



**HELLENIC REPUBLIC
UNIVERSITY OF IOANNINA
SCHOOL OF SCIENCES
DEPARTMENT OF MATERIALS SCIENCE AND ENGINEERING**

**Computational Methods for Atherosclerotic Plaque
Characterization**

Lambros S. Athanasiou

PhD THESIS

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«Η έγκριση της διδακτορικής διατριβής από το Τμήμα Μηχανικών Επιστήμης Υλικών της Σχολής Θετικών Επιστημών του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνώμων του συγγραφέα Ν. 5343/32, άρθρο 202, παράγραφος 2».

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Επιβλέπων

Δημήτριος Ι. Φωτιάδης - Καθηγητής Μηχανικών Επιστήμης Υλικών, Παν/μιο Ιωαννίνων

Μέλη

Λάμπρος Μιχάλης - Καθηγητής Ιατρικής, Παν/μιο Ιωαννίνων

Αντώνιος Χαραλαμπίδης - Αναπλ. Καθηγητής ΣΕΜΦΕ, ΕΜΠ

Ημερομηνία ορισμού θέματος: 8-7-2009

ΔΙΟΡΙΣΜΟΣ ΕΠΤΑΜΕΛΟΥΣ ΕΞΕΤΑΣΤΙΚΗΣ ΕΠΙΤΡΟΠΗΣ :

- | |
|---|
| 1. Καθ. Δημήτριος Φωτιάδης, Τμήμα Μηχανικών Επιστήμης Υλικών, Σχολή Θετικών Επιστημών, Παν/μιο Ιωαννίνων |
| 2. Καθ. Καρδιολογίας Λάμπρος Μιχάλης, Τμήμα Ιατρικής, Σχολή Επιστημών Υγείας, Παν/μιο Ιωαννίνων |
| 3. Αναπλ. Καθ. Αντώνιος Χαραλαμπίδης, Σχολή Εφαρμοσμένων Μαθηματικών και Φυσικών Επιστημών, Εθνικό Μετσόβιο Πολυτεχνείο |
| 4. Αναπλ. Καθ. Συμεών Αγαθόπουλος, Τμήμα Μηχανικών Επιστήμης Υλικών, Σχολή Θετικών Επιστημών, Παν/μιο Ιωαννίνων |
| 5. Αναπλ. Καθ. Κωνσταντίνος Παπαλουκάς, Τμήμα Βιολογικών Εφαρμογών και Τεχνολογιών, Σχολή Επιστημών Υγείας, Παν/μιο Ιωαννίνων |
| 6. Αναπλ. Καθ. Καρδιολογίας Χρήστος Κατσούρας, Τμήμα Ιατρικής, Σχολή Επιστημών Υγείας, Παν/μιο Ιωαννίνων |
| 7. Επίκ. Καθ. Λεωνίδας Γεργίδης, Τμήμα Μηχανικών Επιστήμης Υλικών, Σχολή Θετικών Επιστημών, Παν/μιο Ιωαννίνων |

Έγκριση Διδακτορικής Διατριβής με βαθμό « **Άριστα** » στις 15-6-2015

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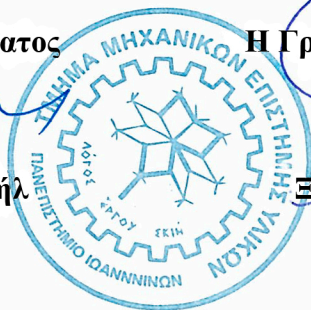
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**Καρακασίδης Μιχαήλ
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Ξανθή Τουτσουζόγλου



Dedication

To the memory of my grandfather.

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List of Abbreviations

<i>Acronym</i>	<i>Meaning</i>
2D	Two Dimensional
3D	Three Dimensional
CA	Calcium
CAD	Coronary Artery Disease
CP	Calcified Plaque
CTA	Computed Tomography Angiography
DC	Dense Calcium
DICOM	Digital Imaging and Communications in Medicine
FD-OCT	Frequency Domain Optical Coherence Tomography
FFT	Fibro Fatty Tissue
FN	False Negative
FP	False Positive
FT	Fibrous Tissue
GLCM	Gray Level Co-occurrence Matrix
GMM	Gaussian Mixture Model
HCP	Hard Calcified Plaque
HNCP	Hard non Calcified Plaque
HU	Hounsfield Units
IVUS	Intravascular ultrasound
LBP	Local Binary Patterns
LT	Lipid Tissue
M	Media
micro-CT	Micro-Computed Tomography
MIFR	Maximum Image Formation Error
MRI	Magnetic Resonance Imaging
MT	Mixed Tissue
NC	Necrotic Core
NCP	Non Calcified Plaque
NIRS	Near Infrared Spectroscopic
NN	Neural Networks
NT	Normal Tissue
OCT	Optical Coherence Tomography
PET-CT	Positron Emission Tomography–Computed Tomography
RF	Random Forests
RIP	Radial Image Projection
ROI	Region of Interest
SP	Soft Plaque
SVM	Support Vector Machines
TA	Tissue Area
TP	True Positive
VH-IVUS	Virtual Histology - Intravascular ultrasound
WRP	Wrapper Feature Selection

Abstract

This thesis focuses on the atherosclerotic plaque characterization using invasive and non-invasive imaging modalities. The outermost aim is the development and implementation of atherosclerotic plaque characterization methodologies using Intravascular Ultrasound (IVUS), Optical Coherence Tomography (OCT) and Computed Tomography (CT) images of coronary vessels. The research was initiated after studying the limitations of existing automated plaque characterization methodologies, applied to widely used imaging modalities. In order to overcome these limitations, several methodologies have been developed.

In the first chapter, the anatomy and physiology of the cardiovascular system is presented. It is described in detail and the relevant literature is provided for the following topics: heart functionality along with the vascular system, blood circulation and atherosclerosis. The imaging methods, invasive and non-invasive, for the investigation of plaque composition are presented also in detail. In the second chapter the up to date literature and their limitations are highlighted. The methodologies developed for segmenting and characterizing atherosclerotic plaque using IVUS, OCT and CT images are presented. In the third chapter two novel methodologies for the automated identification of different plaque components in grayscale IVUS images, are presented. The first methodology is based on a hybrid approach that incorporates both image processing techniques and classification algorithms. The methodology allows classification of the plaque into three different categories, providing reliable results when compared to experts' annotations. The second IVUS plaque characterization methodology classifies the plaque into four classes. In order to train and validate the methodology, we used IVUS frames acquired from a commercially available software. In the fourth chapter a novel methodology that processes OCT images in a fully automated manner is presented. The proposed methodology is able to detect the lumen borders in OCT frames, identify the plaque region and detect four plaque types. The efficiency of the developed methodology was evaluated using experts' annotations. In the fifth chapter a new methodology for automated three-dimensional (3D) reconstruction of coronary arteries and characterization of plaque morphology in CT images, is presented. The methodology

detects the inner - outer wall vessel borders, detects the calcium plaque and reconstructs the vessel and plaque into 3D surfaces. The methodology was validated using the estimations of the recently presented IVUS plaque characterization methodology. In the sixth chapter a study for propagating the imaging errors and image segmentation errors in plaque characterization methodologies applied to 2D vascular images, is presented. The maximum error that can be propagated to the plaque characterization results is calculated, assuming worst case scenarios. The proposed error propagation methodology is validated using methods applied to real datasets, obtained from IVUS and OCT systems. Finally, in the seventh chapter, a framework for the inflation of histology/micro-CT images based on IVUS is presented. The proposed methodology inflates the deformed histological/micro-CT images, which are the current gold standard in plaque characterization, based on the IVUS and micro-CT/histological lumen contour difference. In order to validate the proposed image inflation methodology plaque areas in the inflated micro-CT and histological images are compared with the ones in the IVUS images.

The contribution of this thesis is related to the following: (i) the implementation of new plaque characterization methodologies which overcome the literature limitations, (ii) the emergence of producing plaque characterization methodologies that can be applied to recently developed imaging systems, (iii) the presentation of methodologies able to produce 3D arterial and plaque models for investigating the evolution of the plaque, (iv) the study and analysis of the error propagation of imaging systems to plaque detection, and (v) the automated image registration of intravascular images with histology.

Περίληψη στα Ελληνικά

Η παρούσα διδακτορική διατριβή συμβάλλει στο πρόβλημα του χαρακτηρισμού της αθηρωματικής πλάκας χρησιμοποιώντας επεμβατικές και μη επεμβατικές απεικονιστικές μεθόδους. Απώτερος σκοπός είναι η ανάπτυξη και η εφαρμογή μεθοδολογιών για το χαρακτηρισμό της αθηρωματικής πλάκας χρησιμοποιώντας εικόνες ενδοαγγειακού υπερηχογραφήματος (IVUS), οπτικής συνεκτικής τομογραφίας (OCT) και αξονικής τομογραφίας (CT). Η έρευνα ξεκίνησε κατόπιν μελέτης των περιορισμών στις υφιστάμενες αυτοματοποιημένες μεθοδολογίες χαρακτηρισμού αθηρωματικής πλάκας, οι οποίες εφαρμόζονται σε ευρέως χρησιμοποιούμενες απεικονιστικές μεθόδους. Για να ξεπεραστούν αυτοί οι περιορισμοί, αναπτύχθηκαν νέες μεθοδολογίες. Επιπλέον, αναπτύχθηκαν και παρουσιάζονται για πρώτη φορά μεθοδολογίες για τον χαρακτηρισμό της αθηρωματικής πλάκας σε προσφάτως ανεπτυγμένα συστήματα απεικόνισης.

Στο πρώτο κεφάλαιο, παρουσιάζεται η ανατομία και η φυσιολογία του καρδιαγγειακού συστήματος. Συγκεκριμένα, περιγράφεται με λεπτομέρεια και παρέχεται η σχετική βιβλιογραφία για τα ακόλουθα θέματα: λειτουργία της καρδιάς και του αγγειακού συστήματος, κυκλοφορία του αίματος και αθηροσκλήρωση. Τέλος, παρουσιάζονται λεπτομερώς όλες οι μέθοδοι απεικόνισης, επεμβατικές και μη επεμβατικές, για τη διερεύνηση της σύνθεσης πλάκας. Στο δεύτερο κεφάλαιο παρουσιάζεται η μέχρι σήμερα βιβλιογραφία και επισημαίνονται οι περιορισμοί της σχετικά με τις μεθοδολογίες που αναπτύχθηκαν για την κατάτμηση και τον χαρακτηρισμό αθηρωματικής πλάκας με τη χρήση εικόνων IVUS, OCT και CT. Στο τρίτο κεφάλαιο, παρουσιάζονται δύο νέες μεθοδολογίες για την αυτόματη κατηγοριοποίηση των διαφορετικών ειδών αθηρωματικής πλάκας χρησιμοποιώντας εικόνες IVUS. Η πρώτη μεθοδολογία βασίζεται σε μια υβριδική προσέγγιση που συνδυάζει τεχνικές επεξεργασίας εικόνας και αλγορίθμους ταξινόμησης. Η μεθοδολογία αυτή επιτρέπει την κατάταξη της πλάκας σε τρεις διαφορετικές κατηγορίες, παρέχοντας αξιόπιστα αποτελέσματα σε σύγκριση με τις επισημάνσεις ειδικών. Η δεύτερη μεθοδολογία χαρακτηρισμού πλάκας χρησιμοποιώντας εικόνες IVUS, κατατάσσει την πλάκα σε τέσσερις κατηγορίες. Για την εκπαίδευση και την αξιολόγηση της μεθοδολογίας, χρησιμοποιήθηκαν εικόνες IVUS προερχόμενες από ένα εμπορικά διαθέσιμο λογισμικό. Στο τέταρτο κεφάλαιο παρουσιάζεται για πρώτη

φορά μια νέα μεθοδολογία που επεξεργάζεται OCT εικόνες με ένα πλήρως αυτοματοποιημένο τρόπο. Η προτεινόμενη μεθοδολογία είναι σε θέση να ανιχνεύσει τα σύνορα αυλού στις εικόνες OCT, να προσδιορίσει την περιοχή της πλάκας και να εντοπίσει τέσσερις διαφορετικούς τύπους πλάκας. Η αποτελεσματικότητα της μεθοδολογίας που αναπτύχθηκε αξιολογήθηκε χρησιμοποιώντας επισημάνσεις ειδικών. Στο πέμπτο κεφάλαιο, παρουσιάζεται μια νέα μεθοδολογία για την αυτοματοποιημένη τρισδιάστατη (3Δ) ανακατασκευή των στεφανιαίων αρτηριών και της μορφολογίας της πλάκας χρησιμοποιώντας εικόνες CT. Η μεθοδολογία ανιχνεύει τα εσωτερικά και εξωτερικά σύνορα του αγγείου, ανιχνεύει την πλάκα ασβεστίου και ανακατασκευάζει το αγγείο και την πλάκα σε 3Δ επιφάνειες. Η μεθοδολογία, αξιολογήθηκε χρησιμοποιώντας τα αποτελέσματα της IVUS μεθοδολογίας χαρακτηρισμού πλάκας. Στο έκτο κεφάλαιο, παρουσιάζεται μελέτη για την διάδοση των σφαλμάτων απεικόνισης και των λαθών κατάτμησης της περιοχής της πλάκας στις μεθοδολογίες χαρακτηρισμού πλάκας. Υπολογίζεται το μέγιστο σφάλμα που μπορεί να διαδοθεί στα αποτελέσματα του χαρακτηρισμού πλάκας. Η προτεινόμενη μεθοδολογία διάδοσης των σφαλμάτων αξιολογήθηκε με τη χρήση μεθόδων που εφαρμόζονται σε πραγματικά δεδομένα που προέρχονται από IVUS και OCT συστήματα απεικόνισης. Τέλος, στο έβδομο κεφάλαιο, παρουσιάζεται μεθοδολογία για τη εμφύσηση των εικόνων ιστολογίας/micro-CT χρησιμοποιώντας IVUS. Η προτεινόμενη μεθοδολογία διογκώνει το παραμορφωμένο τοίχωμα των εικόνων ιστολογίας/micro-CT, οι οποίες αποτελούν τη χρυσή τομή για το χαρακτηρισμό πλάκας, βασιζόμενη στη διαφορά των ορίων του αυλού στις εικόνες ιστολογίας/micro-CT. Για την αξιολόγηση της προτεινόμενης μεθοδολογίας περιοχές πλάκας στις διογκωμένες micro-CT και ιστολογικές εικόνες συγκρίνονται με εκείνες των εικόνων IVUS.

Η συνεισφορά της παρούσας διατριβής εντοπίζεται στους ακόλουθους συγκεκριμένους τομείς: (i) στην εφαρμογή νέων μεθοδολογιών χαρακτηρισμού αθηρωματικής πλάκας οι οποίες ξεπερνούν τους περιορισμούς της υπάρχουσας βιβλιογραφίας, (ii) στην ανάγκη ανάπτυξης μεθοδολογιών χαρακτηρισμού αθηρωματικής πλάκας ικανές να εφαρμοστούν σε προσφάτως ανεπτυγμένα συστήματα απεικόνισης, (iii) στην παρουσίαση μεθοδολογιών ικανών να παράγουν 3Δ μοντέλα αρτηριακής πλάκας, για τη διερεύνηση της εξέλιξης της αθηρωματικής πλάκας, (iv) στην μελέτη και την ανάλυση της διάδοσης

των σφαλμάτων των συστημάτων απεικόνισης στην αντίχρεωση της πλάκας, και (v) στην αυτοματοποιημένη ευθυγράμμιση των εικόνων ιστολογίας με ενδοαγγειακές εικόνες.

Chapter 1: Introduction

1.1 Physiology of the Cardiovascular System

1.2 Atherosclerosis

1.3 Imaging of Atherosclerosis: Invasive and Noninvasive Techniques

1.1 Physiology of the Cardiovascular System

1.1.1 Introduction

In the human body, blood flows through the arteries which are connected to small arterioles and smaller capillaries. Heart is the organ responsible for pumping the blood through the arteries to the entire body. Nutrients, dissolved gases, waste products and electrolytes are exchanged between the surrounding tissues and blood in the heart. 7,000 l of blood is daily pumped by the heart. In order blood to return to the heart, capillaries return blood to the venules and then venules to the larger veins. The series of vessels that transport blood to and from the heart consist of the circulation system (Figure 1.1).

1.1.2 The Heart

The heart [1] is a muscular organ at the size of a fist and its responsibility is to keep blood circulating properly. Heart is located in the mediastinum, the area between the lungs in the thoracic cavity. It is a cone-shaped organ and the tip of the heart points downward, therefore the strongest beat can be heard on the left side of the thoracic cavity. The size of heart of an average adult is about 14 cm long and 9 cm wide. Pericardial membranes enclose the heart are classified to three layers: fibrous pericardium, parietal and visceral (epicardium) as it is shown in Figure 1.2. The outer layer is the fibrous pericardium (pericardial sac) which is a loosefitting sac composed of strong fibrous connective tissue. The pericardium extends over the diaphragm and over the bases of the large vessels that enter and leave the heart. The serous pericardium is a two layer membrane; the parietal and visceral (epicardium) and between serous fluid exists for friction prolepsis due to the heart beating. Four chambers exist in the heart and

their walls are made of cardiac muscle called myocardium. The chambers are covered with endocardium (Figure 1.2).

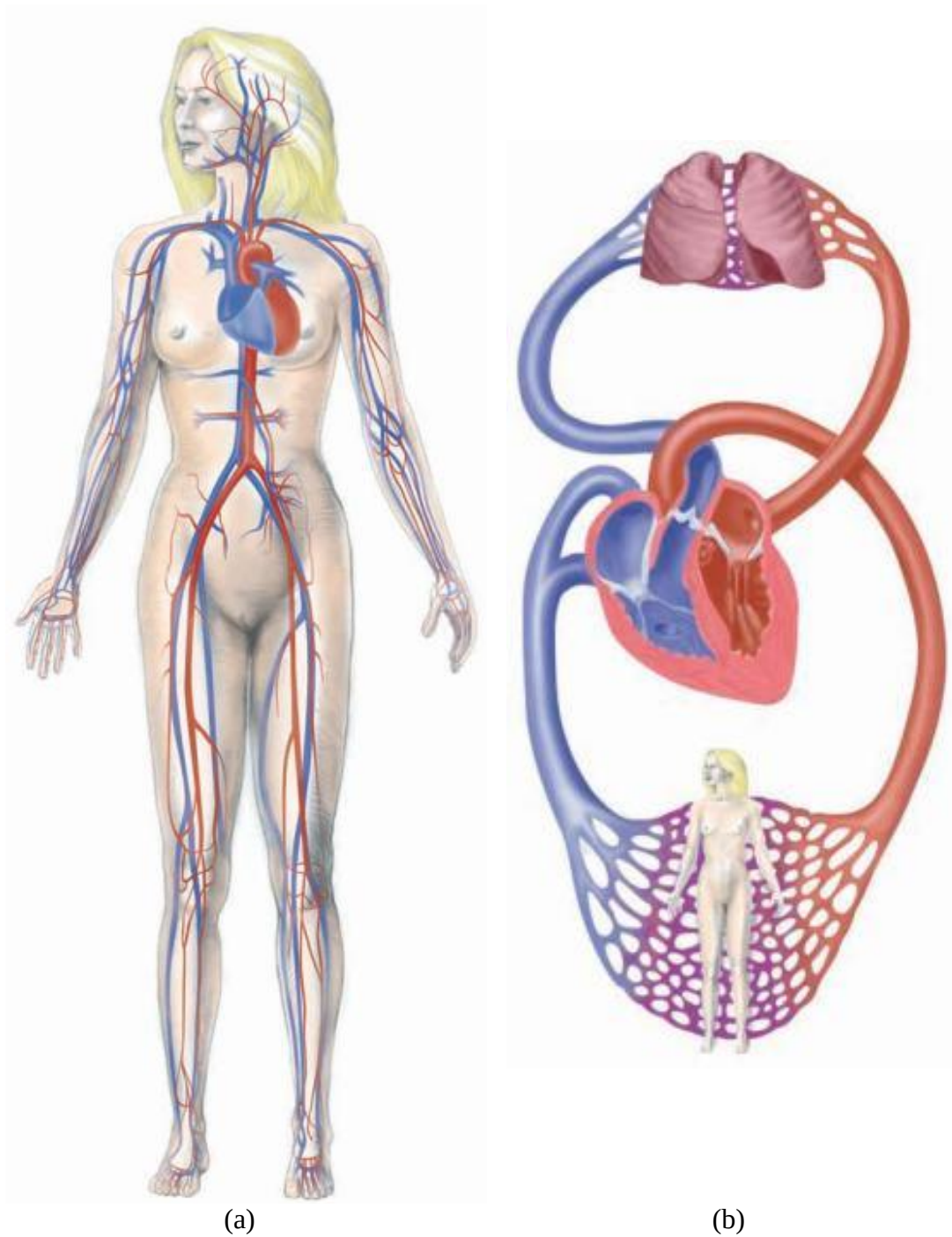


Figure 1.1: The circulation system. Image taken from Moini J [1].

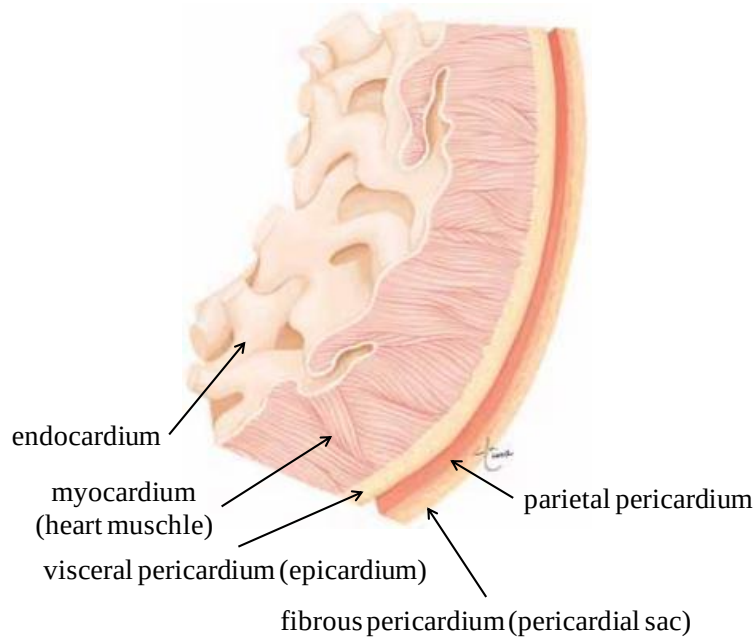


Figure 1.2 Layers of the wall of the heart and the membranes enclosing the heart. Image taken from Steele [2].

The upper chambers are divided to right and left chambers which are called atria. Atria receive the blood returning to the heart and sent it to the lower chambers called ventricles. Ventricles pump out the blood into the arteries (Figure 1.3). A wall-like structure called septum separates the left and right ventricle and the left and right atrium. The role of septum is to keep the blood from left of the heart from mixing with blood from the right of the heart and vice versa. The atrioventricular valve (AV valve) consists of the tricuspid valve on the right and the mitral valve on the left. Atrioventricular valves ensure that the blood flows one-way from the ventricles to the atria (Figure 1.3).

The right atrium receives blood from two large veins: one coming from the head and one coming from the body, and from a smaller vein called coronary sinus and drains blood from the heart's myocardium. The first vein coming from the head is called the superior vena cava and the second vein coming from the body is called inferior vena cava (Figure 1.3). The tricuspid valve lies between the right atrium and ventricle and allows blood to move only from the right atrium into the right ventricle and prevents the backflow of the blood. The muscular wall of the right ventricle is thinner than that of the wall of the left ventricle. This is due to its operation: to pump blood to the lungs having low resistance to blood flow. The left ventricle is thicker as it forces blood to all body organs and has

higher resistance to blood flow. When the right ventricle contracts, the blood pressure in the right ventricle increases it, passively closes the tricuspid valve and the blood can only exit through the pulmonary trunk. The pulmonary trunk is divided into the right and left pulmonary arteries that supply the lungs with blood and has a pulmonary valve with three cusps. This pulmonary valve at the base of the pulmonary trunk allow blood to leave the right ventricle and prevents the backflow of the blood into the ventricle (Figure 1.3).

The left atrium is supplied with blood from four pulmonary veins, two from each lung. Blood enters the left atrium and passes into the left ventricle through the mitral valve (bicuspid valve). The mitral valve prevents the backflow of blood from the ventricle into the left atrium. When the mitral valve closes the blood is directed to the large artery named aorta (Figure 1.3). At the base of the aorta is the aortic valve, with three cusps. This valve opens to allow blood to leave the left ventricle. When the ventricle relaxes, the valve closes preventing the backflow of the blood into the ventricle. Blood flows through the aortic valve into the aorta. The right and left coronary arteries (the first two aortic branches) supply blood to the heart tissue. Coronary artery branches supply with blood many capillaries into the myocardium.

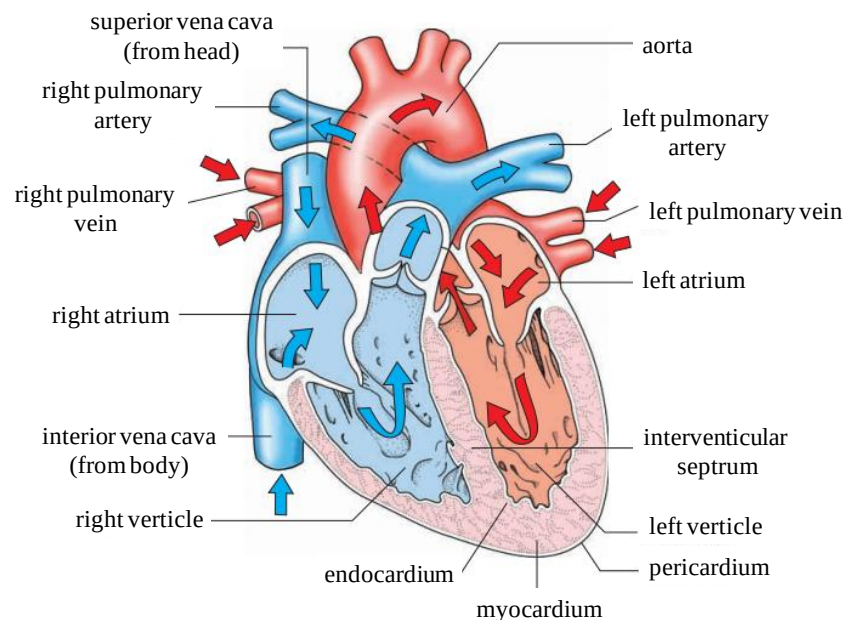


Figure 1.3: Cross-section of the heart showing the four chambers and the major vessels and valves. Image taken from Moini J [1].

1.1.2 The vascular system

The vascular system [2] consists of the arteries, capillaries, and veins. The blood is carried from the heart to the capillaries through arteries. Arterioles are small blood vessel that extend and branch out from an artery and they lead to capillaries. By examining a cross section of an artery three layers of tissues exist (Figure 1.4). The interior layer is called the tunica intima and is the part of the vessel that is in contact with blood and is made of endothelium. The endothelium produces nitric oxide (NO) which is a vasodilator. The middle layer is called tunica media and is made of elastic connective tissue and smooth muscle cells which are responsible for blood pressure maintenance. Relaxation of the smooth muscle cells makes the vessel to dilate as the smooth muscle is the tissue affected by the vasodilator NO. The outer layer of an artery is the tunica adventitia and is composed by fibrous connective tissue forms which very strong preventing the rupture of larger arteries that carry high pressure blood.

Blood returns to the heart from capillaries though veins. Veins are composed of the same three tissue layers as arteries and the smaller veins are called venules. However, there are some differences when compared to the layers of the arteries. First of all, the internal layer of the veins is folded and forms valves (Figure 1.4). Similarly to the heart valves, the veins valves prevent backflow of blood. The number of valves is larger in the veins of the legs as the blood return to the heart against the force of gravity. The middle layer of veins is a very thin layer of smooth muscle as veins do not control blood pressure. The outer layer of veins is thinner than the outer layer of arteries because blood pressure in veins is very low. Blood is transferred from arterioles to venules though capillaries (Figure 1.4).

