchida Y, Hiruta N. Histological characteristics of glistening yellow coronary plaques seen on angioscopy: with special reference to vulnerable plaques. Circ J. 2011; 75: 1913-1919.
[7] Matsuura E, Hughes GR, Khamashta MA. Oxidation of LDL and its clinical implication. Autoimmun Rev. 2008; 7: 558-566.
[8] Chen JH, Riazzy, Smith EM, Proud CG, Steinbrecher UP, Duronio V. Oxidized LDL-mediated macrophage survival involves elongation of factor-2 kinase. Arterioscler Thromb Vasc Biol 2009; 29: 92-98.
[9] Katsuda S, Okada Y, Minamoto T, Matsui Y, Nakanishi I. Collagens in human atherosclerosis: Immunohistochemical analysis using collagen type-specific antibodies. Atheroscler Thromb. 1992; 12:494-502.
[10] Murata K, Motoyama T. Collagen species in various sized arteries, their changes with intimal proliferation. Artery. 1990; 17: 96-106.
[11] Murata K, Kotake C, Motoyama T: Collagen species in human aorta: with special reference to basement membrane-associated collagens in the intima and media and their alteration with atherosclerosis. Artery. 1987; 14: 229-247.
[12] Shah PK, Falk E, Badimon JJ, et al. Human monocyte-derived macrophages induce breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-metalloproteinases and implication for plaque rupture. Circulation 1995; 92: 1565-1569.
[13] Takano T, Itabe H, Mori M, et al. Molecular pathology in atherosclerosis: the mechanisms by which cholesteryl ester accumulates in atherosclerotic aorta. Yakugaku Zasshi. 2008; 128: 1383-1401.

[14] Li W, Yuan XM, Olsson AG, Brunk UT. Uptake of oxidized LDL by macrophages results in partial lysosomal enzyme inactivation and relocation. Arterioscler Thromb Vasc Biol. 1998; 18: 177-184.
[15] Grandl M, Bared SM, Liebisch G, Werner T, Barlage S, Schmitz G. E-LDL and ox-LDL differentially regulate ceramide and cholesterol raft microdomains in human macrophages. Cytometry A. 2006; 69: 189-191.
[16] Tardif JC. Emerging high-density lipoprotein infusion therapies: fulfilling the promise of epidemiology? J Clin Lipidol. 2010; 4: 399-404.
[17] Negi S, Ballantyne CM. Insights from recent meta-analysis: role of high-density lipoprotein cholesterol in reducing cardiovascular events and rates of atherosclerotic disease progression. J Clin Lipidol. 2010; 4: 365-370.
[18] Vilani SS, Nambi V. The role of lipoprotein-associated phospholipase A_2 as a marker of atherosclerosis. Curr Atheroscler Rep 2007; 9: 97-103.
[19] Erdogan A, Schaefer MB, Kulmann CR, Most A, Hartmann M, Mayer K, Renner FC, Schaefer C, Abdallah Y, Hoelschermann H, Schaefer CA. Activation of Ca2+-induced potassium channels is involved in lysophosphatidylcholine-induced monocyte adhesion to endothelial cells. Atherosclerosis. 2007; 190: 100-105.
[20] Matsubara M, Hasegawa K. Benipine, a dihydopyridine-calcium channel blocker, prevents lysophosphatidylcholine-induced injury and reactive oxygen species production in human aortic endothelial cells. Atherosclerosis. 2005; 178: 57-66.
[21] Matsumoto T, Kobayashi T, Kamata K. Role of lysophosphadidylcholine (LPC) in atherosclerosis. Curr Med Chem. 2007; 14: 3209-3220.
[22] Stollenberk MM, Svensson O, Schiopu A, Jansson B, Arnebrant T, Fredrikson GN. A desorption of low-density lipoprotein, its oxidation, and subsequent binding of specific recombinant antibodies: an in situ ellipsometric study. Biochim Acta. 2011; 1810: 211-217.
[23] Kanani FH, Alam JM. Apolipoprotein B in type 2 diabetes—a cross sectional study in a tertiary care set-up. J Pak Med Assoc. 2010; 60: 653-656.
[24] Holewijn S, den Heijer M, Swinkels DW, Stalenhoef AF, de Graaf J. Apolipoprotein B, non-HDL cholesterol and LDL cholesterol for identifying individuals at increased cardiovascular risk. J Intern Med. 2010; 68: 567-677.
[25] Enkhmaa B, Annurad E, Zhang Z, Pearson TA, Berglund L. Usefulness of apolipoprotein B/ apolipoprotein A-1 ration to predict coronary artery disease independent of the metabolic syndrome in African Americans. Am J Cardiol. 20210; 106: 1264-1269.
[26] Kappelle PJ, Gansevoort RT, Hillege JL, Wolffenbultel BH, Dullaart RP. Apolipoprotein B/A-1 and total cholesterol/high-density lipoprotein cholesterol rations both predict cardiovascular events in the general population independently of nonlipid risk factors, albuminuria and C-reactive protein. J Interm Med. 2011; 269: 232-242
[27] Lee YH, Choi SH, Lee KW, Kim DJ. Apolipoprotein B/A1 ratio is associated with free androgen index and visceral adiposity and may be an indicator of metabolic syndrome in male children and adolescence. Clin Endoclinol. 2011; 74: 579-588
[28] Rosenson RS, Carlson DM, Kelly MT, Setze CM, Hirshberg B, Stolzenbach JC, Williams LA. Achievement of lipid targets with combination of rosuvastatin and fenofibric acid in patients with type 2 diabetes mellitus. Cardiovasc Drugs Ther. 2011; 25: 47-57
[29] Labreuche J, Touboul PJ, Amarenco P. Plasma triglyceride levels and risk of stroke and carotid atherosclerosis: a systematic review of the epidemiological studies. Atherosclerosis. 2009; 203: 331-345.
[30] Gandotra P, Miller M. The role of triglycerides in cardiovascular risk. Curr Cardiol Rep. 2008; 10: 505-511.