1.1.3 Blood circulation

Blood is a multifaceted fluid which contains red blood cells called erythrocytes, white blood cells called leukocytes, and plasma. Hormones, proteins, ions, nutrients, gases and waste products compose plasma solution in which are dissolved. In all body cells, blood is used both as supply line and as waste disposal system. Circulation of blood is divided

to pulmonary and systemic [3]. Pulmonary circulation starts at the right ventricle while systemic circulation starts at the left ventricle.

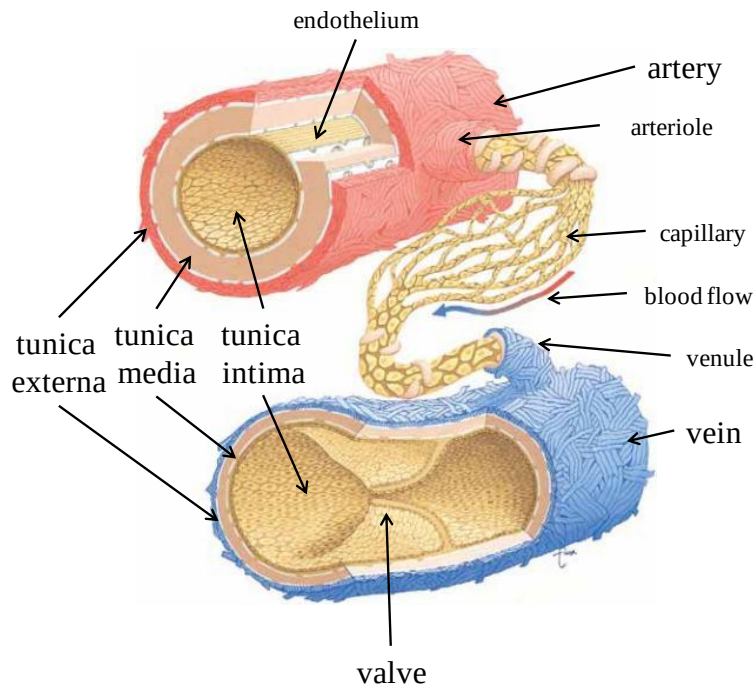


Figure 1.4: Structure of an artery, arteriole, capillary network, venule, and vein. Image taken from Steele [2].

The right ventricle of the heart pumps blood into the pulmonary artery. The pulmonary artery conducts the blood to the right and left pulmonary arteries which are going to the right and left lung, respectively. Within the lungs the artery is divided into smaller arteries and even smaller arterioles and capillaries. The capillaries surround the alveoli of the lungs and the oxygen and carbon dioxide exchange takes place. Then the capillaries are united to form the venules which are united to form veins. Finally veins are merged to the two pulmonary veins and the oxygenated blood returns to the left atrium.

The left ventricle pumps blood into the aorta which is the largest artery of the body. The blood from the branches of the aorta is flowing to the arterioles and the capillary network. The capillaries merge and form venules and veins. The blood is transferred to the inferior vena cava through the veins from the lower body and to the superior vena cava through the veins of the upper body and returns to the right atrium. The aorta is anatomically divided to: the ascending aorta, aortic arch, descending thoracic aorta, and abdominal aorta. The

ascending aorta comes out from the top of the left ventricle. Ascending aorta creates an arch that curves posteriorly over the heart. The thoracic descending aorta is descending to the chest cavity and through the diaphragm continues to the abdominal aorta where it is divided into the two common iliac arteries. The ascending aorta supplies blood to the myocardium through the two branches: the right and left coronary arteries (Figure 1.5). The aortic arch supply blood to the head and arms and has three branches: the brachiocephalic artery, left common carotid artery, and left subclavian artery. The brachiocephalic artery is further divided into the right common carotid artery and right subclavian artery. The right and left common carotid arteries are divided into an internal carotid artery and external carotid artery supplying the head with blood. Both the right and left subclavian arteries supplies the arms.

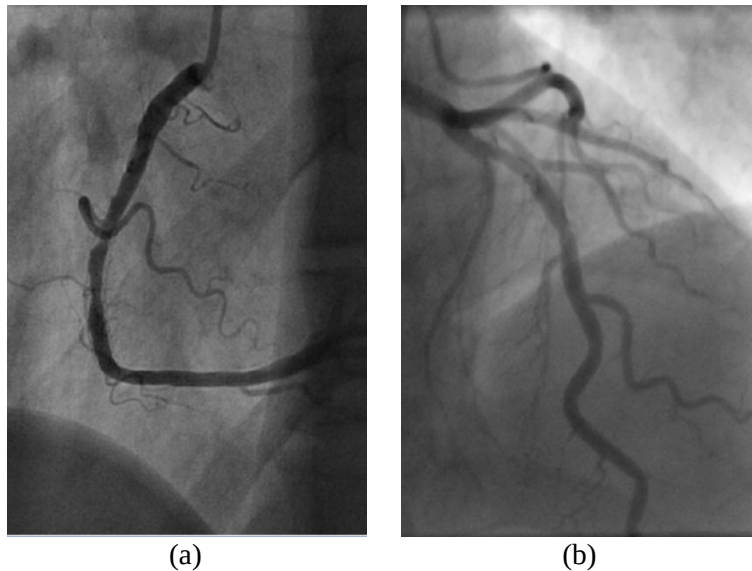


Figure 1.5: The ascending aorta of the heart that supply blood to the myocardium is divided to: the right (a) and left (b) coronary arteries.

1.2 Atherosclerosis

1.2.1 Introduction

Atherosclerosis [4] is an inflammatory disease caused by high concentrations of low-density lipoprotein (LDL) cholesterol [5]. It starts by lipid retention and oxidation [6] which provokes chronic inflammation, resulting in plaque growth which can cause vessel thrombosis or vessel stenosis. Atherosclerotic lesions (atherosclerotic plaques) can cause

occlusion of major conduit arteries, supplying the heart, brain and legs [7]. Atherosclerotic lesions are initially created in the intima of the arteries and gradually affect the entire arterial wall. The first atherosclerotic lesions have been found in mummies and demonstrated the same pathologic features observed today [8]. Among the common risk factors that promote atherosclerosis are smoking, hypertension, hypercholesterolemia, obesity, diabetes mellitus and genetic predisposition. Human atherosclerosis has been studied mainly in the coronary arteries, the carotid arteries, and the aorta.

1.2.2 Development of atherosclerosis

Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is a disease which is developed progressively. The development of the disease with lipid accumulation and inflammation varies significantly among individuals. Atherosclerosis starts with the accumulation of cholesterol-rich lipids and the wall's inflammatory response. This evolution has been observed in the histopathology of plaques in both human and animal studies and is similar for the coronary arteries, the carotid arteries and the aorta.

Atherogenesis starts with a qualitative change in the monolayer of endothelial cells (inner arterial surface) [9]. A normal artery is composed by three layers: the inner layer, the middle layer and the adventitia (outer layer) as it is shown in Figure 1.6 - a. Endothelial cells of an artery, usually oppose white blood cells attachment and express adhesion molecules which capture leukocytes on their surfaces (Figure 1.6 - b) when influenced by irritative stimuli. Both the composition of the extracellular matrix beneath the endothelium and the changes in endothelial permeability help the cholesterol-having low-density lipoprotein (LDL) particles to enter the arterial wall [10]. When monocytes which are the most numerous white blood cells in plaques, reside in the arterial wall, they differentiate into macrophages. Inside the growing atheroma, the mononuclear phagocytes surround the lipoprotein particles and are transformed to foam cells (lipid-laden macrophages). The formation of atheroma involves the recruitment of smooth muscle cells (SMCs) from the middle layer of the arterial wall into the tunica intima (Figure 1.6 - c). The proliferation of resident intimal SMCs and the synthesis of

extracellular medium macromolecules. These macromolecules include elastin, proteoglycans and collagen. SMCs and macrophages die in advanced lesions by apoptosis. The extracellular lipid which is derived from the dead cells can build up in the plaque composing the lipid pool or necrotic core of the plaque [11]. Plaque lesions generally cause stenoses and produce limited blood flow which can lead to tissue ischaemia (Figure 1.6 - d). Thrombi are generated when the fibrous cap of a plaque ruptures and enables blood coagulation components to come into contact with the thrombogenic plaque.

Several risk factors can affect the atherogenesis process such as: hypertension, angiotensin II hormone, cigarette smoking, diabetes and cholesterol. Hypertension can cause increased arterial wall tension leading to the formation of aneurysm. Angiotensin II which is a pressor hormone alters the endothelial function helping the adhesion of leukocytes. Diabetes and cigarette smoking can affect the biology of the vessel. The role of cholesterol plays central role in the atherogenesis and has been studied extensively [12].

Lipids have an important role in the development of atherosclerotic plaque lesions. However, the direct connection between lipids and atherogenesis remains uncertain. LDL levels are associated with the risk of cardiovascular disease and satisfy modified Koch's (disease criteria) [13]. LDL levels correlate with cardiovascular disease in humans, and accumulation of individual vulnerability to atherosclerosis. Monogenic disorders can increase the cardiovascular risk and several studies have shown that the reduction of LDL levels reduced the likelihood of cardiovascular events.

1.2.3 Treatment of atherosclerosis

Atherosclerosis can affect any artery in the body, especially coronary arteries of the heart, carotid arteries, brain arteries, peripheral arteries in arms and legs. The result is the development of different diseases which are classified based on which arteries are affected. Atherosclerosis can lead to serious problems, including mainly heart attack and stroke. The coronary artery disease is the leading cause of death in both men and women in the developed world [5]. Coronary artery disease occurs when plaque builds up in the

coronary arteries. Plaque development narrows the coronary arteries reducing the blood flow to the heart muscle. Plaque development makes blood to clot blocking partially or completely the blood flow. When blood flows to the heart, the muscle is reduced or blocked, angina symptoms (chest pain) occur leading to plaque rupture and heart attack. The carotid artery disease occurs if plaque is developed in the carotid arteries which supply blood to the human brain [14]. The reduction or the block of the blood flow to the brain can cause stroke. The peripheral Arterial Disease occurs if plaque builds up in the major arteries that supply blood to the legs or arms of the body [15]. Once the blood flow to these parts of the body is reduced, numbness, pain, and dangerous infections occur.

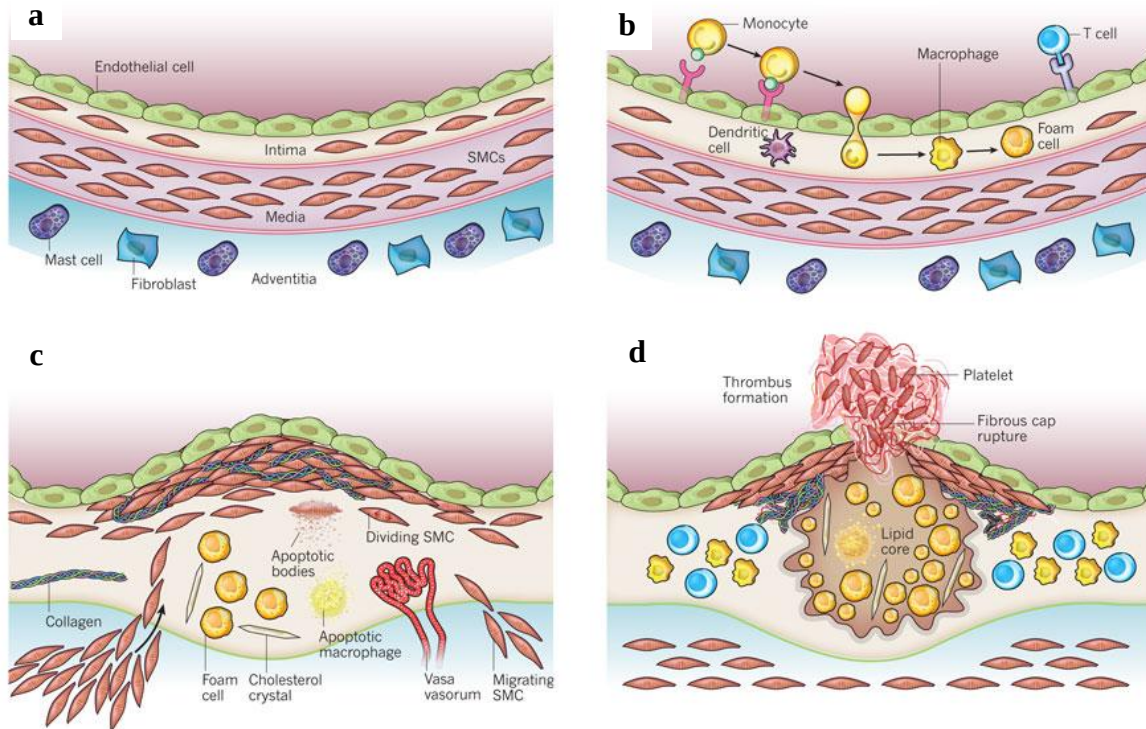


Figure 1.6: Stages in the development of atherosclerotic lesions: (a) The normal artery having a three layer structure: the inner layer, the middle layer and the adventitia. (b) Initial steps of atherosclerosis: adhesion of blood leukocytes to the activated endothelial monolayer, directed migration of the bound leukocytes into the intima, maturation of monocytes into macrophages, and their uptake of lipid, yielding foam cells. (c) Lesion progression involving the movement of SMCs from the media to the intima and the heightened synthesis of extracellular medium macromolecules. (d) Thrombosis process in atherosclerosis. Image taken from [9].

Atherosclerotic disease has been a major focus for clinical and basic investigation in the last half of the past century. Therefore, advances have been made in the development of methods to prevent or treat coronary artery disease [16]. The development of lowering lipids, thrombolytic, antithrombotic or even catheter-based therapies have reduced the percentage of death, and disability from atherosclerotic disease. A lipid-lowering medicine can reduce the amount of cholesterol deposits in the arteries and prevent the progression of the disease [17]. Additionally, other treatments such as low-dose aspirin can even prevent blood clots. Medicines for coronary artery disease reduce the heart's need for oxygen by lowering the blood pressure. Finally, drugs can help in reducing the symptoms from a poor blood supply to the heart. However, there exist severe narrowings and blockages due to the plaque development which can be treated with interventions such as coronary angioplasty, bypass operation and endarterectomy. Coronary angioplasty (balloon dilation) is the dilation of a narrowed coronary artery with a balloon. In order to achieve a good long-term result a metal stent is placed within the artery to keep the artery open. Stents sometimes are overlaid with a drug (drug eluted stents) [18] to prevent restenosis of the artery. Bypass operation is an procedure in which a vessel is taken from another part of the body and is anastomosed to the diseased coronary artery distally to the stenosis to bypass the obstruction to blood flow [19]. Usually the blood vessel used for the bypass operation is a vein from the leg. The internal mammary artery or an artery from the arm is used very often (the radial artery). Endarterectomy is a surgical operation which is used to scrape the lipid rich plaques from inside the arteries and is performed mainly in the carotid arteries.

Atherosclerotic plaques are responsible for the different outputs of the atherosclerotic disease [20]. Clinical investigations for atherosclerosis focus on quantifying the degree of stenosis caused by plaques and on plaque characterization. Many of the plaque features which are related to high-risk plaques such as thin fibrous cap, large amount of necrotic core, neovascularization, macrophage infiltration and intra-plaque hemorrhage, can now be studied using imaging techniques [21, 22]. However, each technique has its own advantages and disadvantages and can be classified to invasive or noninvasive techniques.

1.3 Imaging of Atherosclerosis: Invasive and Noninvasive Techniques

Acute thrombus formations result to life threatening consequences such as stroke or myocardial infarction. It is well known that the high risk to thrombus plaques is not necessarily the plaque types which cause the most significant stenoses. The degree of stenoses and the different clinical outcomes of atherosclerosis are dissociated by several studies. Initially it was revealed by angiography [23] and it was further demonstrated by randomized controlled trials [24, 25]. In order to investigate these changes in the composition of the plaque and evaluate accurately the arterial pathology the visualization of the vessel wall is needed. Several invasive [intravascular ultrasound (IVUS), optical coherence tomography (OCT), Near-infrared spectroscopy (NIRS)] and non-invasive imaging modalities [22] [computed tomographic angiography (CTA), positron emission tomography computed tomography (PET-CT) magnetic resonance imaging (MRI)] are nowadays available and allows assessment of luminal pathology, quantification of plaque burden and characterization of the type of the plaque.

1.3.1 Invasive imaging of atherosclerotic plaques

IVUS is currently the most widely used intravascular imaging modality. It requires the insertion of a catheter with a transducer on its tip that emits ultrasound signal perpendicular to its axis at a frequency from 20-70MHz. The reflections of the emitted signal are received by the transducer and are analyzed to generate cross sectional images of the vessel. In these images an expert observer can identify the luminal, stent, and media-adventitia borders, quantify the plaque burden and characterize the composition of the plaque with a moderate accuracy [26]. A typical IVUS image is shown in Figure 3.1. To increase the accuracy of IVUS in characterizing the type of the plaque, several methodologies that rely on the radiofrequency analysis of the IVUS backscatter signal (RF-IVUS) have been developed [27]. These techniques take into consideration not only the amplitude but also the frequency of the reflected signal and are the only approaches that are able to quantify in a semi-automated fashion the composition of the plaque types. However, although these approaches appear superior to grayscale IVUS for plaque characterization, recent reports have questioned their reliability especially in stented segments and behind calcium deposits [28].

Other limitations of IVUS are the numerous artifacts seen in IVUS images such as the ring down effect, the Non Uniform Rotational Distortion, the guidewire artifact and the signal drop-out behind calcium, which prohibits the visualization of deep vessel structures, as well as its moderate resolution which does not permit visualization of the microstructures of the plaque that are associated with an increased vulnerability such as the presence of neo-vessel, micro-calcification and macrophages and the measurement of the thickness of the fibrous plaque [29, 30]. Finally, IVUS cannot provide any information regarding the 3D vessel geometry and detect the exact location of the plaque in the artery.

OCT imaging uses near infrared light instead of acoustic waves and relies on measuring the time delay and the magnitude of the backscattered light to generate the final images. Compared to IVUS, OCT has a higher axial resolution (12-18 μ m versus 150 μ m) which allows detailed imaging of the vessel wall, better plaque characterization, detection of plaque erosion and rupture and visualization of plaque micro-features related to increased vulnerability (i.e., the presence of microcalcifications, neovascularization, and plaque hemorrhage) [31, 32]. In stented segments, OCT appears more sensitive than IVUS in detecting struts' coverage, struts' malapposition, neointimal hyperplasia, thrombus and vessel wall trauma [33].

Limitations of OCT imaging are its poor signal penetration (2-3mm), which often inhibits visualization of the outer vessel wall, and its inability to penetrate lipid-rich cores as well as the fact that it cannot provide any information about vessel geometry and the distribution of the plaque into the artery wall [34].

NIRS relies on the principle that different organic molecules absorb and scatter NIRS light to different degrees and at various wavelengths. The spectral analysis of the reflected NIRS light allows evaluation of the chemical composition of the plaque and identification of the lipid component. In contrast to RF-IVUS, NIRS is restricted to the identification of superficial, wide lipid cores (with cap thickness <450 μ m, circumferential extent >60° and plaque thickness >200 μ m) but it appears more reliable for the detection of lipid-rich plaques located behind calcific deposits [35]. The main limitation of NIRS is

its inability to visualize the lumen and outer vessel wall, quantify the atheroma burden and give information about the 3D vessel geometry and plaque distribution.

Apart from the abovementioned intravascular imaging modalities that have already been used in clinical practice, several other intravascular techniques [22] have been introduced, such as the intravascular magnetic resonance spectroscopy that allows detections of the lipid component, the intravascular magnetic imaging that has been used only in experimental models and appears as the only imaging technique that can visualize both calcific and lipid rich plaques, the Raman spectroscopy which is capable of discriminating the esterified from the non-esterified cholesterol rich plaques, the intravascular photoacoustic (IVPA) imaging which can detect the presence of neo-vessel macrophages and the composition of the atheroma, the NIRF that is able to identify vascular inflammation and the TRFS which can characterize the composition of the superficial plaque. These techniques which are under-development have unique strengths but cannot provide a holistic representation of coronary anatomy and pathology.

1.3.1 Non-invasive imaging of atherosclerotic plaques

MRI is an imaging technique with a high potential to visualize vessel anatomy. The use of multi-contrast MRI provides sufficient information for the qualitative assessment, mostly of carotid plaque, by taking advantage of the different signal intensities of the components on the various image sequences [36]. In particular, four MRI image sequences, Time of Flight (TOF), T1-weighted (T1W), T2-weighted (T2W) and proton density weighted (PDW), can be combined to extract conclusions about the "vulnerability" of the plaque. On all sequences, the image intensities of the plaque regions are compared with the corresponding adjacent sternocleidomastoid muscle [37]. After the classification of plaque regions as hyper-, iso - or hypo-intense, plaque components are detected using the unique combinations of signal intensity of each one in different contrast weightings [38]. Studies have reported that MRI is an effective tool to evaluate plaque regression following lipid - lowering medication [39]. Nevertheless, a plaque characterization including the identification of high-risk plaques, still remains difficult.

CT is an imaging tool that allows noninvasive visualization of atherosclerosis. Since the initially introduced 4-slice scanners, the technique has developed producing 64-slice and 320-slice systems. The spatial resolution has been improved resulting in higher quality images and diagnostic accuracy for the detection of atherosclerotic plaque lesions. CT resolution for detecting coronary plaques remains inferior to that of invasive imaging modalities. However, studies have demonstrated high diagnostic accuracies for the detection coronary plaques [40]. CT is not only used to identify artery stenosis, but also to provide information about plaque morphology and composition. CT can image three different plaques: calcified, non-calcified and mixed. Calcified plaques can easily identified due to their higher density values. Detection of different non-calcified plaque types is not so easy due to the more subtle difference in attenuation. While CT may be suitable for the non-invasive detection of plaque and characterization of its composition several limitations remain as the technique is associated with exposure in radiation and as the resolution remains inferior compared to invasive atherosclerosis imaging techniques [41, 42].

The combination of positron emission tomography and computed tomography (PET-CT) has proven to be promising for the evaluation of atherosclerotic plaques and inflammation in coronary arteries [43]. PET-CT uses a device which combines both a positron emission tomography (PET) scanner and an x-ray computed tomography (CT) scanner. The produced images, acquired simultaneously from both devices are combined into one superimposed image. The combined image has the advantages of both PET imaging, which depicts the spatial distribution of metabolic and biochemical activity in the body, and CT, which depicts the correct anatomy of the artery. However further investigation is necessary in order to acquire more precise and reliable images and advance the use of this imaging technique [44].

Chapter 2: Literature Overview

2.1 Introduction

2.2 Segmentation

2.3 Plaque Characterization

2.4 Contribution of this Thesis

2.1 Introduction

Cardiovascular disease is the most common cause of death in western societies and is due to the built up of atherosclerotic plaques that obstruct the coronary anatomy and cause ischemic events [45, 46]. Several studies over the last years have shown that the accurate assessment of coronary artery disease progression requires imaging of the lumen and of the vessel wall which is not provided by coronary angiography that allows only visualization of the luminal silhouette in two dimensions (2D) [47].

Intravascular ultrasound (IVUS) [48] imaging was the first imaging modality introduced to overcome the inherited limitations of X-ray imaging. IVUS imaging involves the advancement of a miniaturized ultrasound probe to the coronaries. The probe is attached to the distal end of the catheter and is able to emit ultrasound signal perpendicular to its axis allowing visualization of the lumen, outer vessel wall and plaque.

Optical coherence tomography (OCT) [32, 49] is a recently developed invasive imaging technique and is regarded as the optical analogous of IVUS. This imaging technique relies on the analysis of the reflected light that is emitted by an OCT probe which is inserted in the coronaries. A unique advantage of this technique is its high resolution that permits visualization of vessels' microstructures that are unseen by IVUS (OCT: axial resolution: 12-18 μ m, lateral resolution: 20-90 depending on the depth of focus, 30-40MHz IVUS transducers axial resolution: 80-100 microns and lateral resolution: 200-250 microns) [50]. Thus, OCT not only provides evaluation of the luminal dimensions, and assessment of vessel wall morphology but it also permits identification of features that are associated with increased vulnerability such as: the thickness the of fibrous cap,

the presence of micro-calcifications, neo-vessels, and macrophages which cannot be evaluated by any other intravascular imaging technique [51].

However, both IVUS and OCT are invasive imaging techniques which have risks, cannot be used in the contemporary practice for the study of the entire arterial tree and are not indicated in asymptomatic individuals. Therefore Coronary Computed Tomography (CT) angiography has been introduced as a non-invasive imaging modality to visualize the coronary arteries.

In parallel an effort has been made to develop methodologies that would allow automated fast, reliable, and reproducible analysis of the all three imaging methods: IVUS, OCT and CT. The aim of this chapter is to present the available methodologies developed for the segmentation of the lumen, and outer vessel wall borders and the characterization of the composition of the plaque using IVUS, OCT and CT images.

2.2 Intravascular ultrasound - IVUS

2.2.1 Segmentation of IVUS images

The automated processing of the IVUS images is not an easy task as the developed algorithms had to face several challenges mostly caused by the high noise content, the low image resolution, the non – uniform luminance etc. Dhawale *et al.* [52] presented the first semi-automated method for detecting the lumen and media-adventitia borders in IVUS images. The region between these borders is called Region of Interest (ROI). Their approach required an expert observer to approximate the regions of interest in each frame and then these estimations were used to guide a 1D dynamic search algorithm. Although this approach had significant limitations such as the fact that it was semi-automated and time consuming as it requires expert's annotations in each frame, it showed for the first time that IVUS imaging segmentation is possible.

Since then several methodologies have been developed that minimize manual interaction allowing automated border detection [53-58]. Sonka *et al.* [59] presented a fully automated segmentation algorithm for IVUS images which is used until today. The algorithm used heuristic graph searching and global image information. Cost functions were applied to re-estimate the initial contours of the lumen and outer vessel wall. The

method was validated in 38 IVUS images which were manually annotated by experts; the obtained results demonstrated an excellent agreement of the computer-detected and observer-defined lumen areas ($r=0.96$, $y=1.02x+0.52 \text{ mm}^2$). The main limitation of this method is the approximation that the distance between media-adventitia and luminal border is at least 0.3mm.

Lobregt and Viergever [57] were the first who employed the deformable models for medical image segmentation and IVUS was used among the tested medical images. Few years later Shekhar et al. [58] modeled the IVUS frames into a 3D object and applied a 3D deformable model while Kovalski *et al.* [56] added a variable in the energy function of the snake. The energy function tended to inflate the snake (balloon snakes) and thus the proposed approach was able to address the shrinkage of the snake around the IVUS catheter that was often seen in previous approaches. Plissiti *et al.* [60] implemented a Hopfield neural network to expedite the minimization of the deformable model's energy function. They implemented image processing techniques to address common artifacts seen in IVUS images such as the blood speckle the calcific shadowing and the guide wire artifact, and to reduce the noise seen in IVUS images. Validation of the method showed that the estimations of the proposed segmentation approach were close to those derived by experts (linear regression analysis for the lumen area between the estimations of the proposed approach and the annotations of the experts: $y=0.97x+6.13\text{mm}^2$, $r=0.99$ and for the plaque area: $y= 1.01x-5.47\text{mm}^2$, $r = 0.99$). However, a significant limitation of the segmentation approaches that relied on snakes was the fact that the detection of the borders in the first IVUS frame was done manually. Giannoglou *et al.* [53], overcome this problem proposing the first fully automated snake-based segmentation methodology. In this approach, the luminal border of the first frame is estimated by using the intensity difference between the lumen area the plaque area and the media-adventitia border using low-pass filtering that simplifies the delineation of the outer vessel wall. Validation of the method using experts' annotations showed mean differences of: $0.70\pm 2.68 \text{ mm}^2$, $0.17\pm 4.58 \text{ mm}^2$, $-0.53\pm 3.50 \text{ mm}^2$ for the luminal, media-adventitia and plaque areas, respectively.

Iskurt *et al.* [61] implemented for the first time level sets for IVUS processing and later on, Yousheng *et al.* [62] combined the level set method with B-spline models. Taki *et al.*

[63] implemented a fully automated method for segmenting the two borders. The authors presented two different methods, one using active contours and one using level sets. They validated the two approaches using expert annotations on 60 images derived from 7 patients and concluded that the geometric deformable models (active contours) have advantages over the parametric ones (level sets). The above methodologies that are based on the level set models are fast and they do not require increased computational effort. However, significant disadvantages of the level set methods are the weak noise resistance and the large errors in edge detection as they involve multidimensional curved surfaces evolvment. These limitations render them less useful in IVUS segmentation than the active contour models which do not require parameterization, provide good numerical stability and topological flexibility and can be straightforward extended from the 2D formulation to 3D. However, the fact that the level set models have been recently introduced in IVUS processing leaves plenty of space for improvement.

From the above it is apparent that, none of the available methodologies can be considered as the gold standard for IVUS processing. This is due to the increased noise and artifacts seen in IVUS which often distract the developed algorithms leading to false estimations. In addition most of the described methodologies operate in a semi-automated manner requiring an initial approximation of the regions of interest, while others appear time consuming and require an expert to make corrections whenever they fail. Therefore further effort should be made to develop new algorithms but also to improve the image quality of the obtained IVUS image minimizing the noise and artifacts and hence facilitating the detection of the regions of interest.

2.2.2 Plaque characterization in grayscale IVUS images

Grayscale IVUS imaging allows characterization of four main plaque components [64, 65]: Dense Calcium (hard calcified plaque), Necrotic Core (soft plaque), Fibrotic Tissue (hard non calcified plaque) and Fibro-Fatty Tissue (mixed plaque). Dense Calcium (DC) has a high echoreflectivity in grayscale IVUS images and is composed of compact calcium crystals. The necrotic Core (NC) has a low echoreflectivity in grayscale IVUS images consisting of lipids tissue while the fibrotic Tissue (FT) has a medium echoreflectivity and is composed of bundles of collagen fibres [66]. Finally, Fibro-Fatty Tissue (FFT) (mixed

plaque with also medium echoreflectivity) consists of loosely collagen fibres with lipid accumulations. Initially plaque characterization was performed by expert observers. However, this process is laborious, time consuming and has a low reproducibility. Zhang *et al.* [67] were the first who proposed an automated plaque characterization algorithm. The authors extracted texture based and fractal based features from IVUS images and by using a classifier they classify the plaque components to hard and soft plaques. Afterwards, the hard plaque was divided to hard and hard calcified plaque based on the acoustic shadow of the calcium. They have tested their method using the estimations of experts in 12 IVUS images and the overall accuracy of the proposed approach was 89.9%. Brunenberg *et al.* [68], proposed an alternative methodology that relied on the extraction of texture based features and then they used adaptive boosting to classify pixels in three different plaque types: DC, NC and FT. The classification results were post-processed using a new developed snake called "Stop & Go Snake". The method was tested on 30 IVUS frames achieving an overall accuracy 75.82%.

Filho *et al.* [69] presented a method that was able to detect only the calcium in IVUS images. They applied Otsu's thresholding method in IVUS images and analyzed the acoustic shadow that follows the calcium. The method was tested using the annotations of experts as gold standard and the reported sensitivity and specificity was 84% and 72%, respectively. In a similar attempt Zhang *et al.* [70] used active contours and the contourlet transform to detect the calcific tissue. Each image was decomposed with the contourlet transform into low-pass and band-pass bands image. Then adaptive thresholding was applied on the low-pass bands and the regions of interest were detected. In these regions the calcium was identified by examining the presence of acoustic shadow. Testing the method on 80 IVUS frames using experts' annotations they reported sensitivity and specificity 87.16% and 87.35%, respectively.

Finally, Taki *et al.* [71] used gray scale data from IVUS frames and classified the atherosclerotic plaque composition into three classes: DC, NC, and FT_FFT, (the FT and FFT classes into one class the FT_FFT). According to the above method the ROI is detected first, using a border detection algorithm [72]. Then the shadowed regions of the ROI are detected and features are extracted for each pixel of the ROI. Finally a Support Vector Machine classifier [73] is used to classify each pixel of the ROI (except the pixels

corresponding to the shadow regions) into one of the three classes DC, NC, and FT_FFT with overall classification accuracy 75%.

A significant limitation of the abovementioned techniques was the fact that they were relied on the processing of grayscale IVUS images that are generated from the amplitude of the backscatter signal. However, apart from the amplitude it has been shown that the frequency of the IVUS backscatter also provides useful information about the composition of the plaque. Therefore over the last years several methodologies have been developed that are able to process the backscattered IVUS signal, taking advantage not only of the information given by the amplitude of the backscatter signal but also of the frequency data allowing more accurate plaque characterization [27].

The first methodology developed for this purpose integrated backscatter analysis of IVUS [74, 75]. This approach relies on the spectral analysis of the scattered signal implementing fast Fourier transform and is able to differentiate 3 different tissues types for fibrous, lipid-rich, calcium and fibrocalcific. Validation of this methodology using histology as gold standard demonstrated an excellent accuracy of IB-IVUS for the detection of different tissues (rang 93% - 96%), while Sano *et al.* [76] were the first that used IB-IVUS to assess the composition of the plaque in 160 non-obstructing lesions and demonstrated that lipid rich plaques - as these were detected by IB-IVUS - were associated with future events.

Virtual histology IVUS (VH-IVUS) [77] is the most widely accepted methodology for the backscatter analysis of the IVUS signal. It relies on the use of histological data for the creation of IVUS backscattering spectral maps for the different plaque types and then autoregressive analysis is used to classify the radiofrequency backscatter signal in 4 different tissue types i.e., NC, FF, FT, DC that are portrayed in the IVUS images with a red, light green, green and white color, respectively. Validation of this methodology using histological data has shown that the proposed method is capable to detect different tissue types with high accuracy: 96.7%, 95.8%, 93.5% and 94.1% for DC, NC, FT and FFT, respectively [78]. The high accuracy of VH-IVUS and the fact that it was easy to use and allowed quantification of the atheroma and its composition has permitted its broad application in the research arena to study the effect of different medical therapies on

plaque characteristics and atherosclerotic evolution [79, 80]. In addition several studies referring to natural history of atherosclerosis, such as the PROSPECT [80] and the VIVA [79] studies have demonstrated that the composition and the phenotype of the plaque as this was assessed by VH-IVUS is associated with plaque vulnerability and future adverse events.

Finally Sathyanarayana *et al.* [81] implemented a pattern recognition algorithm for the characterization of the composition of the plaque from the IVUS radiofrequency data. The training of the pattern recognition algorithm was performed into 1200 histological images. Similar to the previous methodologies i-Map is able to identify 4 different tissue types (necrotic, lipidic, fibrotic and calcified). The validation of the proposed approach in histological data yielded high accuracies (range 95% - 98%) for all the four tissue types.

Although, the proposed approaches have evidence about their efficacy some recent reports have casted doubts about their capability to detect the different tissue types, especially in complex lesions in stented segments and behind the calcific tissue. Moreover, it appears that there is a high discrepancy between the estimations of different methodologies for the same tissues [82]. Nevertheless, what it is beyond any question is the value of these modalities in research arena - especially in the detection of vulnerable, high risk lesions – as they are able to portray in a comprehensive manner the different tissue types quantify their burden and characterize the phenotype of the plaque [64].

2.3 Optical coherence tomography - OCT

2.3.1 Segmentation of OCT images

OCT has limited tissue penetration which often does not allow imaging of the entire plaque and thus the media and adventitia are not clearly visible. Thus, all the segmentation methodologies that have been presented up to date focus only on the automated lumen border detection [83-86]. Sihan *et al.* [83] and Tanimoto *et al.* [84] applied edge detection methods to automatically segment the lumen. They both validated their method using commercially available software (CURAD vessel analysis, CURAD BV, Wijk bij Duurstede, The Netherlands) and the obtained results demonstrated that their methods are able to detect with a high accuracy the lumen borders. Most recently

Tsantis *et al.* [85] and Ughi *et al.* [86] made a step forward presenting automated methods that allowed not only detection of the lumen borders but also of the stent struts permitting estimations of the strut apposition and coverage. Tsantis *et al.* [85] segmented the lumen using an Markov random field model (MRF) clusters and compared their results with expert's annotations. Ughi *et al.* [86] processed the A-lines of the OCT image and by computing the rise and fall of the image intensity at each line they detected the lumen border. They validated their findings using experts' annotations and high Pearson's correlation was obtained (0.96).

2.3.2 Plaque characterization in OCT images

OCT imaging allows detection of four different plaque components [51, 87]: calcium (CA) which appears as a low back-scattering heterogeneous region that is fully bordered, lipid tissue (LT), which is a heterogeneous back-scattering area and in contrary to the calcific regions it has diffuse borders, fibrous tissue (FT) that appears as high back-scattering homogeneous areas and mixed tissue (MT) that combines different tissue characteristics.

The first plaque characterization methodology was presented by Xu *et al.* [88] who attempted to identify cutoff values of the backscattering and attenuation coefficients of the reflected light beam that would allow characterization of different plaque components. Similarly, van Soest *et al.* [89] used the attenuation coefficient of the reflected light to detect healthy vessel wall, intimal thickening, LT and macrophage infiltration. Both approaches made a significant step forward towards the identification of different tissue components. However, their validation in histological data showed that they are incapable to define fixed cutoff values for the backscattering and attenuation coefficients that would allow accurate differentiation of the plaque components.

In contrary to Xu *et al.* [88] and van Soest *et al.* [89] who analyzed the A-lines of the OCT signal, Wang *et al.* [90] proposed a semi automatic image processing method for detecting the CA in OCT images. They used edge detection to identify possible CA regions and then they applied an active contour model to delineate the CA tissue. The

method was semi-automatic as it requires the removal of the false detected CA, and was able to detect only the calcific component.

2.3 Computed Tomography - CT

During the last 15 years, CT scanners became widely available for general purpose scanning. This imaging technique offers excellent spatial and temporal resolution. As a result, scientific interest soon turned on the potential use of this technology on the treatment of cardiovascular disease (CAD). Moreover, as plaque "vulnerability" gradually became a more important factor in the prognosis of cardiovascular events, in comparison to vessel stenosis, researchers soon focused on the characterization of plaque components using CT images. Several authors have proposed that plaque characterization using CT images, is feasible using density measurements. Calcifications and lipid cores are the main components of interest, while there is still controversy on whether hemorrhage detection is feasible [91].

Identification of calcifications was well established even at the early stages of CT usage in terms of calcium score [92]. The work of Schroeder *et al.* [93] was one of the first to evaluate the accuracy in characterizing plaque lesions other than calcium by multislice computed tomography. The results were compared with IVUS findings. First, 12 plaques were classified on IVUS as soft, intermediate and calcified. Using MSCT, soft plaques showed a mean density of 14 ± 26 Hounsfield units (HU), intermediate plaques of 91 ± 21 HU and calcified plaques 419 ± 194 HU. The authors concluded that there was no overlap in the mean density values among the three groups of plaques. According to them, plaque characterization could be performed through MSCT under the assumption that "soft" plaques correspond to lipid cores and "intermediate" to mixed plaque.

Identification of lipid rich plaques was at the centre of scientific interest in the last years. As computer capabilities increased dramatically at the same period, there was an increased interest on computer aided methods on plaque characterization using CT images. Dey *et al.* [94] developed a software to identify lipid-rich plaque with non-contrast electron beam tomography (EBT) and MDCT. Based on the results of Prokop *et al.* [95] on the attenuation of various plaque components on spiral and multislice CT, the authors assumed that only calcifications could be detected with the use of a fixed

threshold value. On the contrary, they suggested that lipid tissue should be detected by the use of an adaptive threshold.

First, possible regions were drawn by a trained observer to identify a normal blood pool region in the ascending aorta. Afterwards, possible regions were drawn on the coronary arteries. Histograms were considered for all artery regions. All of them were compared with a Gaussian function that was iteratively fitted on the normal blood area and used as a calibration standard. The patient specific lipid threshold was defined as the mean intersection of all the coronary artery image histograms with the normal blood-fitted curve. The authors validated their method through a reproducibility test on interscan measurements in 25 patients. They reported that the value of lipid threshold exhibited a variation 9.65%. Also, in five patients, the locations of lipid-rich plaque correlated well with available IVUS findings.

Several studies [96, 97] have demonstrated that coronary CT derived metrics such as the luminal dimensions, plaque burden and the composition of the plaque can provide useful prognostic information [98], while recently Papadopoulou *et al.* [96, 99] demonstrated that coronary CT has a value in assessing changes in the composition of the plaque in patients admitted with an acute coronary event. Moreover, an accurate 3D reconstruction of the coronaries can provide models that allow comprehensive visualization of the vessel geometry and assessment of the distribution of different plaque types in space and permit blood flow simulation and evaluation of the role of the local hemodynamic forces on plaque progression regression [100].

Several methods have been presented in the literature which have been developed to assess plaque burden and characterize the composition of the plaque from the CT [101]. The outcome of the proposed approaches was compared with the estimations of IVUS which was treated as the reference gold standard. Leber *et al.* [102] manually detected CP in both CT and IVUS and compared their volume. Fiducial points such as: side branches, calcified plaques and the origin of the stents were manually selected and used in order to identify matching between CT and IVUS. Following the same rationale Voros *et al.* [103] studied the correlation between CP plaques detected by IVUS and CT and compared both their volume and areas. In a similar attempt Brodoefel *et al.* [104] compared the CP

volume by using VH software and a software (SUPERPlaque software) for characterizing the plaque in IVUS and CT, respectively. Recently Graaf *et al.* [105] attempted to correlate the plaque volumes of CTA and IVUS using for plaque characterization QAngio CTA software (QAngio CT 1.1, Medis medical imaging systems) and VH-IVUS. However, all the above mentioned methods were semi-automated requiring increased manual interaction for the extraction of the lumen and outer vessel wall borders and did not present any classification or plaque reconstruction method. They only correlated the plaques using manual estimations [96, 102, 103, 106] or commercially available softwares [104, 105, 107]. In addition they focus only on volume comparison ignoring the spatial location of the volumes within the studied IVUS and CT segments.

2.4 Contribution of this Thesis

The majority of the reported works in the literature focused on developing methodologies that process imaging data, in order to detect and characterize the various atherosclerotic plaque types. In this thesis we focused on developing new methodologies for processing imaging data in order to either enhance the produced results and overcome the literature limitations or for processing imaging data derived from novel imaging systems.

The presented IVUS plaque characterization methods reported results which are compared favorably to those reported in the literature. The first methodology is based on a hybrid approach that incorporates both image processing techniques and classification algorithms. The methodology allows the classification of the plaque into three different categories: the Soft Plaque, the Hard Non-Calcified Plaque and the Hard Calcified Plaque. Annotations by two experts on 8 IVUS examinations were used to train and test the methodology. The combination of an automatic thresholding technique and active contours coupled with a Random Forest classifier provided an overall classification accuracy of 86.17%. The methodology achieved higher overall classification accuracy than the methods reported in the literature, and it was validated using a larger number of images. Additionally, it can detect more accurately the Hard Calcified Plaque. The second IVUS plaque characterization methodology presented in this thesis is validated using a commercially available software that is widely used in the clinical arena: VH-IVUS. However, its operation can only be performed once per cardiac cycle (ECG-gated

technique) which significantly decreases the number of characterized IVUS frames. Also, atherosclerotic plaques in images, been acquired by machines which are not equipped with the VH software cannot be characterized. These limitations are addressed by the presented methodology which is based on a three step approach. In the first step the plaque area (ROI) is detected semi-automatically. In the second step a set of features is extracted for each pixel of the ROI and in the third step, a Random Forest classifier is used to classify these pixels into four classes: Dense Calcium, Necrotic Core, Fibrotic Tissue and Fibro-Fatty Tissue. In order to train and validate our method, we used 300 IVUS frames acquired from Virtual Histology examinations from 10 patients. The overall accuracy of the proposed methodology was 85.65% suggesting that our approach is reliable and may be further investigated in the clinical and research arena.

The presented OCT plaque characterization methodology, to our knowledge, is the first attempt to develop a fully automated method for the characterization of the composition of the plaque using OCT images which permits accurate border detection and reliable characterization of the different plaque types in a fully automated manner. The overlapping areas ratio between the methodology and experts' annotations for CA, LT, FT and MT was 0.81, 0.71, 0.87 and 0.81, respectively. The methodology relies on the combination of medical knowledge, image processing and machine learning techniques. The innovative aspects of the presented methodology are: (i) the fact that it focuses on the analysis of the OCT images and not on the OCT signal, (ii) the ability to detect accurately the lumen borders and (iii) the definition of a region of interest and the fact that it can characterize with a high accuracy different tissue types with a typical morphology.

The presented CT study is an a new automated methodology for the 3D Reconstruction of coronary arteries and plaque morphology. In order to validate the 3D plaques detected by our method we used as gold standard the results of the VH-IVUS based plaque characterization methodology. Validation was performed on calcium, since non-calcium plaque areas contains various plaque types and a direct comparison with IVUS findings could not be performed. The detected 3D calcific plaques were extensively validated and a significant correlation was observed between CT and IVUS. Additionally the reconstructed inner and outer wall of the proposed method were compared with the ones

reconstructed using the IVUS borders providing an exceptional agreement. The innovative aspects of the proposed method are the fact that it focuses on the reconstruction of both lumen, outer vessel wall and calcific plaques and the methodology is automated with user intervention only in the plaque parameters adaptation step. Additionally, the presented approach is able to reconstruct the vessel geometry taking into consideration its curvature allowing assessment of the distribution of the plaque in 3D space. Finally, the validation is performed in 3D space by comparing the volumes of the lumen, outer vessel wall and calcium, derived by CT and by IVUS after a spatial co-registration of the IVUS onto the CTA data.

All the plaque characterization methods have a serious drawback: plaque characterization and segmentation steps are validated independently. This leads to overestimated results as the segmentation error computed in the validation step and the acquired system image formation errors are not propagated in the final result. To overcome this limitation, for the first time, we present a methodology to study the error propagation of both segmentation step and image formation. We calculate the maximum error that can be propagated to the plaque characterization result to present a more thorough and realistic validation.

Concluding, all the images acquired from both micro-CT and histology are deformed, due to the lack of blood pressure. Therefore expert's intervention is required in order for the micro-CT/histological images to be used for validation of plaque characterization methods. To overcome this, a framework for the inflation of micro-CT and histology data using intravascular ultrasound (IVUS) images, is presented for the first time. The proposed methodology consists of three steps. In the first step the micro-CT/histological images are manually co-registered with IVUS by experts, using fiducial points as landmarks. In the second step the lumen of both the micro-CT/histological images and IVUS images are automatically segmented. Finally, in the third step the micro-CT/histological images are inflated by applying a transformation method on each image. The transformation method is based on the IVUS and micro-CT/histological contour difference. In order to validate the proposed image inflation methodology, plaque areas in the inflated micro-CT and histological images are compared with the ones in the IVUS images. The proposed methodology for inflating micro-CT/histological images increases

the sensitivity of plaque area matching between the inflated and the IVUS images (7% and 22% in histological and micro-CT images, respectively).

Chapter 3: Intravascular Ultrasound Imaging (IVUS)

3.1 Introduction

3.2 First approach

3.3 Second approach

3.4 Discussion

3.1 Introduction

IVUS is considered as one of the most useful modalities for the study of coronary atherosclerosis as it provides real time, high resolution two dimensional (2D) cross-sectional images of the coronary arteries, which allow accurate estimation of the luminal and outer vessel wall dimensions and computation of the plaque burden. A typical OCT image is shown in Figure 3.1. Furthermore, IVUS provides qualitative information about the composition of the plaque. This is of great importance since it is well known that prognosis and management depends not only on the degree of the stenosis but also on the type of the atheroma [108, 109].

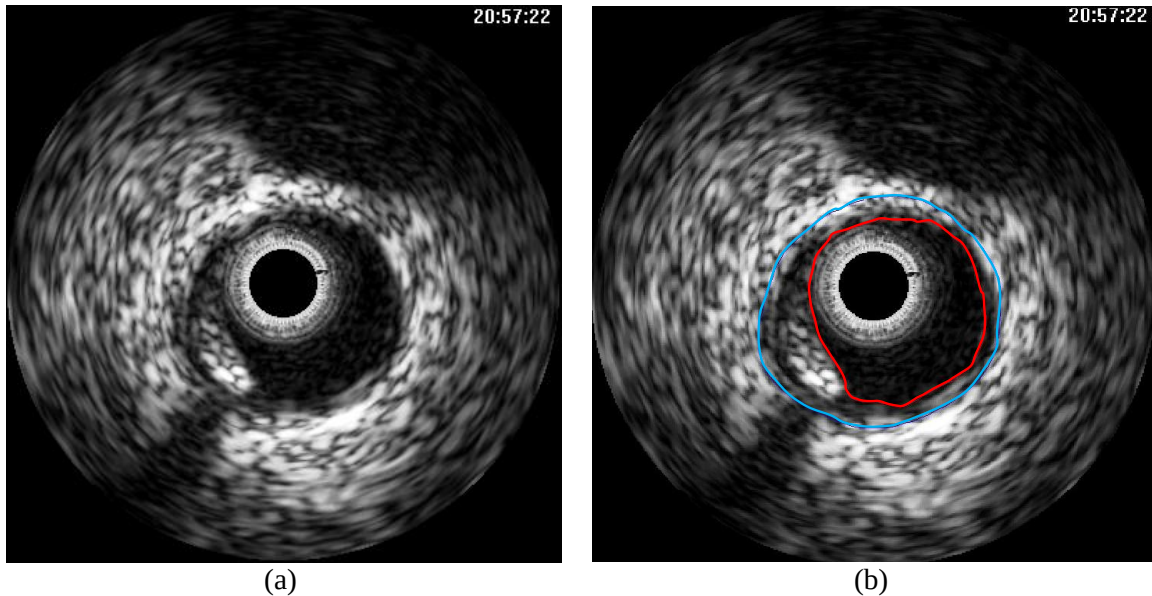


Figure 3.1: A typical IVUS image: (a) initial image, (b) annotated image with the lumen and media-adventitia borders marked with red and blue, respectively.

Based on tissue echoreflectivity three different plaque types can be identified in grayscale IVUS frames: the Soft Plaque (SP), the Hard Non-Calcified Plaque (HNCP) and the Hard Calcified Plaque (HCP) [48, 110]. SPs consist mainly of soft lipid tissue with loose connectivity; they have low echoreflectivity, are texturally homogeneous and are often associated with unstable lesions. HNCPs consist of fibrous tissue, have medium echoreflectivity which is similar to the adventitia and are texturally heterogeneous. HCPs, finally, have high echoreflectivity and obstruct the visualization of the underlying vessel wall, resulting in an acoustic shadow [48]. Traditionally the characterization of the type of the plaque was performed manually. However, this process is time-consuming and limited by high inter- and intra-observer variability [111]. In order to overcome these pitfalls several plaque characterization [67, 68, 70, 77, 112-115] and plaque segmentation [116] methodologies have been introduced.

The most prominent methodologies rely on the radio frequency (RF) analysis of the backscattered IVUS signal. In contrast to the grayscale IVUS images that are constructed based on the amplitude of the backscatter signal these data entail also information about the signal which also depends on the imaged tissue. Several methodologies have been proposed to process these data and numerous histology based studies have demonstrated that these techniques permit reliable identification of different plaque components [77, 112-114, 117]. IVUS Virtual Histology (VH-IVUS) [77] is a new technique which provides automated plaque characterization in IVUS frames, using the ultrasound backscattered RF-signals. VH-IVUS can classify the plaque to four different types: Dense Calcium, Necrotic Core, Fibrotic Tissue and Fibro-Fatty Tissue [113]. Dense Calcium (DC) (hard calcified plaque with high echoreflectivity in grayscale IVUS images) is composed by compact calcium crystals. Necrotic Core (NC) (soft plaque with low echoreflectivity in grayscale IVUS images) consists of high levels of lipids with many necrotic cells. Fibrotic Tissue (FT) (hard non calcified plaque with medium echoreflectivity) is composed by bundles of collagen fibres [66]. Finally, Fibro-Fatty Tissue (FFT) (mixed plaque with also medium echoreflectivity) consists of loosely collagen fibres with lipid accumulations. Additionally, VH-IVUS can also detect the media (M) (normal tissue and media) similarly to histological cross sections. However, its computation can only be performed once per cardiac cycle (ECG-gated technique) which

significantly decreases the number of characterized IVUS frames. Also atherosclerotic plaques in images been acquired by machines which are not equipped with the VH software cannot be characterized. Taki et al. [71] proposed a method that addresses the previous described limitations of VH. Although the method addresses most of the VH limitations, some deficiencies should be mentioned: (i) the plaque is classified only into three plaque types instead of four (VH), (ii) the overall classification accuracy is low (75%) when compared to VH, (iii) the method cannot detect the plaque in the shadow regions of the region of interest (ROI) as VH technique does and (iv) the Media class been recognized by VH in the ROI cannot be detected [71].

Two different semi-automated methodologies for characterizing the atherosclerotic plaque using IVUS images are proposed. The first methodology is a hybrid approach that allows complete plaque characterization and combines the advantages of classification methods [67, 68] and image filtering techniques [70, 115]. The methodology is a 4 step approach for the detection of different plaque components in grayscale IVUS images. The first step includes the identification of the plaque region (ROI) and the enhancement of the plaque signal. Then, a set of features is extracted for each pixel of the ROI. Based on these features, the pixels are classified into three types: SPs, HNCPs and HCPs. Finally, in the fourth step post processing is performed by using active contours to enhance the classification accuracy of the HCPs. Several classification algorithms are tested: Support Vector Machines (SVM) [73], Artificial Neural Networks (ANN) [118] and Random Forests (RF) [119]. To validate the performance of the proposed methodology we compared its estimations with the annotations of two experts in 40 IVUS frames. The use of active contours and an automatic thresholding technique combined with the RF classifier provided an overall classification accuracy of 86.14%.

The second methodology characterizes the plaque formations of an IVUS grayscale frame into four classes. Briefly, the method detects the ROI and extracts a set of features for each pixel of the ROI in the grayscale IVUS frames. This set of features consists of texture based features such as co-occurrence matrices [120] and local binary patterns [121, 122] and of intensity based features [123]. In addition to the texture and intensity based features two geometrical features are extracted. The first one describes the distance of each pixel from the media-adventitia border and the second describes the relative position of the

pixel from the media-adventitia and the lumen border. Then using the Random Forests classifier [119], the atherosclerotic plaque is classified into four plaque types, DC, NC, FT and FFT and into the Media class. The proposed method does not require the ECG – gated acquisition and therefore all IVUS frames can be characterized. Furthermore, as our method utilizes only grayscale data it can be used in IVUS data been acquired when VH-IVUS software is not available.

3.2 First approach

3.2.1 Proposed method

The proposed approach consists of the following stages.

3.2.1.1 Plaque Segmentation

The first stage of the proposed method is related to the detection of the region where the atherosclerotic plaques are developed and to image enhancement.

a. Detection of the Region of Interest

As Region of Interest (ROI) we denote the region between the lumen border and the media adventitia border, where the atherosclerotic plaques are developed. To be able to detect the ROI, we use the method proposed by Plissiti *et al.* [60]. This method is based on deformable models [124]. Furthermore the approach entails an initial contour of the lumen border and the media adventitia border to be provided at the first frame of the IVUS series. In this way the borders are exported.

b. Image enhancement

To achieve this, we apply contrast enhancement [123] to the ROI of each IVUS image, consisting of three steps:

- compute the histogram H of the IVUS image,
- saturate the 1% of the lower and higher values of H ,
- map the remaining values to the $[0, 2^8]$ range.

3.2.1.2 Feature extraction

In the second stage of the proposed method, a set of features (texture and intensity based features) is calculated for each pixel of the ROI.

a. Texture based features

1) Co-occurrence Matrix

The Gray Level Co-occurrence Matrix (GLCM) [120] is a matrix that is defined over an image I as the distribution of co-occurrence values at a given (D_x, D_y) offset. The dimensions of the co-occurrence matrix are $L \times L$ where L is the number of gray-levels of the image I .