[31] Colley CS, Kazarian SG, Weinberg PD, Lever MJ. Spectroscopic imaging of arteries and atherosclerotic plaques. Biopolymers. 2004; 74: 328-335.
[32] Krisko A, Stjepanoic G, Pifat G, Ruysechaert JM, Goormaghtigh E. Detection of apolipoprotein B 100 early conformatical changes during oxidation. Biochim Biophys Acta. 2007; 1768: 2923-2930.
[33] Garg S, Serruys PW, van der Ent M, Schultz C, Mastik F, van Soest G, van der Steen AF, Wilder JE, Regar E. First use in patients of a combined infra-red spectroscopy and intra-vascular ultrasound catheter to identify composition and structure of coronary plaque. Eurointervention. 2010; 5: 755-756.
[34] Li D, Patel AR, Klibanov AL, Kramer CM, Ruiz M, Kang BY, Mehta JL, Beller GA, Glover DK, Meyer CH. Molecular imaging of atherosclerotic plaques targeted to oxidized LDL receptor LOX-1 by SPECT/CT and magnetic resonance. Circ Cardiovasc Imaging. 2010; 3: 464-472.
[35] Briley-Saebo KC, Shaw PX, Mulder WJ, Choi SH, Vucic E, Aguinaldo JG, Wiltztum JL, Fuster V, Tsimikas S, Fayad ZA. Targeted molecular probes for imaging atherosclerotic lesions with magnetic resonance using antibodies that recognize oxidation-specific epitopes. Circulation. 2008; 117: 3206-3215.
[36] Uchida Y, Uchida Y, Kawai S, Kanamaru R, Sugiyama Y, Tomaru T, Maezawa Y, Kameda N. Detection of vulnerable coronary plaques by color fluorescent angioscopy. JACC Cardiovasc Imaging. 2010; 3: 398-408.
[37] Uchida Y, Uchida Y, Sugiyama Y, Kanai M, Sakurai T, Shirai S. Two-dimensional visualization of cholesterol and cholesteryl esters within human coronary plaques by near-infrared fluorescence angioscopy. Clinical Cardiology. 2010; 33: 322-325.
[38] Uchida Y, Uchida Y, Kawai S, Kanamaru R, Kameda N. Imaging of lysophosphatidylcholine in human coronary plaques by color fluorescence angioscopy. Int Heart J. 2010; 51: 129-133.
[39] Uchida Y, Uchida Y, Kameda N. Visualization of lipid components in human coronary plaques using color fluorescence angioscopy. Circ J. 2010; 74: 2181-2186.
[40] Uchida Y. Recent advances in coronary angioscopy. J Cardiol. 2011; 57: 18-30.
[41] Uchida Y. Angioscopy system. In Coronary angioscopy, Uchida Y (ed), Futura Publishing INC, Armonk, NY, 2001; 11-103.
[42] Uchida Y, Nakamura F, Tomaru T. Observation of atherosclerotic lesions by an intravascular microscope in patients with arteriosclerosis obliterance. Am Heart J. 1995; 130: 1114-1117.
[43] Uchida Y, Uchida Y, Sakurai T, Kanai M, Shirai S, Morita T.Characterization of coronary fibrin thrombus in patients with acute coronary syndrome using dye-staining angioscopy. ArteriosclerThromb Vasc Biol. 2011; 31: 1452-1460.
[44] Uchida Y, Uchida H. Therapeutic tool for vascular disease. United States Patent. US7025981 B2, 2006.
[45] Ralph P, Nakoinz I. Environmental and chemical dissociation of antibody-dependent phagocytosis from lysis mediated by macrophages: stimulation of lysis by sulfhydryl-blocking and elastase-inhibitory agents and depression by trypan blue and trypsin. Cell Immunol 1980; 50: 94-105.
[46] Brooks BO, Reed ND. The effect of trypan blue on the early control of Trypanosoma musculi parasitemia in mice. J Reticuloendothel Soc. 1979; 25: 325-328.
[47] Chen ZW, Balamurungan A, Chen SM. Detection of DNA by using bio-conducting polymer-Nile blue composite electrode; Nile blue as an indicator. Bioelectrochemistry. 2009; 75: 13-18.



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The present book gives an exceptional overview of molecular imaging. Practical approach represents the red thread through the whole book, covering at the same time detailed background information that goes very deep into molecular as well as cellular level. Ideas how molecular imaging will develop in the near future present a special delicacy. This should be of special interest as the contributors are members of leading research groups from all over the world.

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