If $I(i, j): 1 \leq i \leq k, 1 \leq j \leq l$, is the initial image, with dimensions $k \times l$, then the co-occurrence matrix $C_{(D_x, D_y)}(i, j)$ is defined as:

$$C_{(D_x, D_y)}(i, j) = \sum_{n=1}^k \sum_{m=1}^l \begin{cases} 1, & \text{if } I(n, m) = i \text{ and } I(n + D_x, m + D_y) = j \\ 0, & \text{otherwise} \end{cases} \quad (3.1)$$

where (D_x, D_y) are defined as:

$$D_x = D \cdot \cos(\theta), \quad D_y = D \cdot \sin(\theta). \quad (3.2)$$

with $\theta = [0^\circ, 45^\circ, 90^\circ, 135^\circ]$ and D is the Euclidean distance of the pixels.

From the co-occurrence matrix $C_{(D_x, D_y)}(i, j)$ for each angle $\theta = [0^\circ, 45^\circ, 90^\circ, 135^\circ]$ and for each pixel of the ROI, we compute the following measures:

Contrast:

$$f_{contrast}^\theta = \sum_{i=1}^L \sum_{j=1}^L (i - j) C_{(D_x, D_y)}(i, j). \quad (3.3)$$

Correlation:

$$f_{correlation}^\theta = \frac{\sum_{i=1}^L \sum_{j=1}^L ((i \cdot j) C_{(D_x, D_y)}(i, j)) - \mu_i \cdot \mu_j}{\sigma_i \cdot \sigma_j}. \quad (3.4)$$

where: $\mu_i = \sum_{j=1}^L i C_{(D_x, D_y)}(i, j)$ and $\sigma_i^2 = \sum_{j=1}^L i^2 C_{(D_x, D_y)}(i, j) - \mu_i^2$.

Energy:

$$f_{energy}^\theta = \sum_{i=1}^L \sum_{j=1}^L C_{(D_x, D_y)}(i, j)^2. \quad (3.5)$$

Homogeneity:

$$f_{homogeneity}^\theta = \sum_{i=1}^L \sum_{j=1}^L \frac{C_{(D_x, D_y)}(i, j)}{1 + |i - j|}. \quad (3.6)$$

Thus, we computed 16 features in total from the co-occurrence matrix (four features for each angle $\theta = [0^\circ, 45^\circ, 90^\circ, 135^\circ]$).

2) Local Binary Patterns

The Local Binary Patterns (LBP) [121] can determine uniform texture patterns into circular neighborhoods. LBPs are based on a circular symmetric neighborhood of P members of a circle with radius R .

Among the various LBP we used the uniform rotation $LBP_{P,R}^{riu2}$ [125], defined as:

$$LBP_{P,R}^{riu2} = \begin{cases} \sum_{p=0}^{P-1} s(g_p - g_c), & \text{if } U(LBP_{P,R}) \leq 2 \\ P+1, & \text{otherwise} \end{cases}, \quad (3.7)$$

where g_c is the gray value of the center pixel and g_p are the gray values of the circularly

symetric neighborhood, $U(LBP_{P,R}) = |s(g_{P-1} - g_c) - s(g_0 - g_c)| + \sum_{p=1}^{P-1} |s(g_p - g_c) - s(g_{p-1} - g_c)|$

and the function $s(x)$, is defined as: $s(x) = \begin{cases} 1, & x \geq 0 \\ 0, & x < 0 \end{cases}$. $LBP_{P,R}^{riu2}$ has a fixed set of discrete

output values from $0, 1, 2, \dots, P+1$.

b. Intensity based features

1) Entropy

Entropy [123] is a statistical measure of randomness which can be used to characterize the texture of the image. The entropy H_I of the image I quantized to L levels, is defined as:

$$H_I = \sum_{i=0}^{L-1} p_i \log_2 \left(\frac{1}{p_i} \right), \quad (3.8)$$

where: $p_i = \frac{\# \text{ pixels with gray level } i}{\text{total \# of pixels}}, 0 \leq i \leq L-1$.

2) Mean Value

The mean value [123] f_{mean} of a pixel (o, q) is defined, over a neighborhood N of the pixel (i, j) , as:

$$f_{mean} = \frac{1}{M} \sum_{(i,j) \in N} I(i, j), \quad (3.9)$$

where M is the number of pixels in the N neighborhood and (i, j) are the positions of pixels of the N neighborhood.

3.2.1.3 Plaque Characterization

In order to classify each pixel of the ROI, we implement the following procedure:

- a) Plaque and normal tissue classification: Each pixel of the ROI is classified in two classes. The first class contains the plaque: SP, HNCP and HCP. The second class contains the Normal Tissue (NT),
- b) Soft and hard plaque classification: The pixels which are classified in the first class (SP, HNCP, HCP) are further classified to SP and Hard Plaque (HP), (where HP contains the HNCP and the HCP),
- c) Hard calcified and hard non calcified plaque discrimination: Finally the pixels that are classified in the HP class are divided to HNCP and HCP.

The above procedure is shown schematically in Figure 3.2. To classify plaque-normal tissue and soft-hard plaque, three different classification algorithms were used: Support Vector Machines (SVM) [73], Artificial Neural Networks (ANN) [118] and Random Forests (RF) [119]. To find the best architecture for the ANN classifier we tested the ANN

using one and two hidden layer architectures [126]. For the one hidden layer architecture we use 14, 16, 18, 20, 22, 24, 26, 28 Neurons and for the two hidden layer we use 8-6, 10-8, 14-12, 16-18, 18-20 for the first and second layer, respectively. To find the best parameters for the RF classifier, we used different number of trees (80, 90, 100, 110, 120, 130, 140 trees) and features (8, 10, 12, 14, 16, 18, 20, 22 features). The RBF kernel was employed in the SVM classifier and to find the best parameters (C, γ) we used GridSearch technique. C, γ are the penalty and kernel parameters, respectively [73].

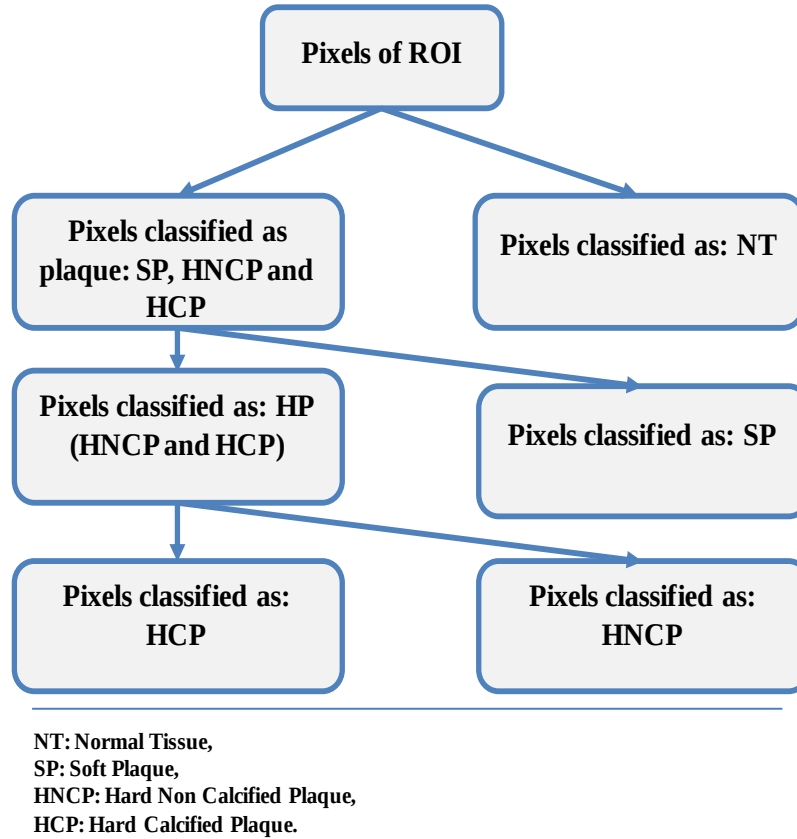


Figure 3.2: Flowchart of the procedure followed to classify each pixel of the ROI to: Normal Tissue, Soft Plaque, Hard Calcified Plaque and Hard Non Calcified Plaque.

a. Plaque and normal tissue classification

The ROI consists of various types of tissue (SP, HNCP, HCP and NT). These types of tissue represent two different classes: the atherosclerotic plaque (SP, HNCP and HCP) and the normal tissue (NT). A classifier (SVM, ANN or RF classifier) was used to classify the ROI to plaque and NT.

b. Soft and Hard plaque classification

The pixels detected as plaque in the previous step are classified into: HP and SP, where HP contains both HNCP and HCP plaques. A very distinctive attribute of the HP class, in IVUS images, is that they appear as very bright sections. Thus, several research studies [70, 115] have used image processing techniques to detect the hard plaque formations. In our method, we use both image processing techniques and classification algorithms [73, 118, 119], to discriminate HP and SP. The procedure consists of the following steps:

- 1) Use a classifier (SVM, ANN or RF classifier) in order to classify the plaque to HP and SP,
- 2) Perform automatic thresholding [127] to the image to detect HP plaque,
- 3) Fuse the results for the HP plaques from step 1 and 2, to exclude possible errors and inconsistencies.

The steps of the automatic thresholding technique (step 2 above), applied to an IVUS frame $I_0(i, j)$, are described below:

- 1) Calculate a threshold th for the image $I_0(i, j)$ using the Otsu's method [128],
- 2) Map the threshold th to the $[0, 2^8]$ range,
- 3) Set the pixels of the image $I_0(i, j)$ that are smaller than th to zero value,
- 4) Go to step 1.

The above described automatic thresholding technique was repeated T_{rep} times. The non – zero pixels of the T_{rep} image that are inside the ROI of the initial image are the Hard Plaques detected by the automatic thresholding technique ($HP_{threshold}$). The above described procedure is shown in Figure 3.3.

Automatic thresholding techniques are considered to be more robust than the data-driven techniques (based on texture characteristics) in detecting HPs [70, 115]. However, both data-driven and thresholding techniques can produce wrong estimations when classifying HP. Thus, to ensure the detection of HPs we have also implemented an automated thresholding.

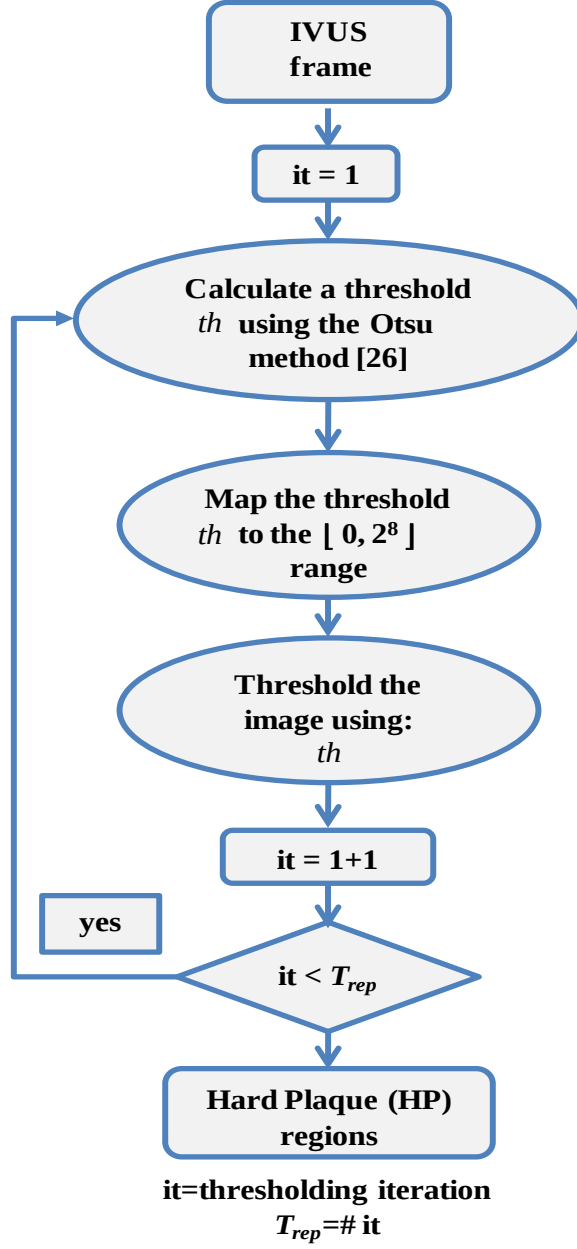


Figure 3.3: Schematic representation of the automatic thresholding technique used for detecting the Hard Plaque (HP) formations

Then the HP areas estimated by the automatic thresholding technique and the classification algorithm are superimposed and a final decision is taken for the HP which is based on the percentage of the overlapping area:

$$HP = \begin{cases} HP_{classification}, & O(HP_{classification}, HP_{threshold}) > 80\% \\ HP_{threshold}, & otherwise \end{cases}, \quad (3.10)$$

where $HP_{classification}$ is the HP detected by the classification algorithm, $HP_{threshold}$ is the HP detected by automatic thresholding technique and $O(HP_{classification}, HP_{threshold})$ is the percentage of their overlapping area.

c. Hard Calcified and Hard non Calcified plaque discrimination

The pixels classified as HP in the previous step are further classified to HNCP and HCP plaques. In an IVUS examination, the HP formations appear as bright regions. As mentioned in previous research studies [70, 115], a HCP has the physical property to block the transmission of the ultrasound signal beyond that point resulting in a shadow behind it which follows the ultrasound signal. This feature is taken into consideration in the process, implemented to discriminate HCP from HNCP, which includes the following steps:

- 1) Transform the IVUS image $I(i, j)$, to polar coordinates $I_p(i_p, j_p)$,
- 2) Calculate each column below HP regions of the $I_p(i_p, j_p)$ image, the median gray level (M_{j_p}) of the column,
- 3) Calculate a threshold T_{HP} for each HP region as:

$$T_{HP} = \frac{1}{l-k} \sum_{j_p=k}^l (M_{j_p}), \quad (3.11)$$

where k is the first column of the HP region and l is the last column of the HP region.

- 4) Compare the threshold values T_{HP} of the HP regions with a threshold T . If $T_{HP} < T$ the HP region corresponds to HCP formation, otherwise it corresponds to a HNCP formation.

3.2.1.4 Post Processing

In the final stage of the proposed method, we perform post-processing in the regions detected as HCP, utilizing active contours [124]. An active contour (snake) ($e(t) = [i(t), j(t)]$, $t \in [0, 1]$) is a curve which has the ability to deform according to specific

constrains. We minimize the energy function E_{active} , in order to obtain a final desirable shape. The energy function is defined as:

$$E_{active} = \int_0^1 (E_{internal}(e(t)) + E_{external}(e(t)))dt, \quad (3.12)$$

where: $E_{internal} = k(s) \left| \frac{\partial e(t)}{\partial t} \right|^2 + \lambda(s) \left| \frac{\partial^2 e(t)}{\partial t^2} \right|^2$ and $k(s)$, $\lambda(s)$ are regulation parameters

that control the snake's tension and rigidity. For the external force $E_{external}$, we use the Gradient Vector Flow method [129]. In each plaque region detected as HCP plaque we apply active contours and the procedure includes three steps:

- Set as initial estimation the contour of each HCP plaque,
- Calculate the active contour's deformation,
- Set as the final HCP region the final deformed active contour.

3.2.2 Dataset

The proposed method is trained and validated in IVUS images obtained from eight patients who underwent IVUS examination for clinical purposes. All participants provided informed consent, while the study was approved by the institution's Ethical Committee. IVUS interrogation was performed using a Volcano Therapeutics, Rancho Cordova, CA system. The catheter used for images acquisition was an Eagle Eye Gold, Volcano Therapeutics catheter with 2.9-F diameter and 20-MHz frequency. The dimensions of the acquired IVUS frame were 384×384 pixels. From each IVUS examination, 15 frames, that contained all the plaque types, were selected and processed by two experts (IFC-CNR institute, Pisa, Italy), who identified the composition of the plaque in each ROI. Any disagreements in experts' annotations were resolved by consensus.

This process resulted to a set of 120 annotated frames. From this set, 66% ($SET_{train}=80$ frames) was used to train and the rest 33% ($SET_{test}=40$ frames) to validate the method. From the training set (SET_{train}) two different balanced training subsets were created ($A-SET_{train}$ and $B-SET_{train}$), of which the first ($A-SET_{train}$) was used to train the

methodology that differentiates the plaque from the NT and the second ($B-SET_{train}$) to train the algorithm that classifies the plaque to SP and HP (HNCP and HCP). In order to create the $A-SET_{train}$ training set, 340 pixels were selected from each frame of the SET_{train} : 50% (170 pixels) of these pixels belonged to NT and the rest (170 pixels) to one of the three plaque types (SP, HNCP and HCP). This process resulted in a balanced training set of 27,200 pixels ($A-SET_{train}$). In order to create the $B-SET_{train}$ training set, 30 SP plaque regions and 30 HP plaque regions (15 HCP plaque regions and 15 HNCP plaque regions) were randomly selected. For each plaque region 340 randomly pixels were selected, from which 50% (170 pixels) of them belong to SP and 50% (170 pixels) to HP. The result of this procedure was a balanced training set of 20,400 pixels ($B-SET_{train}$). The above described procedure is shown in Figure 3.4.

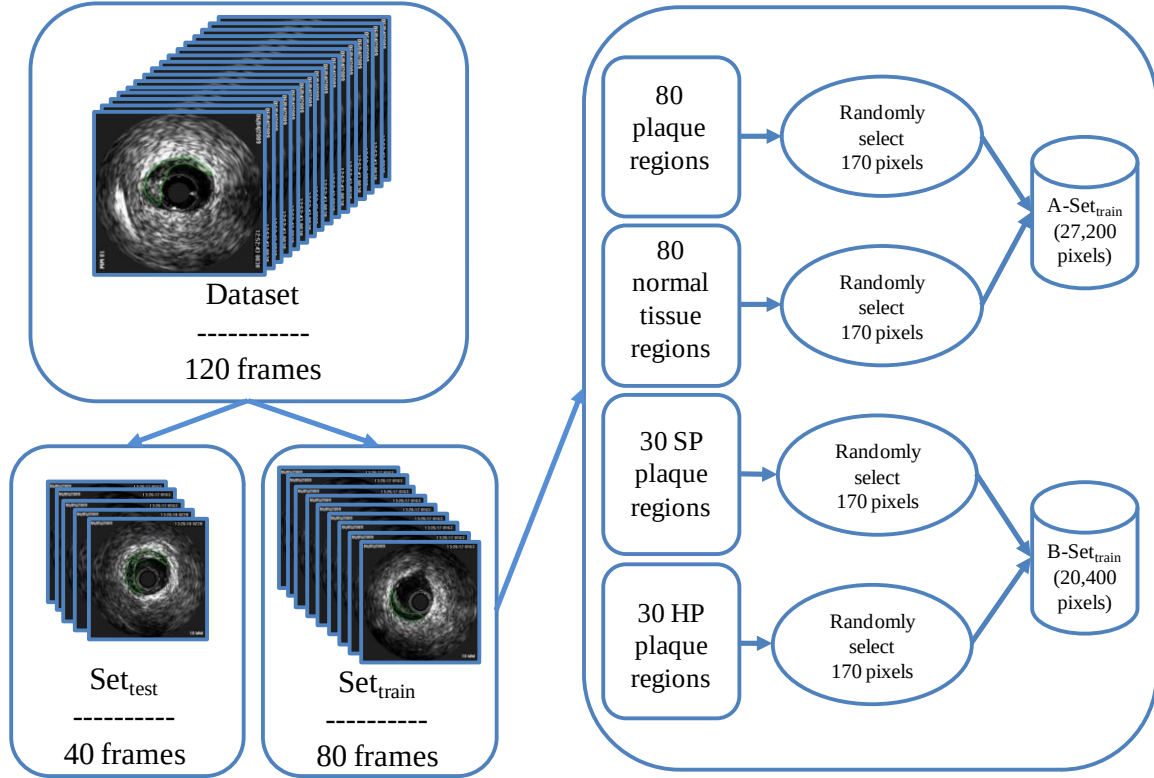


Figure 3.4: Procedure followed for creating the balanced training sets and the test set.

3.2.3 Results

3.2.3.1 Validation measures

In order to measure the performance of our approach we computed the overall accuracy of the method. The overall accuracy is defined as:

$$Ac_{overall} = \frac{\# \text{ pixels correctly classified}}{\text{total \# pixels of the ROI}} \times 100\%, \quad (3.13)$$

Additionally to the overall accuracy, three qualitative evaluation measures are used:

- The area of the detected plaque components as this was estimated by our algorithm and by the experts,
- The overlapping and non overlapping areas between the detections of the algorithm and the annotations of the experts

A schematic presentation of the implemented overlapping area analysis is shown in Figure 3.5.

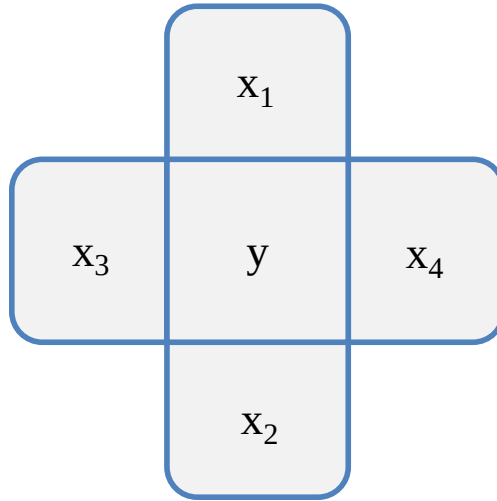


Figure 3.5: Schematic presentation of overlapping and non overlapping areas.

If the estimation of our approach for a specific plaque type defines an area $A_{algorithm} = x_1 + y + x_2$ and the annotations of the experts for the same plaque type an area $A_{expert} = x_3 + y + x_4$, then the overlapping and the non-overlapping areas are:

$$R_{overlapping} = \frac{y}{x_3 + y + x_4}, \quad (3.14)$$

and:

$$R_{non-overlapping} = \frac{x_1 + x_2 + x_3 + x_4}{x_3 + y + x_4}, \quad (3.15)$$

respectively.

3.2.3.2 Feature extraction

Features parameters selection

To select the best feature parameters we computed GLCM and LBP using various parameters. We extracted GLCM using distances: $D=1$, $D=2$, $D=3$, $D=4$, $D=5$ and LBP using the following P/R pairs: $P=4/R=1$, $P=8/R=1$, $P=12/R=1.5$, $P=16/R=2$. The overall accuracy of the method (using only the GLCM or LBP) was the criterion for the selection of the best parameters for the GLCM and LBP. When using only the GLCM features the best overall accuracy (79.55%) was achieved using $D=2$. When using only the LBP features the best overall accuracy (80.68%) was achieved using $P=8/R=1$, therefore we computed 10 features ($0, 1, 2, \dots, P+1$), $f_{lbp}^0, f_{lbp}^1, \dots, f_{lbp}^9$. These 10 LBP features, along with 16 derived from GLCM and the 2 intensity features result to a 28 features set. This feature set was used as input to each classification scheme (ANN, SVM, RF) to classify between plaque and normal tissue and between hard and soft plaque.

Impact of different feature sets on classification accuracy

To examine how different feature sets affect the classification schemes' performance we studied the overall classification accuracy of the method, using the first training set ($A-SET_{train}$) of the dataset and the RF classifier for six different feature sets (FS): GLCM features ($FS1$), GLCM + Intensity features ($FS2$), GLCM + LBP ($FS3$), LBP features ($FS4$), LBP + Intensity features ($FS5$), Intensity features ($FS56$). The overall classification accuracy using the $FS1$, $FS2$, $FS3$, $FS4$, $FS5$ and $FS56$ feature set is 79.55%, 82.72%, 84.71%, 80.68%, 83.08% and 69.42%, respectively. When the GLCM, LBP and Intensity features are used, the overall classification accuracy is 86.17%.

3.2.3.3 Plaque and normal tissue classification

The 10-fold cross validation method was implemented to validate the three different classifiers: SVM [73], ANN [118] and RF [119] over the ($A-SET_{train}$) training set. Random Forests [119] (130 trees and 14 features) reported an accuracy for plaque and NT classification of 89.86% (Table 3.1) while the classification accuracy of the ANN [118] (1 hidden layer with 16 Neurons) and SVM [73] classifiers (RBF kernel with $C=17$ and $\gamma=1$) were 76.56% and 73.42%, respectively. RF was chosen for plaque and normal tissue classification as it outperformed both ANN and SVM (Table 3.1). In order to test the plaque and normal tissue classification step, the SET_{test} (40 frames) was used. In Figure 3.6 (a) the comparison between experts' annotations and the detected plaque area by the plaque and normal tissue step of the method is shown. The overlapping and non-overlapping areas for the plaque regions are shown in Figure 3.6 (b). Furthermore, in the SET_{test} there were 21 regions annotated as HNCP formations, 12 regions annotated as HCP formations and 15 regions annotated as SP formations.

Table 3.1: Accuracy of different classifiers used for classifying Plaque versus Normal Tissue and Soft versus Hard plaque.

Classification Accuracy			
	Support Vector Machines	Artificial Neural Networks	Random Forests
Plaque and Normal Tissue classification	73.42%	76.56%	89.86%
Soft and Hard Plaque classification	89.84%	92.07%	96.34%

3.2.3.4 Soft and Hard plaque classification

Classification

Following the same approach, and applying the 10-fold cross validation method, three different classifiers: SVM [73], ANN [118] and Random Forests [119] were tested using the second training set ($B-SET_{train}$) to classify SP and HP (HNCP and HCP) formations.

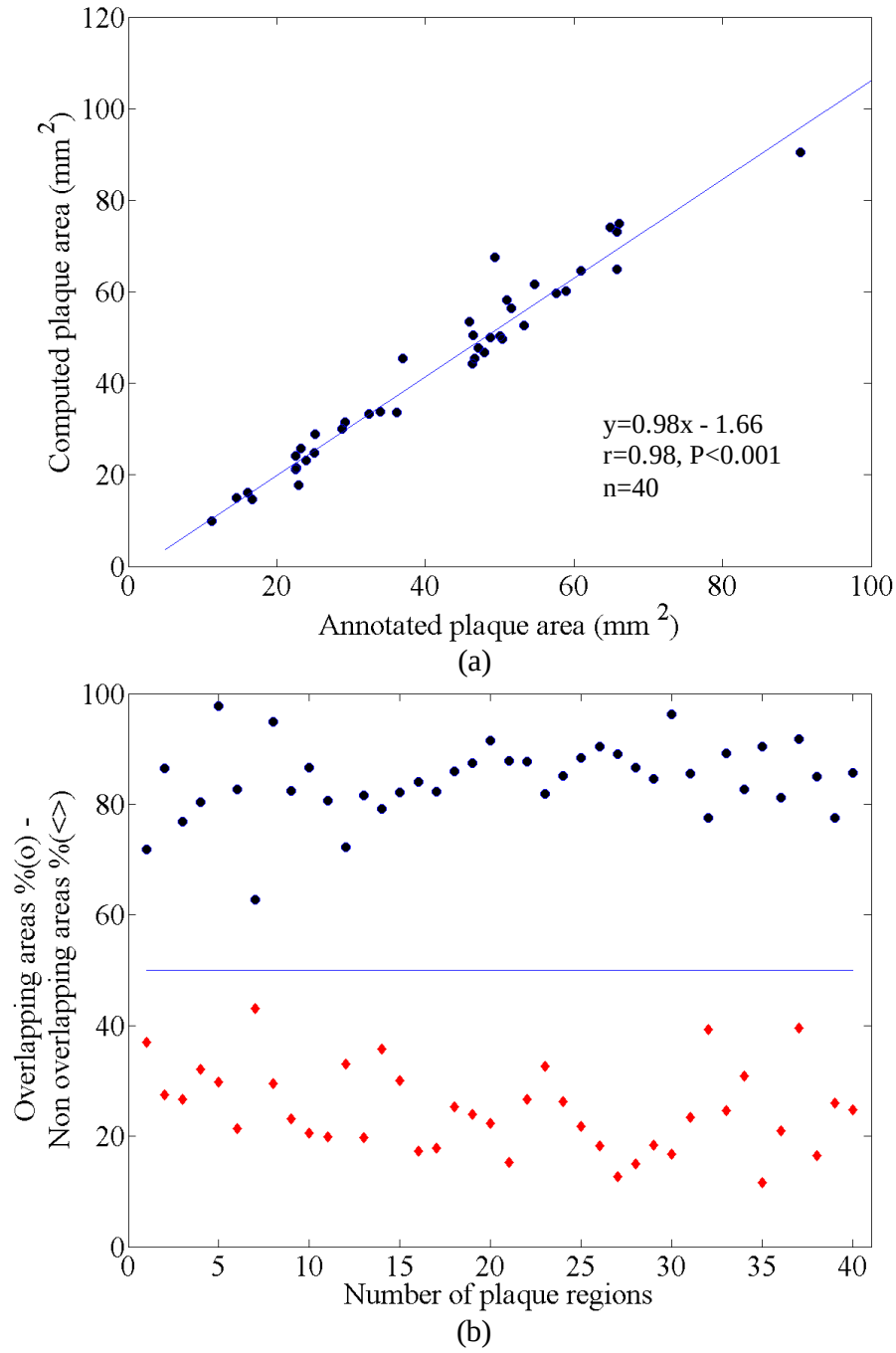


Figure 3.6: (a) Comparison of the plaque area identified by the experts with the one detected by the algorithm. (b) Overlapping and non-overlapping area ratio for plaque regions.

RF classifier (120 trees and 14 features) provided an overall accuracy of 96.34% while the accuracy of the NN (1 hidden layer with 18 Neurons) and SVM (RBF kernel with $C = 17$ and $\gamma = 1$) was 92.07% and 89.84%, respectively. Thus, this classifier was

implemented in the SET_{test} and its estimations were compared to the experts annotations. Figure 3.7 (a) illustrates the linear regression analysis for the SP area between the experts and the proposed method, while Figure 3.7 (b) the overlapping and non-overlapping area analysis for the SP.

Automatic Thresholding

To estimate the optimal number of repetitions (T_{rep}) of the automatic thresholding technique, we calculated the overlapping and non-overlapping areas after applying the automatic thresholding (using Otsu thresholding) method to 12 HP plaques 1, 2, 3 and 4 times. When applying the method once, the mean overlapping area was 93% while the mean non-overlapping areas was 55%, indicating that method overestimates the HCP. When applying the method twice the mean overlapping area was 88% while the mean non-overlapping area remained high (32%). Application of our method for third time yielded a large mean overlapping area and a non-overlapping area 81% and 12%, respectively. Finally, after four repetitions there was a significant reduction of both the mean non-overlapping (3.5%) and overlapping area (68%). Therefore, $T_{rep} = 3$ was selected as the optimal number of iterations for the automatic thresholding technique. We also tested two more thresholding methods: the minimum error thresholding (MET) method [130] and the maximum cross entropy method [131]. When using the entropy-based method we observed no significant change in the overlapping and non overlapping area (80.5% mean overlapping areas and 13 % mean non-overlapping areas) while the MET method reduced the mean overlapping area to 72% and the non-overlapping area to 33 %. In view of these results it was decided to use Otsu's method.

The SET_{test} was also used to compare the HP estimations of the automatic thresholding technique ($HP_{threshold}$) to those derived by the RF classifier ($HP_{classification}$). 33 HP were identified (21 HCP and 12 HNCP) in this set. The obtained results are shown in Figure 3.8.

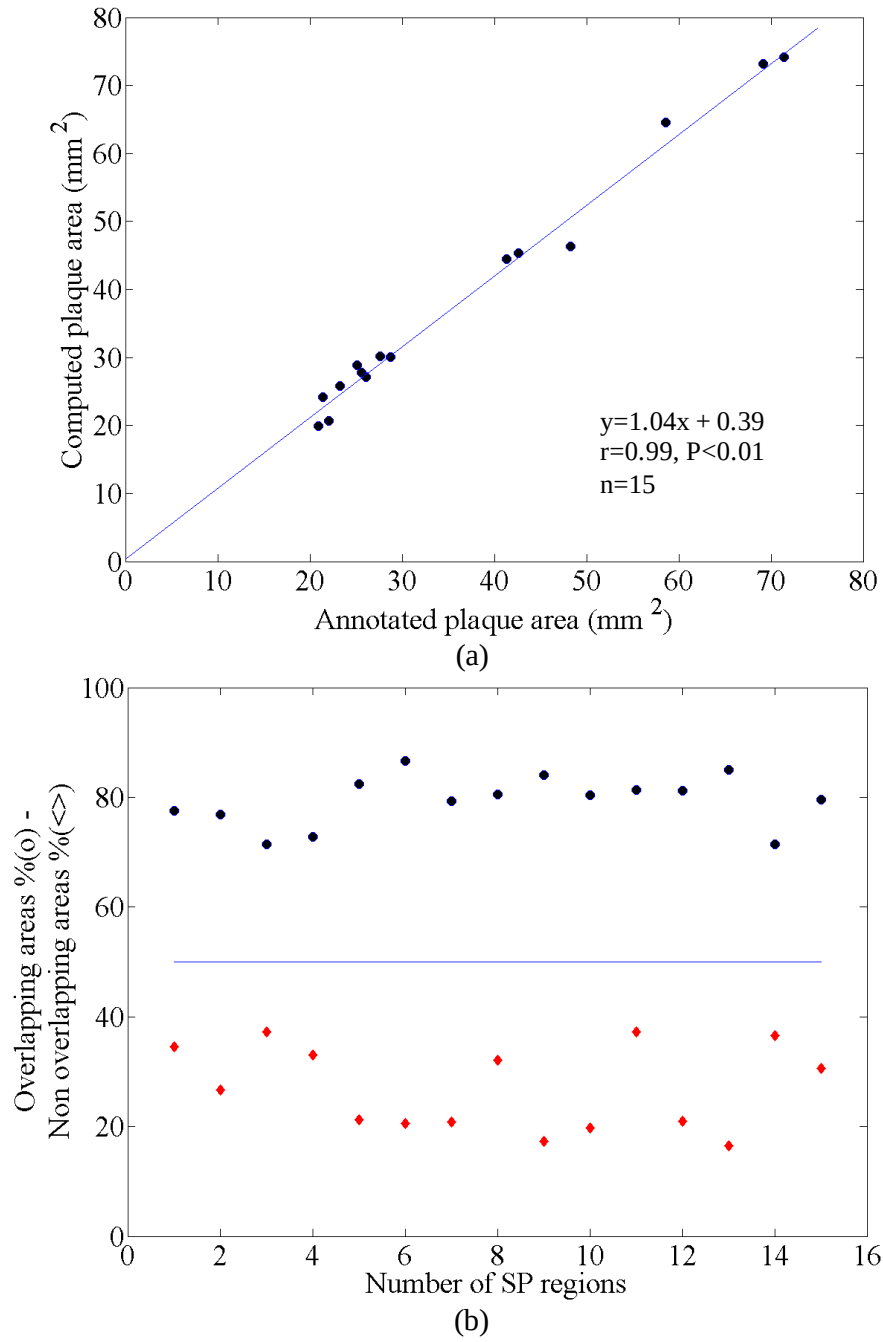


Figure 3.7: (a) Comparison of the soft plaque (SP) area identified by the experts with the one detected by the algorithm. (b) Overlapping and non-overlapping area ratio for SP regions.

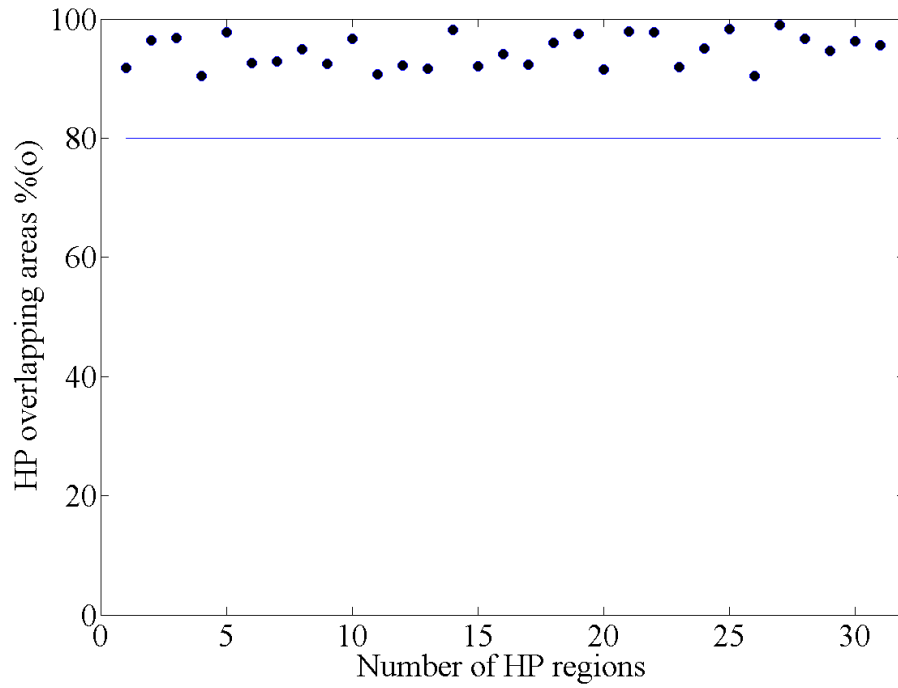


Figure 3.8: Percentages of overlapping areas between the areas detected by the automatic thresholding technique and the ones detected by the Random Forests classifier.

3.2.3.5 Hard Calcified and Hard non Calcified plaque discrimination

Computation of the median threshold value

The threshold value T was computed based on the 65 HP (18 HCP and 47 HNCP regions) identified by the two experts in the test set (SET_{train}). The frames which include these regions were transformed to Cartesian coordinates and then the threshold value of the HP (T_{HP}) was calculated for each frame. The obtained threshold varies from 40 - 50. ROC analysis was performed to find the best threshold. A threshold $T = 45$ was finally selected as it allowed identification of the HP with sensitivity 94% and specificity 93% (Figure 3.9).

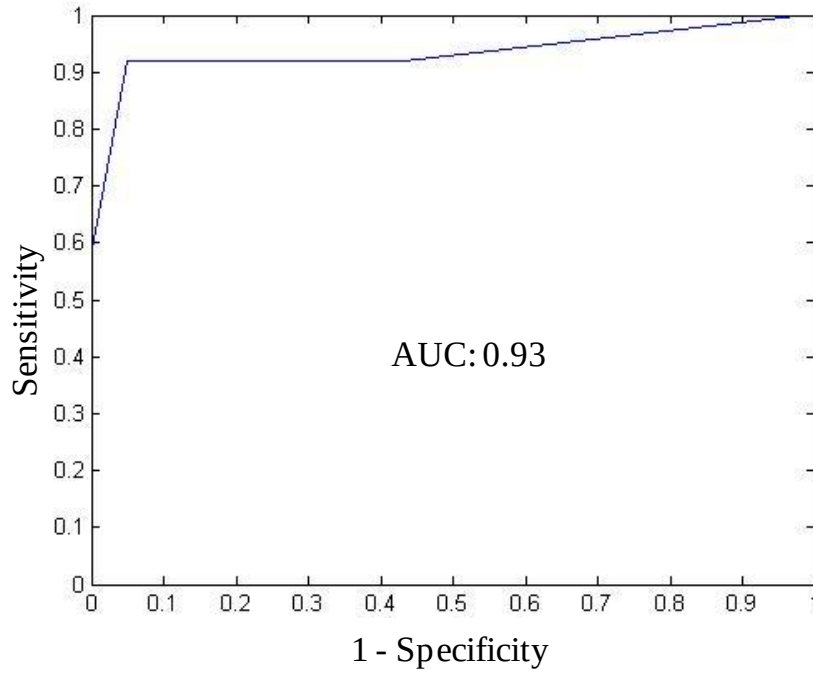


Figure 3.9: Receiver operating characteristic (ROC) analysis for different threshold values T for HCP and HNCP classification.

Classification

HCP and HNCP plaques were tested using the SET_{test} . The linear regression analysis and the overlapping and non-overlapping area analysis between the estimations of our approach and the experts for the HCP is shown in Figure 3.10 (a) and (b) respectively. Figure 3.11 (a) and (b) portrays the linear regression analysis and the overlapping and non-overlapping area analysis between the experts and our method for the HNCP.

The correlation between annotated HCP formations by the experts and the HCP formations detected by the proposed method is shown in Figure 3.10 (a). In Figure 3.10 (b) the overlapping and non-overlapping areas for the HCP regions are shown. The correlation between annotated HNCP formations by the experts and the HNCP formations detected by the proposed method and the overlapping and non-overlapping areas for the HNCP regions are shown in Figure 3.11 (a) and in Figure 3.11 (b).

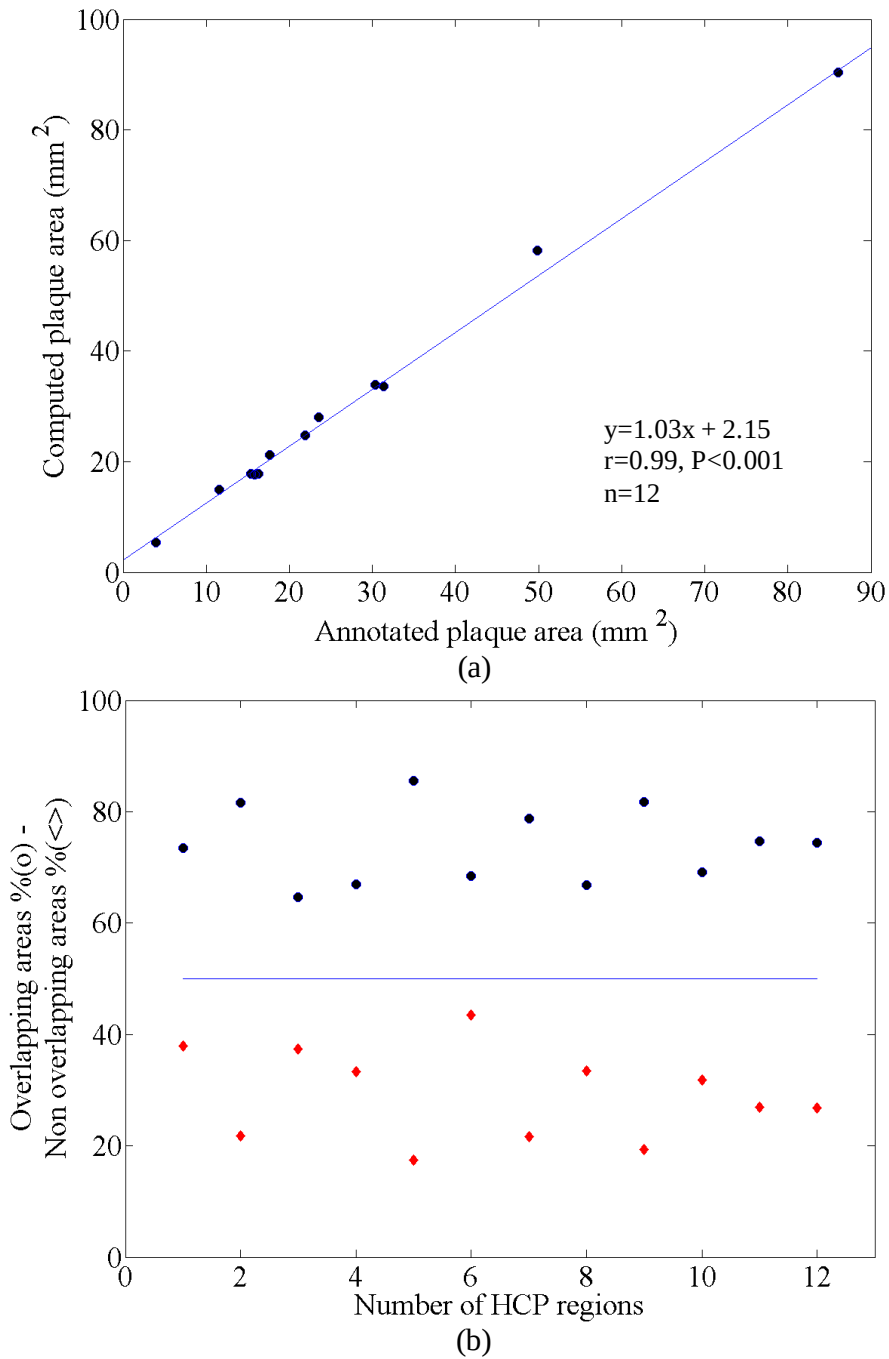


Figure 3.10: (a) Comparison of the hard calcified plaque (HCP) area identified by the experts with the one detected by the algorithm. (b) Overlapping and non-overlapping area ratio for HCP regions.

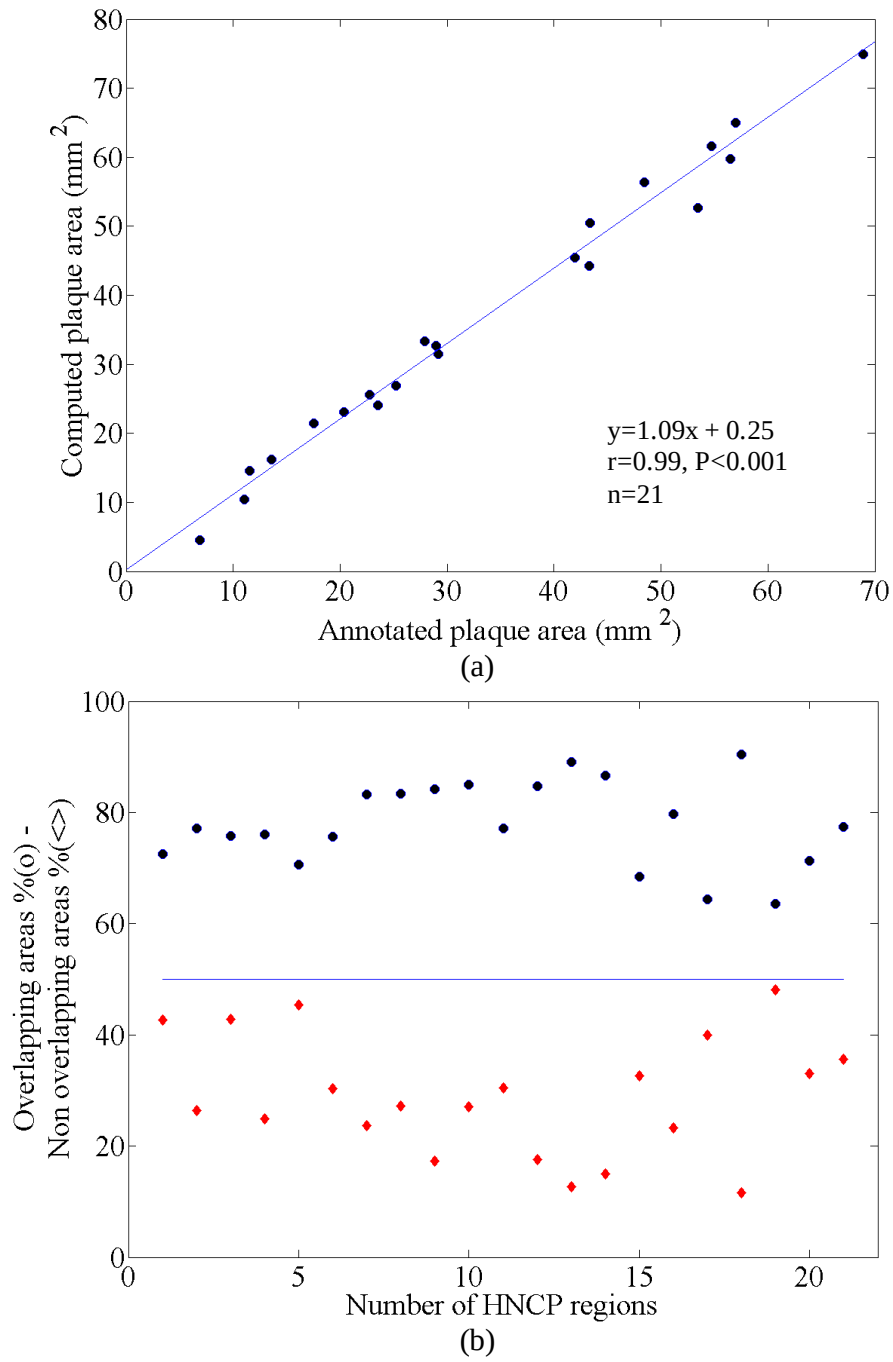


Figure 3.11: (a) Comparison of the hard non-calcified plaque (HNCN) area identified by the experts with the one detected by the algorithm. (b) Overlapping and non-overlapping area ratio for HNCN regions

3.2.3.6 Post Processing

A post-processing step is applied in the results for the HCP regions detected by the proposed method. To optimize the $k(s)$, $\lambda(s)$ regulation parameters that control the snake's tension and rigidity, we tested $k(s)$ in the range 0.05-0.09 and $\lambda(s)$ in the range 0-0.09. The snake deformation was repeated several times (1-40 iterations). Then for each combination of the parameters ($k(s)$, $\lambda(s)$) and the number of iterations we computed the average change of the overlapping area ratio and the average change of the non-overlapping area ratio. The combination of the parameters and the number of iterations which provided the highest and the lower average change in the overlapping and non-overlapping area ratio, respectively, were chosen as the regulation parameters of the snake. More specifically the $k(s)$ and $\lambda(s)$ parameters were set to 0.07 and 0.08, respectively, while the number of iterations for snake deformation was 25. The active contour deformation performed substantially for the HCP formations and increased the average overlapping area ratio by 8.9% between the experts' annotations and the proposed method's results. Figure 3.12 shows the overlapping and non-overlapping area ratio for HCP regions detected by our method before and after applying the post processing step. The post processing step increased the overall classification accuracy of the method by 2.03% (from 84.11% to 86.14%).

3.2.3.7 Application Examples

Figure 3.13 shows application examples of the proposed plaque characterization method in three IVUS frames. Each plaque type detected by our approach is shown in the IVUS images using a color coded map.

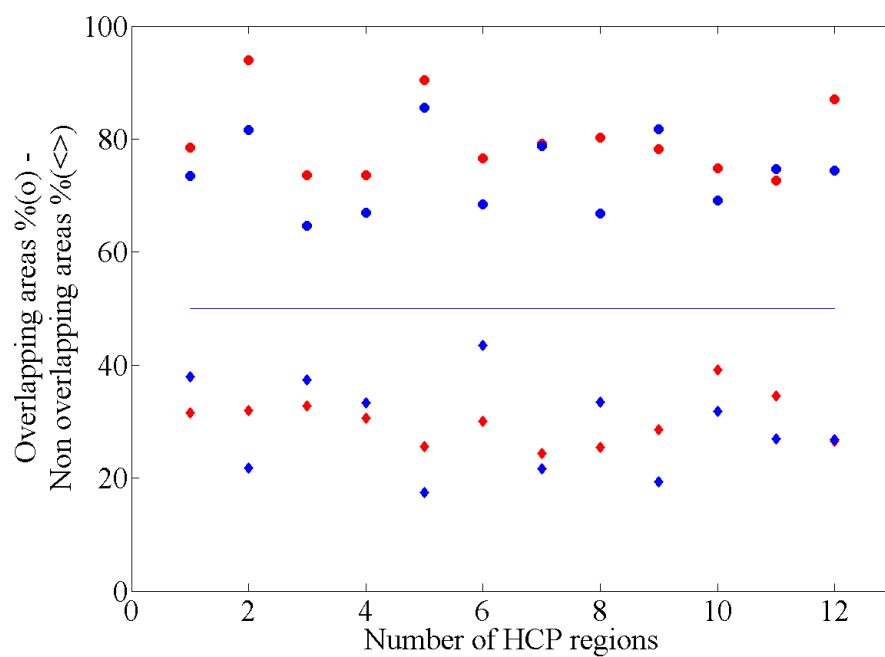


Figure 3.12: Overlapping and non-overlapping area ratio for hard calcified plaque (HCP) regions detected by the algorithm before (blue color points) and after (red color points) applying the post-processing step.

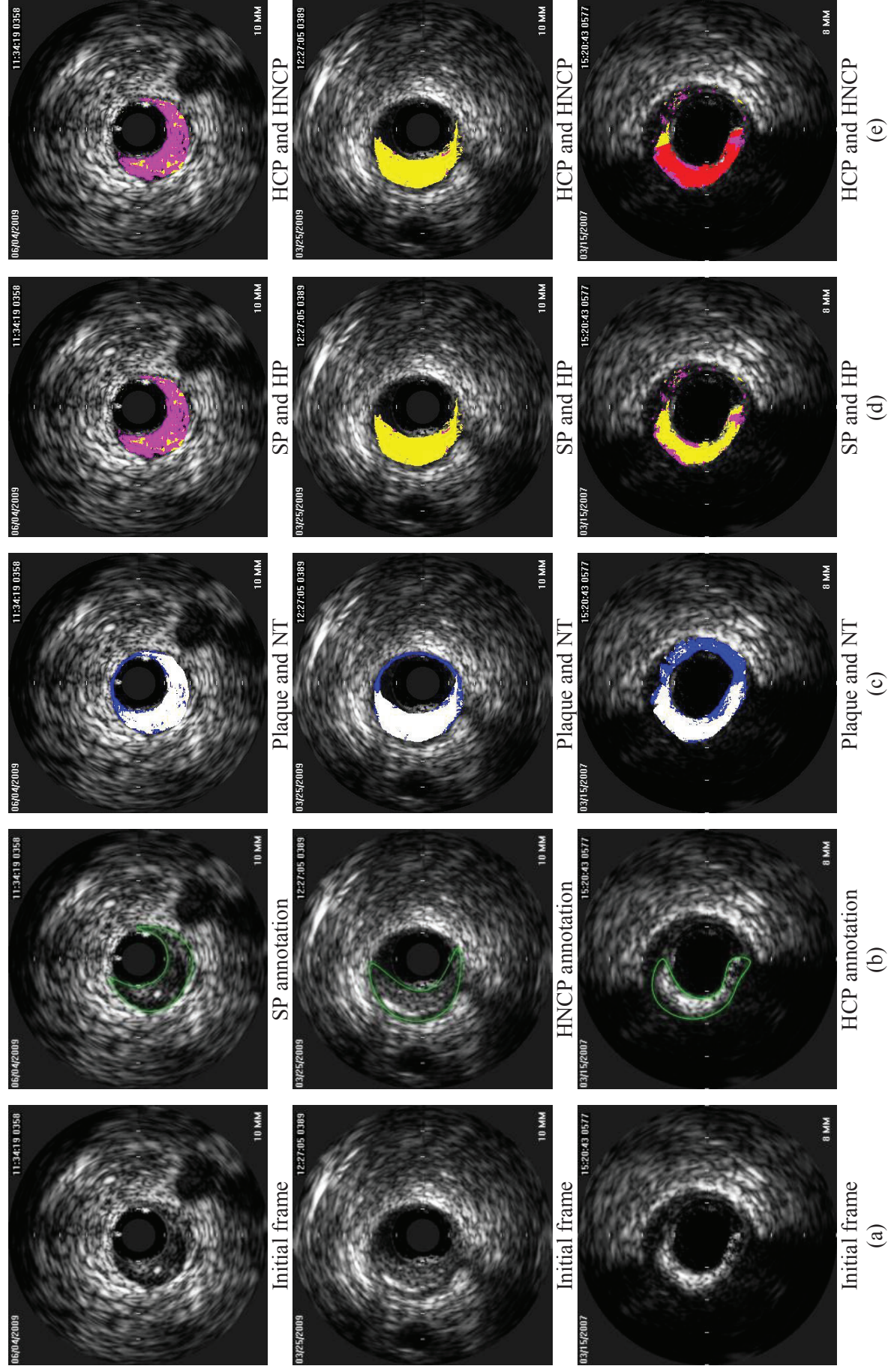


Figure 3.13: Application of the proposed method to three different IVUS frames. (a) The initial frames, (b) the experts' annotations over the initial frames, (c) the plaque (white color) and normal tissue (NT; blue color) classification results, (d) the soft plaque (SP; purple color) and hard plaque (HP; yellow color) classification results, and (e) the hard calcified plaque (HCP; red color) and hard non-calcified plaque (HNCP; yellow color) discrimination results.

3.3 Second approach

3.3.1 Proposed method

The proposed plaque characterization method consists of three steps as it is shown in Figure 3.14. In the first step the ROI is detected. In the second step, a set of features is extracted for all pixels in the ROI. In the third step the pixels of the ROI are classified into one of the four plaque types: DC, NC, FT, FFT.

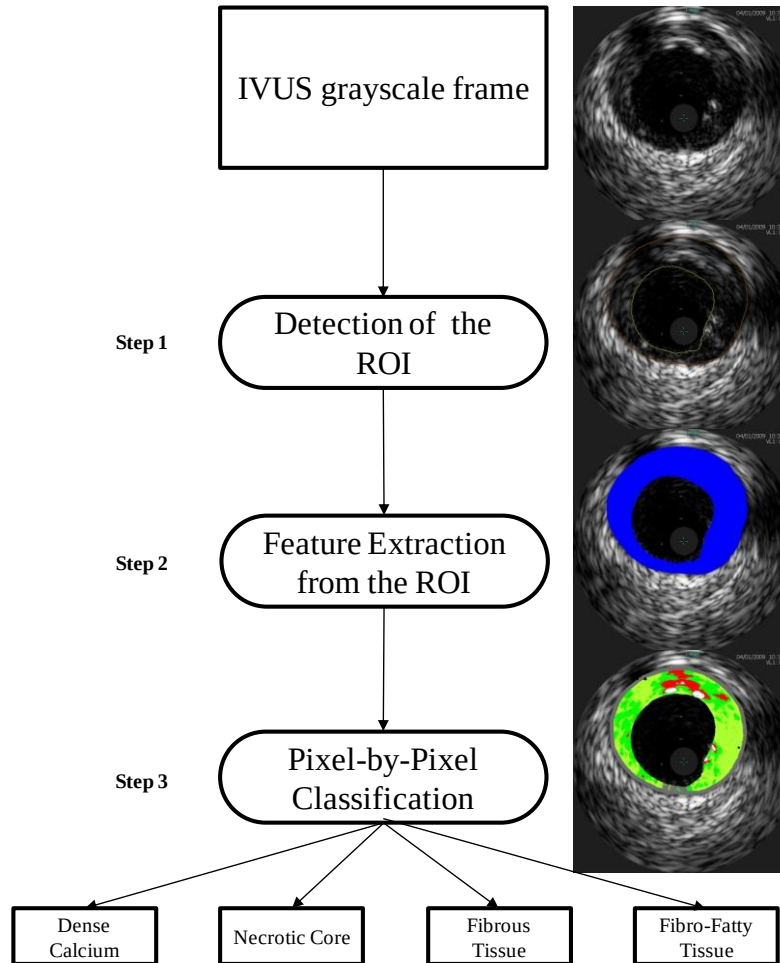


Figure 3.14: Flowchart of our four-class plaque characterization method.

3.3.1.1 Detection of the Region of Interest (ROI)

In order to initialize the automated plaque classification algorithm, the detection of the ROI is required. This is located in the area defined by the lumen and media adventitia borders that are extracted with the implementation of an automated border detection

method, presented by Plissiti *et al.* [60]. The method is based on the use of deformable models [124] and requires an expert observer to provide an initial estimation of these borders in the first frame of each IVUS sequence. Then, the lumen and media-adventitia borders are automatically extracted in the subsequent frames using as first approximation the borders detected in the previous frame. The minimization of the deformable model energy function is expedited with the use of a Hopfield Neural Network (NN) combined with an Simulated Annealing Schema. The method has been extensively validated *in vivo* and the obtained results has confirmed its reliability [132].

If $I(i, j)$ is an IVUS frame (where i, j are the coordinates of a pixel of the image I), $L(i_L, j_L)$ is the lumen border, $MA(i_{MA}, j_{MA})$ the media-adventitia border the ROI, $S(i_s, j_s)$, is defined as:

$$j_L \leq i_s \leq j_{MA} \text{ and } j_L \leq j_s \leq j_{MA}. \quad (3.16)$$

3.3.1.2 Plaque characterization methodology

Feature extraction

A set of features is extracted for each pixel in the ROI for a 11×11 neighborhood. The co-occurrence matrices [120], the Local Binary Patterns [121, 122], the Mean value and the Entropy [123] are calculated (3.2.1.2 Feature extraction). Two geometrical features are also calculated. The first geometrical feature describes the relative position of each pixel from the media-adventitia border and the second the relative position of the pixel from the media-adventitia and the lumen border.

The first geometrical feature f_{geom}^1 , describes the relative position of each pixel from the media-adventitia border:

$$f_{geom}^1 = \frac{D_1}{D_1 + D_2}, \quad (3.17)$$

The second geometrical feature f_{geom}^2 describes the relative position of the pixel from the media-adventitia and the lumen border:

$$f_{geom}^2 = D_1 + D_2, \quad (3.18)$$

D_1 is the perpendicular distance [133] of a pixel $p1$ of the ROI from the media-adventitia border and D_2 is the perpendicular distance of the $p1$ pixel from lumen border. A schematic presentation of the two distances D_1 and D_2 for a pixel $p1$ is shown in Figure 3.15.

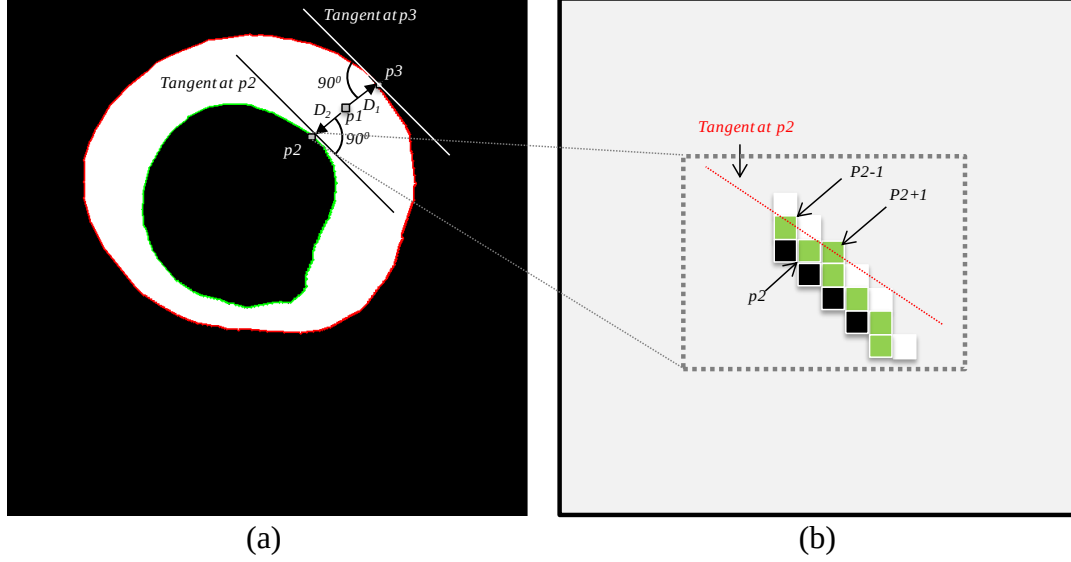


Figure 3.15: (a) The perpendicular distance (D_1) from a pixel $p1$ of the ROI to a pixel $p2$ of the media-adventitia border and the perpendicular distance (D_2) from the pixel $p1$ to a pixel $p3$ of the lumen border, (b) An example of computing the tangent of the pixel $p2$. As tangent we determine the straight line that crosses the previous ($p2 - 1$) and the next pixel ($p2 + 1$) of $p1$.

Feature Selection

To identify the best among the extracted features we examined the correlation between these 30 features. Since the features variables are continuous and are classified to a discrete number of classes (5 classes) we used t-test [134] to study their correlation. T-test gives an indication of the separateness between two different sets of measurements and thus, it was applied to test whether these measurements were essentially different.

For this purpose 900 sets of all the possible pairs of features were created ($\{f1, f1\}_1, \{f1, f2\}_2, \{f1, f2\}_3, ..., \{f30, f30\}_{900}$). If the t-test in a pair of features was greater than the threshold $Th_{cor} = 0.05$, then it was considered that the pair contained correlated features. As it was likely the correlation of these two features to affect the

accuracy of the proposed plaque characterization method, it was decided to select only one feature from the pair. For Q correlated pairs we selected all the possible combinations (2^Q). The feature combination that gives the best accuracy was defined and constituted the final feature set for the proposed plaque characterization method.

Hence, the above described features selection method provided a set of $30 - Q$ features per pixel.

Classification

In order to classify each pixel of the ROI we implemented three classification methods: Neural Networks (NN) [118], Support Vector Machines (SVM) [73] and Random Forests [119].

a) Neural Networks

We have tested several NN architectures [118, 126]. More specifically for the one hidden layer architecture we use 14, 16, 16, 18, 20, 22, 24, 26, 28 Neurons. For the two hidden layer we use 10-8, 14-12, 16-14, 20-18, 24-22, 28-26 Neurons for the first-second layer, respectively.

b) Support Vector Machines

Classification with SVM [73] is performed by mapping the data into a higher-dimensional space where the original problem can be linearly separable. A n dimensional hyperplane is therefore constructed, which optimally separates the data into two categories. The SVM classifier makes use of support vectors, namely the points that are closest to the separator, holding up the separating plane.

Given a training set of instance-label pairs (x_i, y_i) , $i = 1, \dots, q$, where $x_i \in R^n$ and $y \in \{1, -1\}^q$, the SVM classifier requires the solution of the following optimization problem:

$$\begin{aligned} \min_{w, b, \xi} \quad & \frac{1}{2} w^T w + C \sum_{i=1}^q \xi_i \\ \text{subject to} \quad & y_i (w^T \phi(x_i) + b) \geq 1 - \xi_i, \end{aligned} \tag{3.19}$$

where: $\xi_i \geq 0$, $1 \leq i \leq q$, $b \in \mathbb{R}$, C is the penalty parameter of the error term (must be a positive value) and φ is a mapping function [73].

In this problem, the training vectors are mapped into a higher dimensional space by the mapping function φ . A kernel function of the form: $K(x_i, x_j) \equiv \varphi(x_i)^T \varphi(x_j)$ is utilized. This kernel function is used as a measure of similarity between two instances, x_i and x_j in the transformed space. This is a useful method to compute similarity in the transformed space using the original attribute set. We have utilized the radial basis function (RBF) kernel:

$$K(x_i, x_j) = \exp(-\gamma \|x_i - x_j\|^2), \quad \gamma > 0, \quad (3.20)$$

where γ is the kernel parameter. The GridSearch technique is used to optimize the parameters, C, γ .

c) *Random Forests*

Random Forests [119] is an ensemble classifier. In the procedure for generating Random Forests, random vectors (D_r , $r = 1, \dots, t$) from the initial training set are generated. The random vector D_r is generated independently of the random vectors D_s , $s = 1, \dots, r-1, r+1, \dots, t$ but with the same distribution. For each random vector ($D_r, r = 1, \dots, t$), a tree is grown using the random vector resulting in a classifier $h(x, D_r)$ where x is an input vector. After the generation of a large number of trees, each tree of the forest cast a unit vote for the most popular class at input x . The predicted class is the one that most of the decision trees "vote".

3.3.2 Dataset

To train and validate our plaque characterization algorithm 300 VH-IVUS frames and the corresponding grayscale IVUS frames were acquired from ten patients who underwent X-ray angiography and IVUS examination for clinical purposes. All the enrolled patients were suffering from stable angina and did not have previous stent implantation in the studied by IVUS segment. Participants provided informed consent, while the study was

approved by the institution's Ethical Committee. The frames were acquired using an electronic phased array IVUS catheter with synthetic aperture 3.5F and frequency 20MHz (Eagle eye Gold, Volcano Therapeutics, Inc.). The acquired IVUS sequences were stored in DICOM format and transferred to our workstation for further purposes. The pixel size in each sequence was extracted from the DICOM data.

From each patient we selected 30 frames, each one of which included all the plaque types, resulting in a 300 frame dataset. In order to train and validate our classifier we followed the following procedure: we used 70% of the dataset ($Set-A = 210$ frames) to train our classifier and the rest 30% ($Set-B = 90$ frames) was utilized to validate its performance. Between the two sets there was no overlap e.g. no frame in $Set-A$ appeared in $Set-B$ and vice versa. The 210 frames from the $Set-A$ contain the four plaque types (DC, NC, FT and FFT) with an extra class describing the Media [6]. We created 20 training sets ($Set-A_1, Set-A_2, \dots, Set-A_{20}$) by selecting randomly 15 frames from the $Set-A$. From each frame of a training-set, 300 pixels were randomly selected for each plaque type. Thus 20 balanced training sets of 22500 pixels were created ($Set-BA_1, Set-BA_2, \dots, Set-BA_{20}$). The above procedure is shown in Figure 3.16.

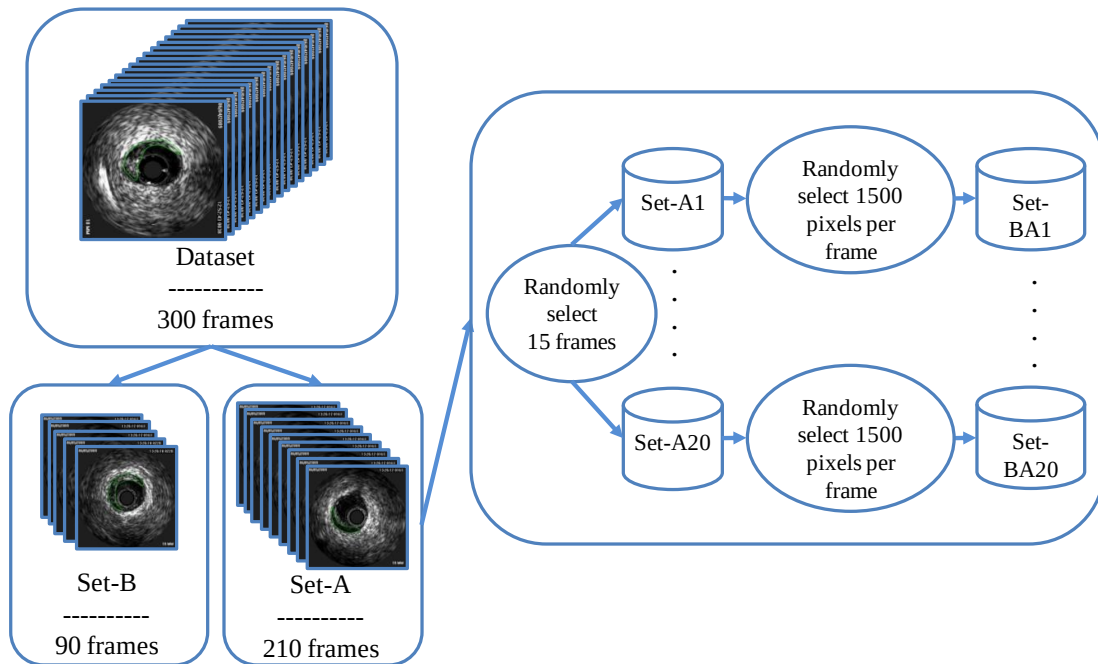


Figure 3.16: Creation of the 20 balanced training sets from the initial dataset.

3.3.3 Results

The performance of our algorithm was evaluated in the (Set – B). The estimations of our approach were compared to those derived by the VH-IVUS. More specifically the following measures were used to quantify the reliability of our method:

- overall accuracy ($AC_{overall}$),
- sensitivity $Sens_a$, for each plaque class a , $1 \leq a \leq 5$ ¹,
- specificity $Spec_a$, for each plaque class a , $1 \leq a \leq 5$ ¹,

which are defined as:

$$AC_{overall} = \frac{\# \text{ pixels correctly classified}}{\text{total \# pixels of the ROI}} \times 100\%, \quad (3.21)$$

$$Sens_a = \frac{\# \text{ pixels correctly classified into class } a}{\text{total \# pixels belong to class } i \text{ according to VH – IVUS}} \times 100\%, \quad (3.22)$$

$$Spec_a = \frac{\# \text{ classified pixels correctly by the method into all classes except } a}{\left(\begin{array}{c} \# \text{ classified pixels by the method} \\ \text{into all classes except } a \\ + \\ \# \text{ pixels wrongly classified by} \\ \text{the method into } i \end{array} \right)} \times 100\%, \quad (3.23)$$

where the “# pixels correctly classified” are the pixels which are correctly classified by our method and belong to the same class with VH-IVUS annotations.

Feature Selection

A balanced training set of 30 features was examined using t-test. Of all possible pairs of features ($\{f1, f1\}_1, \{f1, f2\}_2, \{f1, f2\}_3, \dots, \{f30, f30\}_{900}$), six had t-test value greater than Th_{cor} : $\{f1, f3\}, \{f5, f7\}, \{f9, f11\}, \{f10, f12\}, \{f13, f15\}, \{f14, f16\}$, resulting

¹ $a=1$, is the DC class, $a=2$, is the NC class, $a=3$, is the FT class, $a=4$, is the FFT class, and $a=5$, is the Media class.

in 64 combinations of features. To select the best combination we evaluated the performance of the Random Forests classifier (14 trees-10features) for all the 64 combinations. The 10-fold cross validation method was applied and the obtained results are shown in Figure 3.17. The best accuracy was achieved using the 2nd feature combination: $\{f_2, f_3, f_4, f_5, f_6, f_8, f_9, f_{12}, f_{15}, f_{16}, \dots, f_{30}\}$ and thus this combination was used in the proposed plaque characterization method.

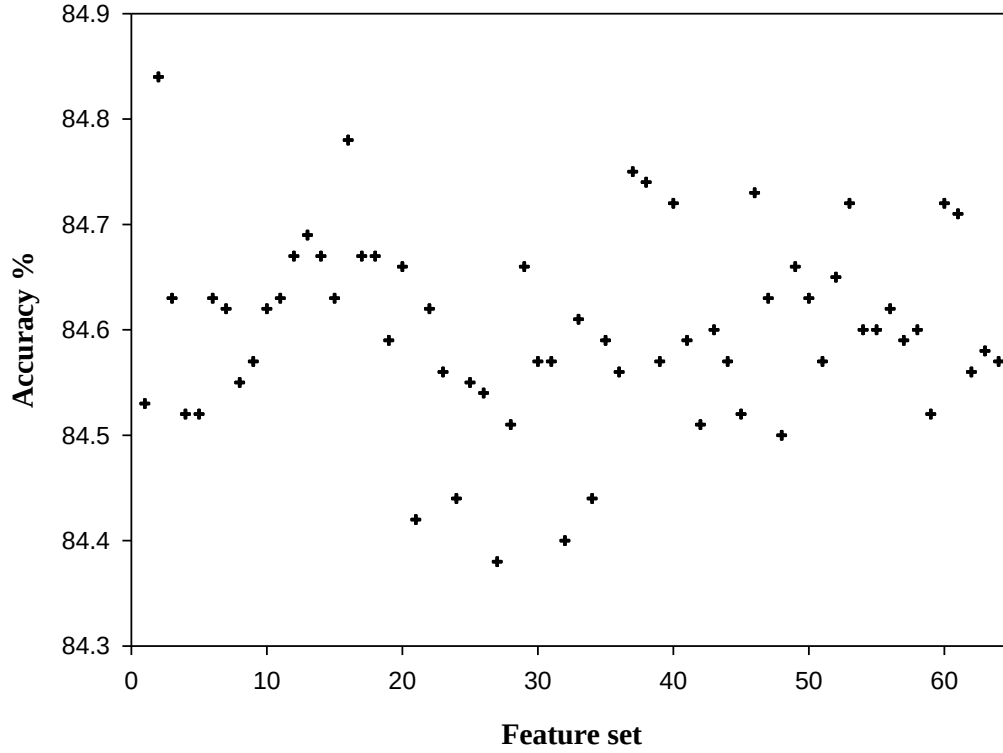


Figure 3.17: Accuracy for all 64 combinations of features using the Random Forests classifier (14 trees-10features).

Neighbourhood Size Selection

To optimize the classification accuracy of our method we evaluated the performance of the different classifiers (Random Forests, NN, SVM) using different neighbourhood sizes of each pixel, during the feature extraction procedure. The neighbourhood sizes used were: 7×7 , 9×9 , 11×11 , 13×13 and 15×15 . In a balanced training set we performed 10-fold cross validation to find the best neighbourhood size in terms overall of accuracy as shown in Figure 3.18.

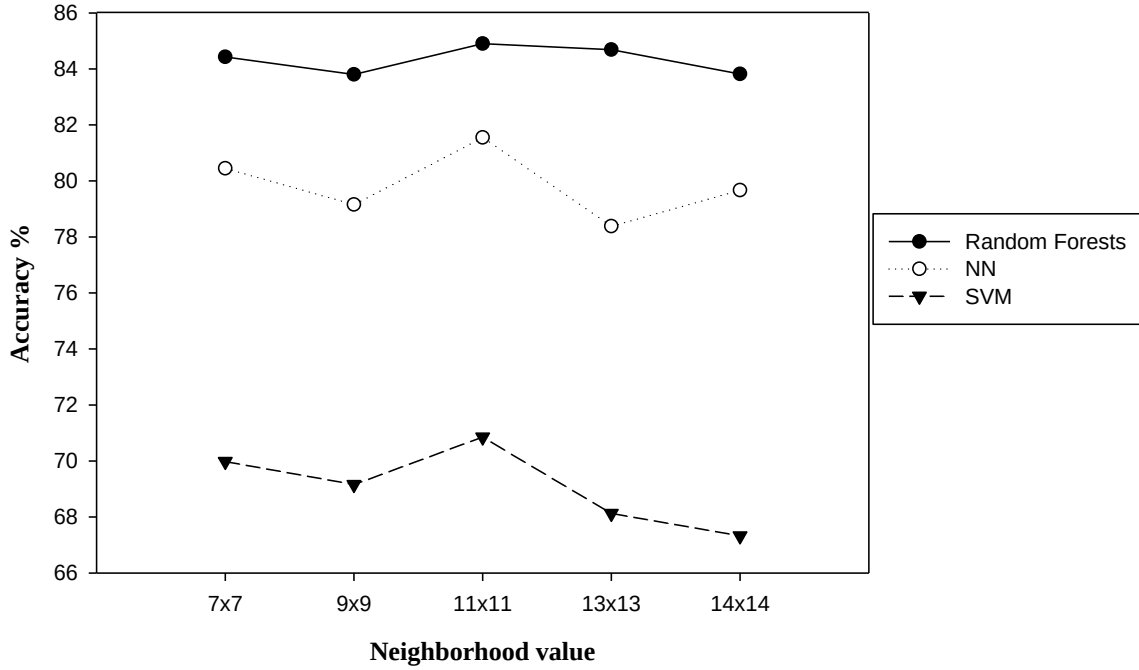


Figure 3.18: Overall accuracy of different classifiers (NN, SVM, Random Forests) using various neighborhood sizes.

Neural Network

The neural network was trained using the back-propagation algorithm [126], using the 10-fold cross validation method. Various architectures of NN were tested using one or two hidden layers. Figure 3.19 presents the overall accuracy ($AC_{overall}$) of the NN classifier for the one and two hidden layer architecture. The best accuracy (80.9%) is achieved, using 1 hidden layer with 22 neurons.

Support Vector Machines

10-fold cross validation was used to train the classifier. The GridSearch technique is employed to optimize the parameters (C, γ), where C is the penalty parameter of the error term (Eq. (14)) and γ is the width of the kernel (Eq. (15)). After the application of the GridSearch technique the parameters are: $C=16$, $\gamma=1$. The maximum overall accuracy was 76.88%.

Random Forests

To train the classifier 10-fold cross validation method was used. Different number of trees and features were used, as we can see in Table 3.2. The maximum overall accuracy ($AC_{overall}$) 84.84%, was achieved using 140 trees and 10 features.

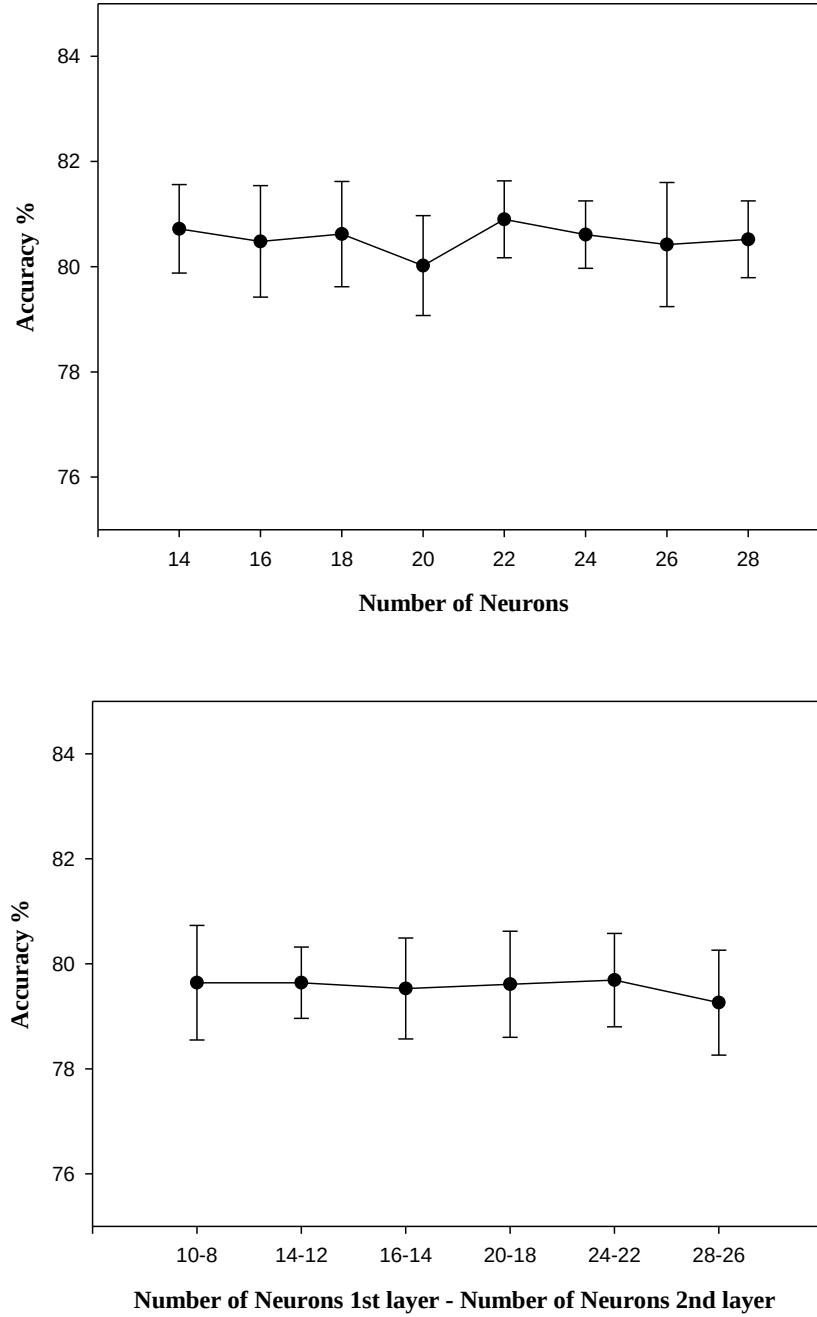


Figure 3.19: Accuracy of the Neural Network for plaque classification, using 1 and 2 Hidden Layer architectures.

Table 3.2: Accuracy of the Random Forests classifier using different number of trees and features.

Accuracy (%)						
# of features						
# of trees	6	8	10	12	14	16
100	84.34	84.58	84.74	84.68	84.7	84.68
110	84.39	84.56	84.79	84.73	84.64	84.66
120	84.45	84.62	84.73	84.72	84.7	84.68
130	84.4	84.61	84.76	84.78	84.7	84.62
140	84.45	84.65	84.84	84.76	84.7	84.4
150	84.66	84.78	84.76	84.62	84.74	84.38
160	84.58	84.59	84.83	84.77	84.67	84.68

Comparison of the Classification Schemes

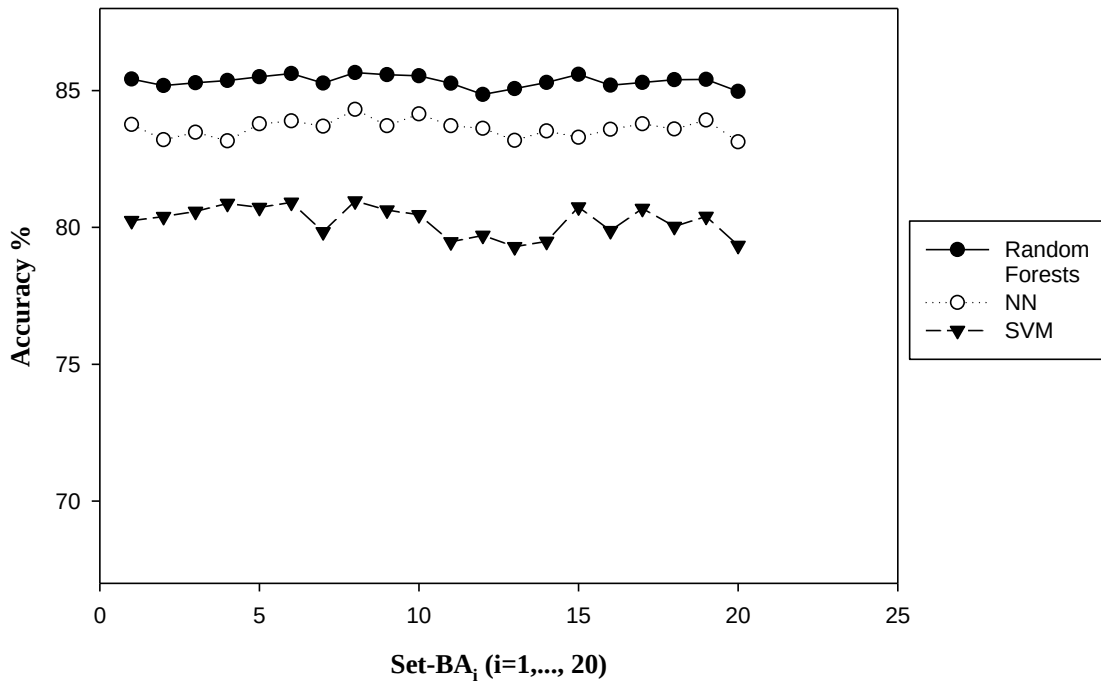
To compare the three classification schemes (NN, SVM, Random Forests) we have used the best parameters for the Random Forests and SVM classifiers and the best architecture for the NN classifier. The best parameters of the SVM, Random Forests, as well as the architecture of the NN classifier are summarized in Table 3.3.

We have used the test set (*Set-B*) in order to test each classification scheme. The test set contained 90 IVUS frames that were not included in the training sets (*Set-BA_i*, $i=1,...,20$). Figure 3.20 presents the classification accuracies over the test set (*Set-B*) using the 20 balanced training sets (*Set-BA_i*, $i=1,...,20$). The Random Forest classifier achieved the highest overall accuracy (85.65%) for the test set *Set-B* using the balanced training set *Set-BA₈*. Since the Random Forests provided the best results it was decided to use this classifier in the proposed plaque characterization method. Table 3.4 presents the classification accuracies of the different classifiers over the test set.

We have also validated our method using the leave one patient out technique. The concept of this technique is to use one patient for testing the classification scheme and the rest of the patients for training. Thus, 30 frames of each patient were used for testing and the rest 270 frames for training. This procedure was conducted for all the patients of the dataset and an average overall accuracy 81.15% is reported.

Table 3.3: The classifiers and the parameters-architectures used for the test set.

Classifier	Parameters / Architecture
Support Vector Machine	$C = 16, \gamma = 1$
Random Forests	# trees=140 # features=10
Neural Networks	1 hidden layer architecture #Neurons=22

**Figure 3.20:** Accuracies of the classification schemes over the test set (*Set – B*) using the 20 balanced training sets (*Set – BA_i*, $i = 1, \dots, 20$).

Contribution of the Geometrical Features

To be able to study the contribution of the geometrical features in the atherosclerotic plaque characterization problem, two experiments were implemented. In the first experiment the Random Forest classifier was trained to classify the four plaque types and the Media using two different feature sets. The first feature set includes all the 24 features (texture, intensity and geometrical features) while the second includes only the 22 texture and intensity features. In the second experiment we employed the same procedure to

classify only the four plaque types using the two feature sets described above. For both experiments the classifier was tested using the test set *Set – B*.

We evaluated the performance of the classifier measuring the class classification accuracy for both the experiments. Figure 3.21, presents the classification accuracy for each plaque type and the Media of the first experiment. Figure 3.22, presents the classification accuracy for each plaque type of the second experiment. The confusion matrices of the two experiments are shown in Table 3.5.

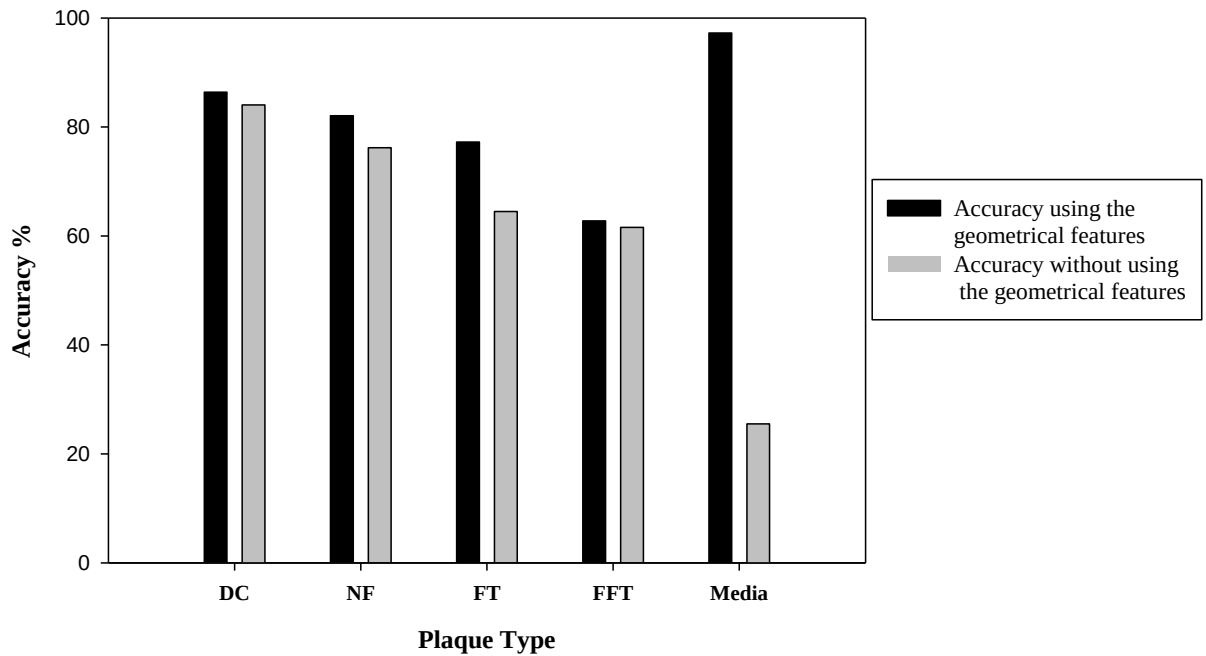


Figure 3.21: Accuracy for each plaque type and Media using two feature sets: the one containing intensity and geometrical features and the other containing only intensity features.

Table 3.4: Classification accuracies of the different classifiers for the test set. $Spec_a$: Specificity of class a , $Sens_a$: Sensitivity of class a , ($1 \leq a \leq 5$).

	Dense Calcium		Necrotic Core		Fibrotic Tissue		Fibro-Fatty Tissue		Media		Overall Accuracy %
	$Sens_1$ %	$Spec_1$ %	$Sens_2$ %	$Spec_2$ %	$Sens_3$ %	$Spec_3$ %	$Sens_4$ %	$Spec_4$ %	$Sens_5$ %	$Spec_5$ %	
Support Vector Machine	90.06	98.59	74.00	97.55	77.44	90.01	47.90	94.35	92.56	93.19	80.96
Neural Network	88.59	98.89	80.71	97.08	74.59	93.96	67.59	91.86	94.74	98.88	84.31
Random Forests	84.87	99.29	80.57	97.66	77.40	93.54	63.47	93.34	97.31	97.74	85.65

Table 3.5: Confusion Matrices for the two experiments that were implemented in order to study the contribution of the geometrical features.

	Using the geometrical features					Not using the geometrical features				
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 1	Class 2	Class 3	Class 4	Class 5
Class 1	34462	4956	168	3	304	33522	5704	35	0	632
9390	74845	5608	784	564		11335	69477	4088	442	5849
393	18084	416008	93629	10442		110	13252	347222	116300	61672
342	19988	65562	157350	7539		204	16509	60096	154444	19528
5213	2677	11351	5620	876063		27074	172354	301673	170047	229776
Using the geometrical features										
Class 1	35008	4711	173	1		Class 1	Class 2	Class 3	Class 4	Class 5
9999	74835	5621	736			33865	5863	157	8	
476	18642	424630	94808			11736	73560	5389	506	
443	20254	68734	161350			530	19486	394802	123738	
						477	20846	68152	161306	

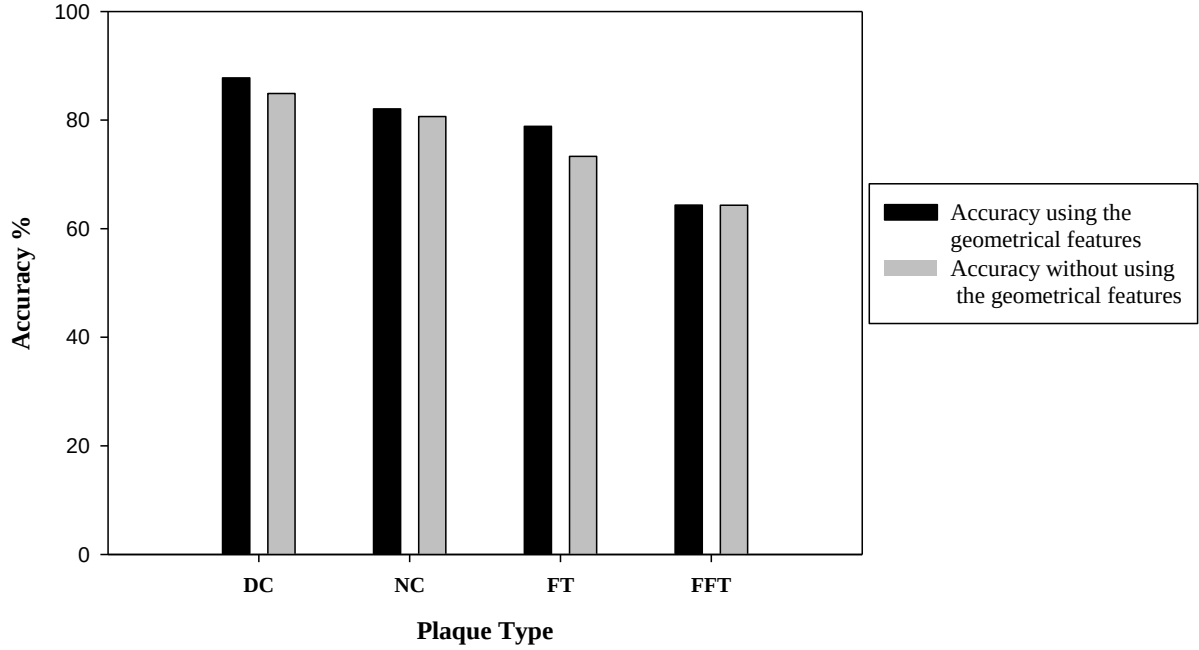


Figure 3.22: Accuracy for each plaque type using two feature sets: the one containing intensity and geometrical features and the other containing only intensity features.

Quantitative validation and application examples

Bland and Altman analysis was applied to examine the agreement between the plaque area detected by the proposed method and by VH for each plaque type in the *Set-B* (90 IVUS frames) using the RF classifier. The obtained results are illustrated in Figure 3.23 while Figure 3.24 shows an application of our methodology in different IVUS frames. It is obvious that there is a good agreement between the estimations of our methodology and VH.

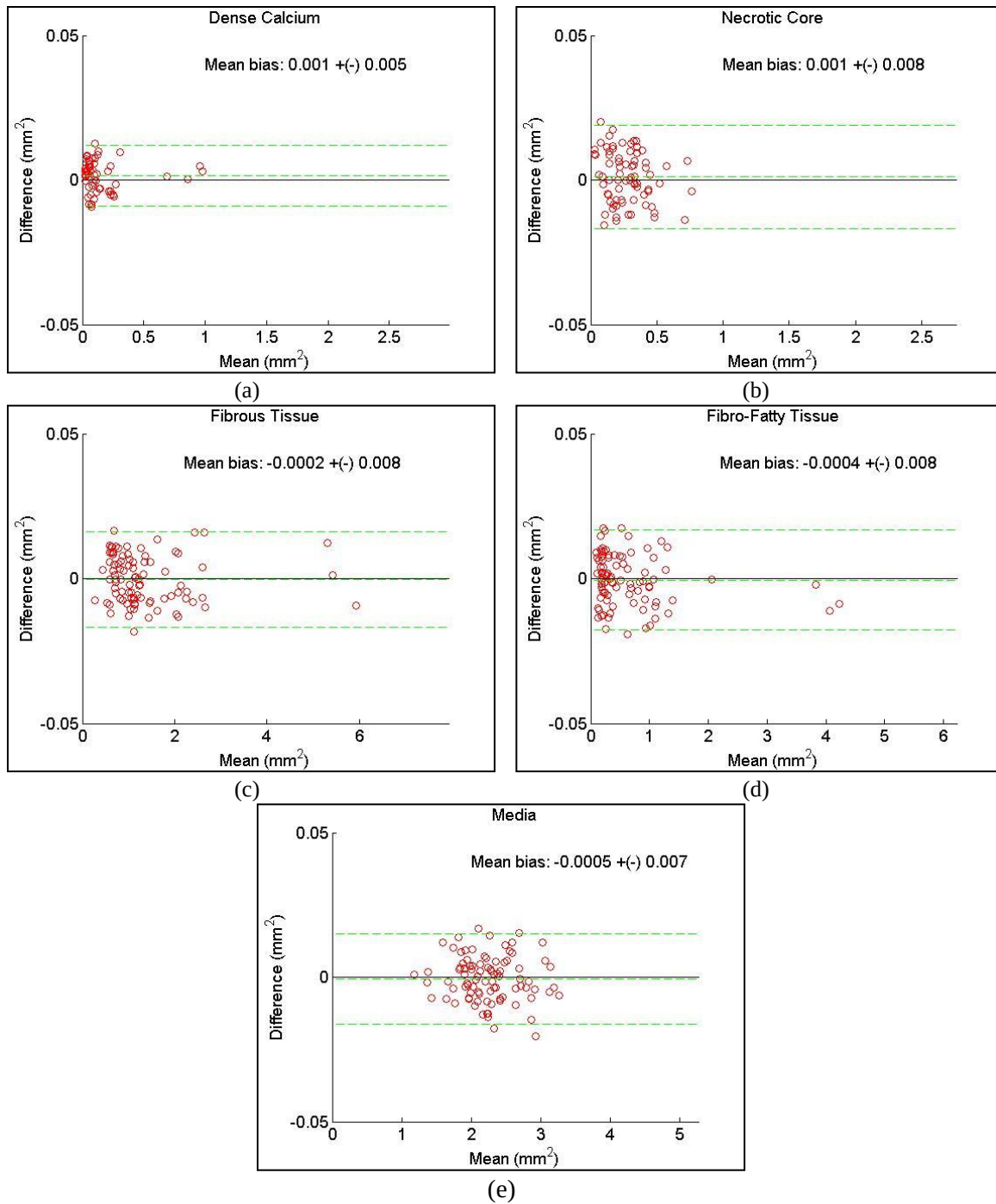


Figure 3.23: (a) Dense Calcium Bland-Altman plot, (b) Necrotic Core Bland-Altman plot, (c) Fibrous Tissue Bland-Altman plot, (d) Fibro-Fatty Tissue Bland-Altman plot and (e) Media Bland-Altman plot.

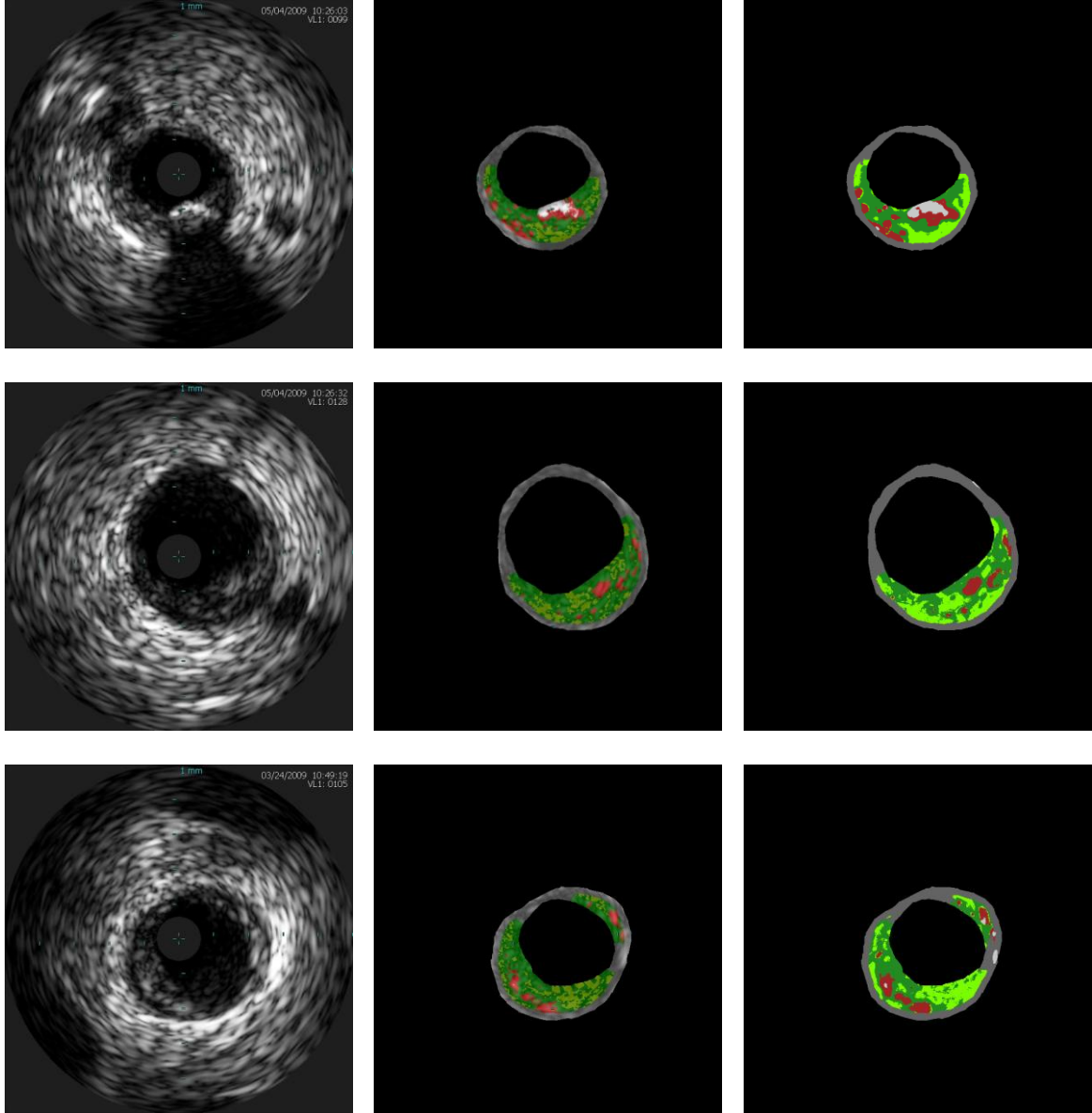


Figure 3.24: The application results of the proposed method applied on three different IVUS frames. The images are from left to right: an initial typical intravascular ultrasound (IVUS) frame, its corresponding virtual histology (VH) frame, the application result of the proposed method applied on the initial frame. The DC, NC, FT, FFT and Media are shown in white, red, green, light green, and gray, respectively.

3.4 Discussion

Currently, two semi - automated methodologies for the characterization of plaque in grayscale IVUS frames are presented. The obtained results demonstrated that both methodologies are reliable (first approach: overall accuracy 86.14% and second

approach: 85.65%) and able to identify the different plaque components with high accuracy.

3.4.1 First approach

Several classification algorithms [73, 118, 119] were tested and the RF classifier [119] appeared to provide the highest classification accuracy. To further improve the results of HP detected by the classification algorithm (RF), we compare its estimations to those derived by an image processing technique (automatic thresholding). After the identification of the HP further analysis is performed to discriminate the HCP from the HNCP. This is achieved with the use of an image processing technique which takes into consideration the presence of acoustic shadow in the HCP. Finally, deformable models are implemented to allow more accurate delineation of the HCP and increase the classification accuracy of our methodology for the HCP [124, 129].

The ability of the proposed methodology to detect accurately the type of the plaque in grayscale IVUS renders it a useful tool in research. Over the last years a number of studies have used IVUS to study the atherosclerotic evolution and assess responses on pharmaceutical treatments [135-138]. Though some of them have used RF-IVUS [136] which provides more accurate characterization of the type of the plaque, a considerable volume of data have been acquired using grayscale IVUS [135, 137, 138]. In addition, numerous catheterization laboratories does not have the necessary software for RF-IVUS analysis and thus rely on grayscale IVUS images for plaque characterization. The methodology introduced in this paper can be used to process these data and provide accurate and reproducible information about the composition of the plaque and the effect of treatment on the atherosclerotic evolution.

3.4.1.1 Comparison with the literature

As it is shown in Table 3.6 the results compare favorably to those reported in the literature [10-13] and only the methodology presented by Zhang *et al.* [67] achieved higher overall classification accuracy. However, it should be stressed that their method was tested in 12 IVUS frames only and thus it is likely the reported results to overestimate the performance of their approach in clinical setting. In addition, our method appears to detect more

accurately the HCP than the approaches that are restricted to the identification of the calcific component.

Table 3.6: Comparison of the proposed hybrid approach method with literature methodologies.

	Research work	Characterized plaque types	Test set	Results
THREE CLASS PLAQUE CHARACTERIZATION PROBLEM	Zhang <i>et al.</i> [67]	• HCP² • HNCP³ • SP⁴	12 IVUS images	Overall accuracy 89.9%
	Brunenberg <i>et al.</i> [68]	• HCP¹ • HNCP² • SP³	30 IVUS images	Overall accuracy 75.82%
	Proposed method	• HCP¹ • HNCP² • SP³	40 IVUS images	Overall accuracy 86.14%
ONE CLASS PLAQUE (HCP) CHARACTERIZATION PROBLEM	Filho <i>et al.</i> [115]	• HCP¹	43 regions(25 HCP)	AUC ⁵ = 0.91 Sensitivity= 84 % Specificity= 72%
	Zhang <i>et al.</i> [70]	• HCP¹	80 IVUS images	AUC ⁴ = 0.907 Sensitivity= 87.16 % Specificity= 87.35 %
	Proposed method	• HCP¹	33 regions(12 HCP and 21 HNCP)	AUC ⁴ = 0.92 Sensitivity= 94 % Specificity= 93 %

3.4.1.2 Limitations

Although accurate and potentially useful the proposed method, has significant limitations. First of all it cannot detect the type of the plaque located behind a calcific deposit; however this disadvantage appears to be an unsolved problem in grayscale IVUS and has

² HCP: Hard Calcified Plaque,

³ HNCP: Hard Non Calcified Plaque

⁴ SP: Soft Plaque

⁵ AUC: Area Under Curve

not been addressed by all the previous plaque characterization methods [67, 68, 70, 115]. Another limitation of our method is that it requires re-training every time that is applied to process data acquired with different types of IVUS catheters. Furthermore, its performance depends on the reliability of the expert observers that were used to train the method. Nevertheless it is well known that experts have a relatively high inter- and intra-observer variability and therefore cannot be considered as the golden standard [111]. This limitation can be addressed with the use of RF-IVUS or histological data for training and validation purposes if available.

3.4.2 Second approach

The second presented methodology is semi - automated approach and requires manual interaction only during the segmentation process where the user has to detect the borders in the first IVUS frame. In this study VH data were used to train and validate our approach. However, after appropriate training (using any RF-IVUS methodology, or even the estimations of expert observers as Gold standard) the proposed methodology can be implemented to process any IVUS sequence. The main difference of the proposed plaque characterization method from the other approaches is that in addition to the texture and intensity features [120-122] that are already used in the literature [67, 68, 139] we extracted two geometrical features. These features can accurately classify the Media [48, 113] (Figure 3.22) and increase the classification precision for all the plaque classes (DC, NC, FT and FFT) providing an overall accuracy of 85.65%. As presented in the Figure 3.22, the geometrical features increase the considerably the classification accuracy for each class type (2.35% for the DC, 5.88% for the NC, 12.77% for the FT, 1.11% for the FFT and 71.73% for the Media). The overall accuracy of the proposed methodology improves further to 94% if we focus in three plaque types: DC, NC and the combination FT and FFT. In this setting the specificity of our approach is close to 100% (>97%) for all plaque types while the sensitivity ranges from 83% (for the NC) to 91% (for the FT-FFT). The ability of the methodology to detect with high accuracy the NC and the DC renders it a useful tool in research and in clinical arena. There are evidence that increased NC volume has been associated with a worse outcome and a higher risk for acute coronary events. Thus, a number of studies have focused on the composition of the

plaque rather than the plaque volume and on the effect of pharmacological treatment on the NC [140]. The proposed methodology can be implemented to process recent grayscale IVUS data, or IVUS obtain in the past when the RF analysis software was not available, and quantify fast and accurately changes in plaque composition and NC burden [141]. In addition, the extent of DC can affect patient management and the planning of a percutaneous coronary intervention as an extensive calcification located in the media may cause inadequate stent expansion which is associated with a poor outcome. Thus, the proposed methodology can be applied in clinical practice to quantify the calcium deposit and guide treatment (e.g. cutting balloon or rotablator in case of increased calcium versus balloon pre-dilation or direct stenting in case of limited calcium deposits) [108]. Another advantage of the methodology is that it allows characterization of the entire IVUS sequence. As it has already been mentioned the VH technique uses ECG – gated acquisition and can characterize only the end-diastolic IVUS frames. Thus, if a patient has a heart rate of 60 beats 20 per minutes, VH will be able to process only 1 of the 30 frames acquired per second [142] discarding the 97% of the data. On the contrary the proposed approach can process this additional data and thus provide more precise information about the composition of the plaque. Finally, another advantage of the methodology is that its estimations are stored in a standardized format allowing recall, portability and data exchange. This will facilitate the future 3-D reconstruction of the plaque and its integration with the hybrid models obtained from the fusion of IVUS and coronary angiography [143]. This synthesis will allow a complete, comprehensive coronary visualization and a reliable evaluation of the distribution of the plaque on vessel's geometry.

3.4.2.1 Comparison with the literature

The proposed methodology was modified in order to classify the plaque formations into three classes (DC, NC and FT_FFT) by combining the FFT and FT plaque types into one class. This modification was employed in order to compare plaque classification method to the one proposed by Taki *et al.* [71]. The Random Forest classifier was employed. The number of features and trees was chosen 140 and 10, respectively. The overall accuracy ($AC_{overall}$) when combining the FFT and FT into one class was 93.85%. A comparison of

our plaque characterization method to the one proposed by Taki *et al.* [71] for the three-class problem is shown in Table 3.7. The improvement of the classification accuracy for each class type: DC, FFT, NC was 8%, 10% and 31%, respectively. Additionally, the overall accuracy improvement was 19%.

Table 3.7: Comparison of the proposed method with the method proposed by Taki *et al.*[71].

$Spec_a$: Specificity of class a , $Sens_a$: Sensitivity of class a , ($1 \leq a \leq 3$), $AC_{overall}$: Overall Accuracy, DC: Dense Calcium, NC: Necrotic Core, FT_FFT: Fibrous Tissue combined with Fibro-Fatty Tissue.

		DC		FT_FFT		NC		$AC_{overall}$ (%)	DATASET		Validation
		$Sens_1$ (%)	$Spec_1$ (%)	$Sens_2$ (%)	$Spec_2$ (%)	$Sens_3$ (%)	$Spec_3$ (%)		# patients	# frames	
Taki <i>et al</i> [71]	Run length matrices	79	85	81	90	52	82	75	10	500	- 5-fold cross validation using 500 frames.
	24 Features	87	99	91	99	83	97	94	10	300	-10-fold cross validation using 210 frames, -method tested using 90 frames, -leave one patient out technique.

3.4.2.2 Limitations

Though the proposed methodology is one of the most accurate approaches presented in the literature it has some limitations. First of all its training is based on VH-IVUS estimations and although VH-IVUS is a valid methodology for plaque characterization it is not the Gold Standard. Thus, the computed in our study accuracy is likely to have been worse if we have compared the estimations of our method with histology. In this context we feel that further validation is required using histological data though we acknowledge that even this approach can lead to false results as it is frequently difficult to determine

the precise correspondence between IVUS images and histological slices [144]. In addition, it needs to be stressed that our methodology can process data that have been obtained only with the same type of IVUS catheter that was used to acquire the training sets. Thus, every time that the catheter changes, the method should be re-trained with a new training set (which can be consisted of RF- or manual estimations) before its implementation.

Chapter 4: Optical Coherence Tomography (OCT)

4.1 Introduction

4.2 Proposed method

4.3 Dataset

4.4 Results

4.5 Discussion

4.1 Introduction

Optical coherence tomography [32, 145] is a relatively new imaging modality which was introduced to overcome the limitation of previous imaging techniques as it provides high resolution cross sectional images of coronary arteries (Figure 4.1). In contrast to intravascular ultrasound (IVUS), OCT is based on the analysis of the reflected light, instead of acoustic waves, allowing visualization of plaque features with a higher analysis (axial resolution: 12-18 μm , lateral resolution: 20-90 depending on the depth of focus) [50]. The unique imaging resolution of OCT has drawn the attention of the scientific community from its early stages. However, its clinical applications were initially limited due to the fact that imaging required blood removal. This drawback was addressed with a proximal balloon occlusion [146] which, however, caused ischemia and possibly vessel wall trauma. A later approach used saline injection for blood removal but the slow image acquisition speed restricted OCT imaging in short segments. The recent introduction of the second generation OCT systems, the so called frequency domain OCT (FD-OCT) [32], permits fast image acquisition (up to 160 frames/sec) and fast pull-back speeds allowing thus visualization of long segments within few seconds [147]. These systems increased considerably the applicability of OCT and today this modality constitutes an indispensable tool in the clinical and research arena.

OCT allows evaluation of the luminal dimensions, assessment of vessel wall morphology and identification of features associated with increased vulnerability such as the thickness of fibrous cap, micro-calcifications, neo-vessels, and the presence of macrophages which

cannot be evaluated by other intravascular imaging modalities [87, 148]. OCT has limited penetration [148], and quite often does not allow imaging of the entire vessel wall, especially in segments with large lumen and increased plaque burden. However, its high resolution permits detailed evaluation of the superficial plaque and nowadays this modality is considered as the gold standard for the *in vivo* characterization of the plaque. According to the recently published expert consensus [51, 87, 148] four different tissue types can be detected in OCT: calcium (CA), that appears as a signal poor region with sharply delineated borders, fibrous tissue (FT), that is portrayed as a high back-scattering homogeneous area, lipid tissue (LT), that is shown as a signal poor region with diffuse delineated borders with a fast drop-off and little or no back-scattering signal, and mixed tissue (MT) that features characteristics from multiple tissue types.

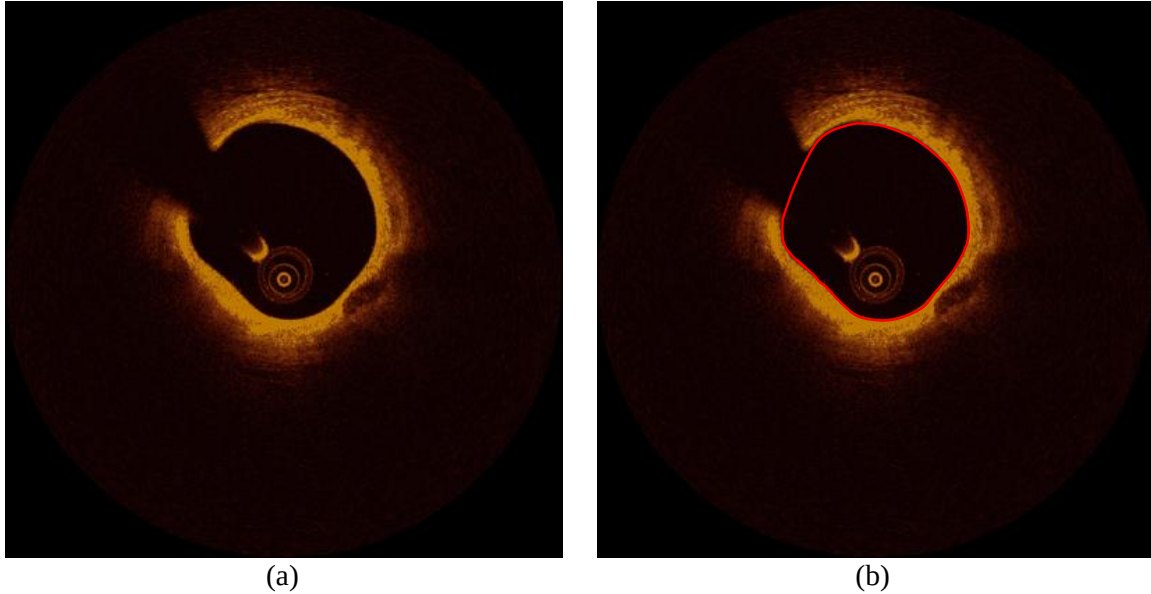


Figure 4.1: A typical OCT image: (a) initial image, (b) annotated image with the lumen and borders marked with red.

Traditionally, OCT lumen detection and plaque characterization [149, 150] was performed manually. However, this process is time consuming and can be unreliable in the hands of non-experienced operators. To overcome these drawbacks and enhance the clinical and research applications of OCT several methodologies have been developed for the automated processing of the OCT images [83, 84]. The most recent ones were proposed by Ughi *et al.* [86] and Tsantis *et al.* [85] who presented an automated methodology that permitted detection of the lumen borders and the stent struts allowing

evaluation of their apposition and coverage. Xu *et al.* [88] were the first who attempted to develop an automated plaque characterization methodology. They used histological images as gold standard and studied the backscattering and attenuation coefficient in 3 tissue types (CA, LT and FT), aiming to determine a cutoff value for each tissue. Similarly the methodology proposed by van Soest *et al.* [89] examined the attenuation coefficients of healthy segments, intimal thickening plaques, LT and fibrous caps infiltrated by macrophages. However, both approaches failed to define fixed cutoff values of the backscattering and attenuation coefficients that would allow reliable plaque characterization. In addition, both approaches analyzed the backscattering and attenuation coefficients across the entire A-lines without being able to identify the plaque region.

For the first time an automated methodology for the segmentation of the lumen border and the characterization of the composition of the superficial plaque in OCT images is introduced. The methodology proposed for the identification of the different tissue types relies on the combination of medical knowledge, image processing and machine learning techniques and is capable to process high quality OCT images in a fully automated manner without user-interaction. The innovative aspects of the proposed methodology are: (i) the fact that it focuses on the analysis of the OCT images and not on the OCT signal, (ii) the ability to detect accurately the lumen borders and define a tissue area and (iii) the fact that it is capable to identify the tissue types with a typical morphology.

The proposed approach was validated against the estimations of expert observers in real patient data. The presented results indicated that the methodology can accurately detect the luminal borders and characterize the composition of the superficial plaque.

4.2 Proposed method

Four steps are followed in order to characterize the composition of the plaque: image acquisition, detection of the Tissue Area (TA), TA segmentation and plaque characterization. Each one of these stages is described in detail in the following sections and are shown schematically in Figure 4.2.

4.2.1 Image acquisition

OCT systems acquire individual A-lines containing the information of the reflected optical energy as a function of time [151]. These A-lines are stored sequentially in a 2D matrix (I_{Matrix}) with each line corresponding to polar intensity data $I(r, \theta)$, with r representing the range dimension and θ the acquisition angle. Each frame corresponds to an $I(r, \theta)$ dataset (grayscale image). In order the true morphology of the tissue to be revealed, the polar 2D data ($I(r, \theta)$ grayscale image) must be converted to Cartesian coordinates ($I(i, j)$). This transformation is performed using: $i = r \cos \theta$, $j = r \sin \theta$.

4.2.2 Detection of the Tissue Area (TA)

A region in each OCT frame is determined in order the plaque characterization methodology to be applied. This region is called Tissue Area (TA) and is located between the lumen border and the expanded (up to 1.5 mm) lumen border (outer border of the TA). The TA detection stage includes two steps: the detection of the lumen border and the extraction of the outer border of the TA.

4.2.2.1 Lumen Detection

Lumen border (L_{mn}) detection is performed in the polar OCT images ($I(r, \theta)$) (Figure 4.3 (a)-(c)) and includes the following steps:

- i. apply a Gaussian filter on the initial image ($I(r, \theta)$),
- ii. perform Otsu's automatic thresholding method [128] in order to reveal binary objects and remove the catheter pixels,
- iii. remove the 8-connected neighborhood non-zero objects (artifacts),
- iv. remove the object close to the catheter with area less than $\frac{1}{5}$ of the image area and mean intensity (of the objects' pixels that correspond to $I(r, \theta)$ image) higher than 200. This area belong to the catheter artifact area A_{cath} ,
- v. scan each column of the image from top to bottom, save the 1st non-zero pixel of the column and connect the non-zero pixels in order to find the lumen contour,

transform the image from polar to Cartesian coordinates.

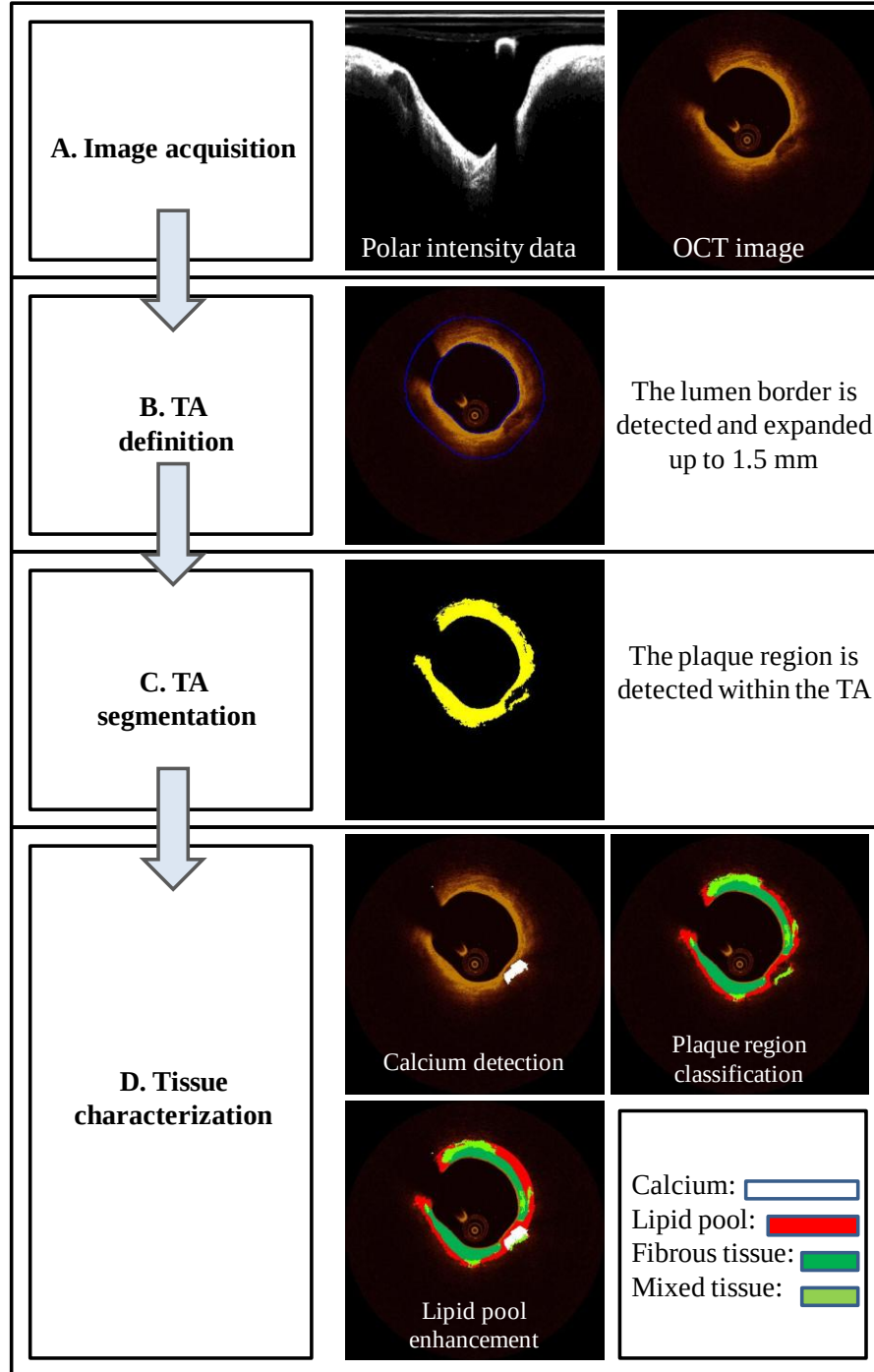


Figure 4.2: Schematic representation of the proposed OCT plaque characterization methodology.

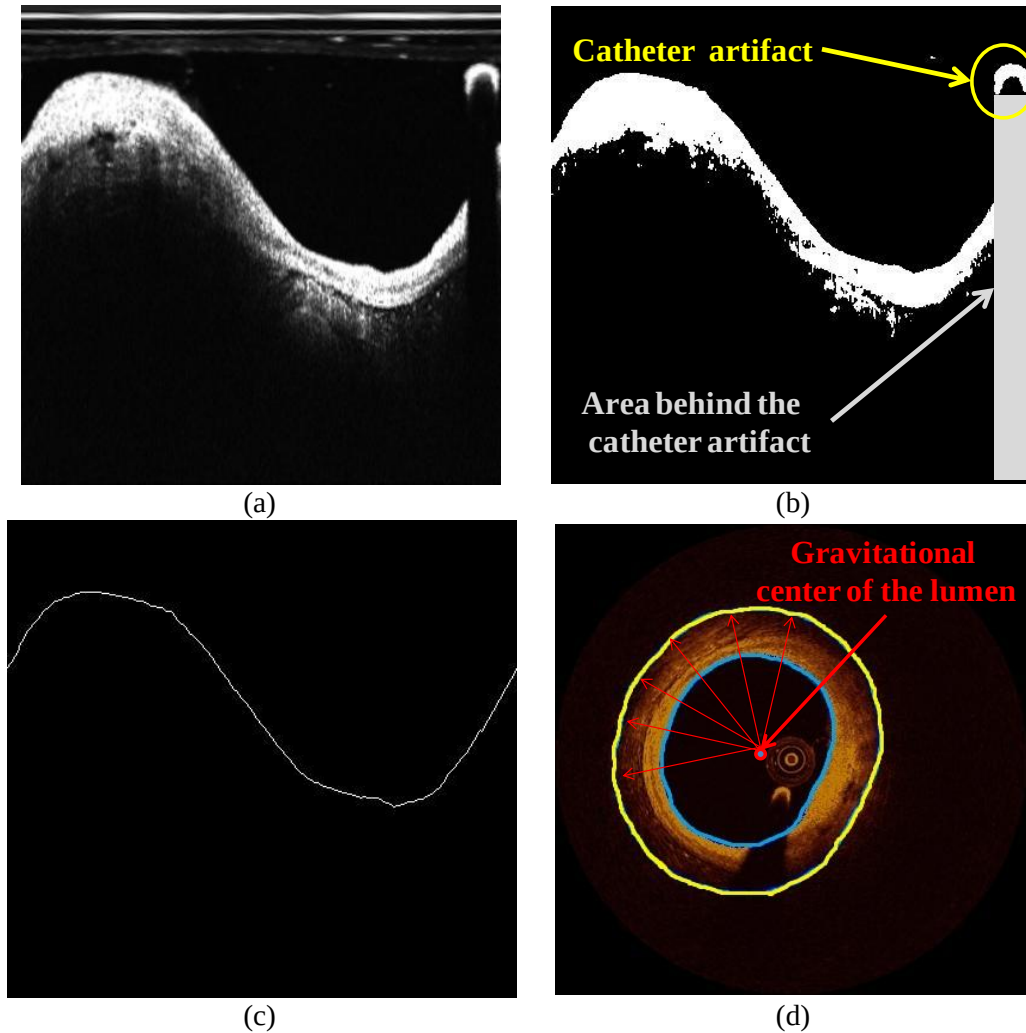


Figure 4.3: Detection of the lumen border and extraction of the Tissue Area (TA): (a) Initial image in polar coordinates, (b) Catheter and catheter artifact removal, (c) The lumen border in polar coordinates, (d) Lumen border marked with blue on the reconstructed OCT image. To extract the outer border of the TA radii are drawn towards the lumen border and extended by 1.5 mm, the curve defined by the distal end of the radius corresponds to the outer border of the TA (yellow curve).

4.2.2.2 Detection of the Outer Border of the TA

The gravitational center of the lumen border is determined in each OCT frame and then 360 radii (with 1° interval) are drawn towards the lumen border and expanded 1.5 mm distally to the detected border. Our decision to focus on the superficial plaque was based on the small tissue penetration of the intravascular OCT systems (1-2 mm) and on the fact that there is an increased signal to noise ratio in the pixels which depict tissues that

are deeply embedded in the plaque [148]. The curve that connects the distal edge of the drawn radius defines the outer border of the TA (L_{mn}^{expn}) (Figure 4.3 (d)).

4.2.3 TA segmentation

TA pixels belong either to plaque or to extravascular tissue. To discriminate the pixels that belong to the plaque from these that image extravascular tissue a *K-means* algorithm is implemented. This approach appeared to outperform to other methods that were tested (automatic thresholding, border detection) in detecting the plaque area and was capable to identify the CA tissue. A *K-means* algorithm partitions a given set of N observations $\{x_1, \dots, x_N\}$, into a fixed number of K clusters $S = (S_1, S_2, \dots, S_K)$ in order to minimize the within cluster sum of squares [126]:

$$\arg \min_S \sum_{l=1}^K \sum_{x_l \in S_l} \|x_l - \mu_l\|^2, \quad (4.1)$$

where μ_l is the mean of points in S_l .

The pixels corresponding to TA are classified to $K=3$ clusters (Figure 4.4 (b)) ($K=2$ and 4 clusters were also tested) resulting to a segmented image $I_{segm}(i, j)$ (Appendix A). The pixels that were classified in the first two clusters (K_1 & K_2) have an intermediate signal intensity and are likely to portray either the superficial LT, FT and MT while the pixels classified in the third cluster (K_3) have a low signal intensity and are likely to portray either the area behind the catheter artifact (A_{Bcath}), or CA, or a signal poor region corresponding to deeply located LT or extravascular tissue.

4.2.4 Plaque characterization

Once the TA is segmented the different plaque types can be detected.

4.2.4.1 Calcium detection

CA appears as a signal poor region with sharply delineated borders. After applying the *K-means* segmentation algorithm the sharply delineated borders belong either to K_1 or K_2

cluster while the signal poor region located between these clusters to K_3 cluster (Figure 4.4 (a) - Figure 4.4 (b)). Therefore to find the CA, each radius of the $I_{segm}(i, j)$ (Figure 4.4 (b)) is scanned from the lumen border to the outer border of the TA. The radial segment which is clustered to K_3 and is enclosed by pixels that belong to the cluster K_1 or K_2 is labeled as calcium (Figure 4.4 (c)). The above procedure is shown schematically in Figure 4.4.

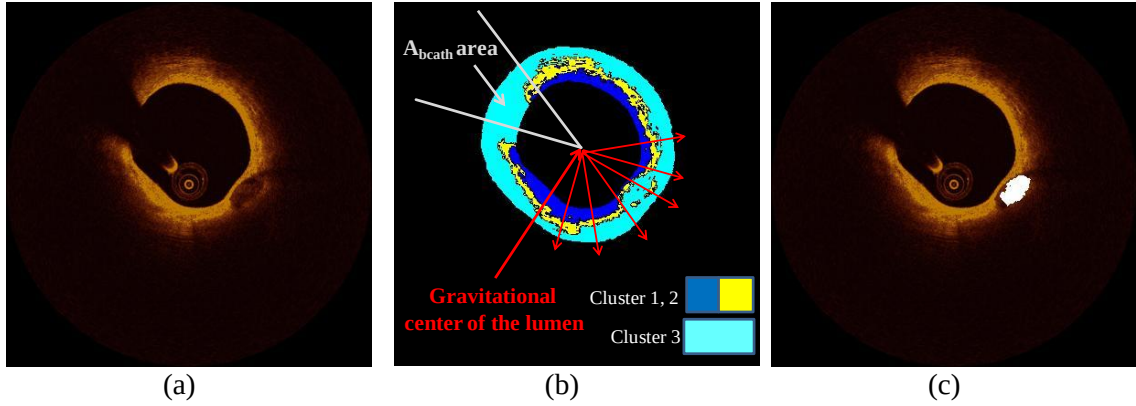


Figure 4.4: (a) Initial image: (b) Segmented image using the K-means clustering method, blue and yellow pixels belong to LT, FT and MT, The light blue pixels belong to: the area behind the catheter artifact, CA, extravascular tissue, or the deeper layers of LT, and (c) the Calcium detected in the initial image is marked with white color.

4.2.4.2 Feature extraction

For each pixel of the area that correspond to clusters K_1 and K_2 (Figure 4.4 (b)), a set of texture and intensity based features is calculated in a $n \times n$ pixel neighborhood (Appendix B). Local Binary Patterns (LBP) and Gray Level Co-occurrence Matrices (GLCMs) are the texture features which are employed.

The Gray Level Co-occurrence Matrix (GLCM) [152] is a matrix with dimensions $L \times L$, defined over an image $I(i, j)$ that calculates how often a pixel with gray-level value i (grayscale intensity) occurs spatially adjacent to a pixel with value j . The spatial relationship of the pixels is defined by an offset (D_x, D_y) as:

$$D_x = D \cdot \cos(\theta), \quad D_y = D \cdot \sin(\theta). \quad (4.2)$$

where θ is the spatial direction and D is the Euclidean distance between the pixels. For the proposed methodology we compute the co-occurrence matrices $C_{(D_x, D_y)}(i, j)$ using all the possible values of $\theta \left\{ \theta = (0^0, 45^0, 90^0, 135^0, 180^0, 225^0, 270^0, 315^0) \right\}$ and $D = 2$ ($D=1, 3$ were also tested). Thus, 8 $C_{(D_x, D_y)}(i, j)$ are computed for each pixel in a $n \times n$ neighborhood. For each co-occurrence matrix $C_{(D_x, D_y)}(i, j)$ the following measures are calculated:

Contrast:

$$f_{contrast}^{\theta} = \sum_{i=1}^L \sum_{j=1}^L (i-j) C_{(D_x, D_y)}(i, j) \quad (4.3)$$

Correlation:

$$f_{correlation}^{\theta} = \frac{\sum_{i=1}^L \sum_{j=1}^L ((i \cdot j) C_{(D_x, D_y)}) - \mu_i \cdot \mu_j}{\sigma_i \cdot \sigma_j}, \quad (4.4)$$

where: $\mu_i = \sum_{i=1}^L i C_{(D_x, D_y)}(i, j)$ and $\sigma_i^2 = \sum_{i=1}^L i^2 C_{(D_x, D_y)}(i, j) - \mu_i^2$.

Energy:

$$f_{energy}^{\theta} = \sum_{i=1}^L \sum_{j=1}^L (C_{(D_x, D_y)}(i, j))^2. \quad (4.5)$$

Homogeneity:

$$f_{homogeneity}^{\theta} = \sum_{i=1}^L \sum_{j=1}^L \frac{C_{(D_x, D_y)}(i, j)}{1 + |i - j|}. \quad (4.6)$$

Therefore 32 features were computed ($f_{glcm}^1, f_{glcm}^2, \dots, f_{glcm}^{32}$), 4 features for each angle $\theta = (0^0, 45^0, 90^0, 135^0, 180^0, 225^0, 270^0, 315^0)$.

LBP [122] determine uniform texture patterns of P members into circular neighborhoods with radius R . Among the various LBP tested we used the rotation invariant $LBP_{P,R}^{riu2}$ [153]:

$$LBP_{P,R}^{riu2} = \begin{cases} \sum_{p=0}^{P-1} s(g_p - g_c), & \text{if } U(LBP_{P,R}) \leq 2 \\ P+1, & \text{otherwise} \end{cases} \quad (4.7)$$

where g_c is the intensity of the center pixel and g_p are the intensities of the circularly symmetric neighborhood, superscript *ri* refers to “rotation invariant” while *u2* means that the pattern has U values (U is the number of spatial transitions in the pattern) of at most 2, $U(LBP_{P,R}) = |s(g_{P-1} - g_c) - s(g_0 - g_c)| + \sum_{p=1}^{P-1} |s(g_p - g_c) - s(g_{p-1} - g_c)|$ whereas the

function $s(x)$, is defined as: $s(u) = \begin{cases} 1, u \geq 0 \\ 0, u < 0 \end{cases}$. $LBP_{P,R}^{riu2}$ output is a fixed set of discrete

values: $0, 1, 2, \dots, P+1$. $P=8$ and $R=1$ are used in the proposed methodology ($P=4, R=1$, $P=12, R=1.5$ and $P=16, R=2$ were also tested but the results were suboptimal) hence 10 LBP features $f_{lbp}^0, f_{lbp}^1, \dots, f_{lbp}^9$ are computed.

The above 42 features (32 GLCMs and 10 LBPs) along with the entropy (f_{en}) and mean value (f_{mv}) [123] of the $n \times n$ neighborhood result to a set of 44 features for each pixel belonging into the two clusters: K_1 and K_2 .

4.2.4.3 Feature selection

Many factors affect the accuracy and success of classification algorithms on a given task. One of these factors is the redundant information given in the classifier. Therefore feature selection is crucial for improving the accuracy of the proposed plaque characterization methodology. We have employed the wrapper feature selection (WRP) [154] to find the best features for the proposed methodology. The WRP method produces feature subsets from all possible feature combinations. Afterwards all the possible feature combinations are used as input to a target classification algorithm and the overall accuracy of each combination is estimated. The feature subset that achieves the highest classification accuracy is chosen as the best feature combination for the specific classification algorithm (Appendix C).

4.2.4.4 Classification

Once the features are extracted, the pixels belonging to the clusters K_1 , K_2 are classified to one of the remaining three plaque types: LT, FT and MT. For this purpose different classification algorithms were tested (Support Vector machines, Neural Networks and Random Forests). Random Forests (RF) provided the best classification results and thus this classification algorithm was used by our methodology. RF [119] is an ensemble classifier that consists of many decision trees RF selects randomly the number of features in order to construct a collection of decision trees with controlled variation. Several numbers of features and trees were tested to find the best combination for the RF classifier (Appendix C).

4.2.4.5 Post processing

In the available IV-OCT imaging configurations, the optical properties of LT limit the ability of OCT to image the full extent of lipid rich plaques. Since the K -means algorithm segments only the pixels portraying FT, MT, and superficial LT the pixels that portray deeper layers of LT cannot be classified (Figure 4.5). When experts manually annotated they LT tissue, they approximate the outer border of LT. In brief they identify the external elastic lamina and draw an arc that connects its lateral extremities (Figure 4.5 (b)). Following the same rationale with experts, we automatically estimate the outer border of LT implementing the following approach:

- transform the segmented image ($I_{segm}(i, j)$) into polar coordinates ($I_{psegm}(i_p, j_p)$),
- scan each column of image ($I_{psegm}(i_p, j_p)$) from the L_{mn}^{expan} to $L_{mn}^{expan} - Th_{post}$ and save the 1st zero pixel of the $I_{segm}(i, j)$,
- the area between the connected zero pixels and the segmented region is considered as LT plaque,
- transform the image from polar to cartesian coordinates.

The above described approach is shown in Figure 4.5 while the sensitivity analysis for applying the post processing threshold ($Th_{post} = 0.75$ mm) is described in Appendix D.

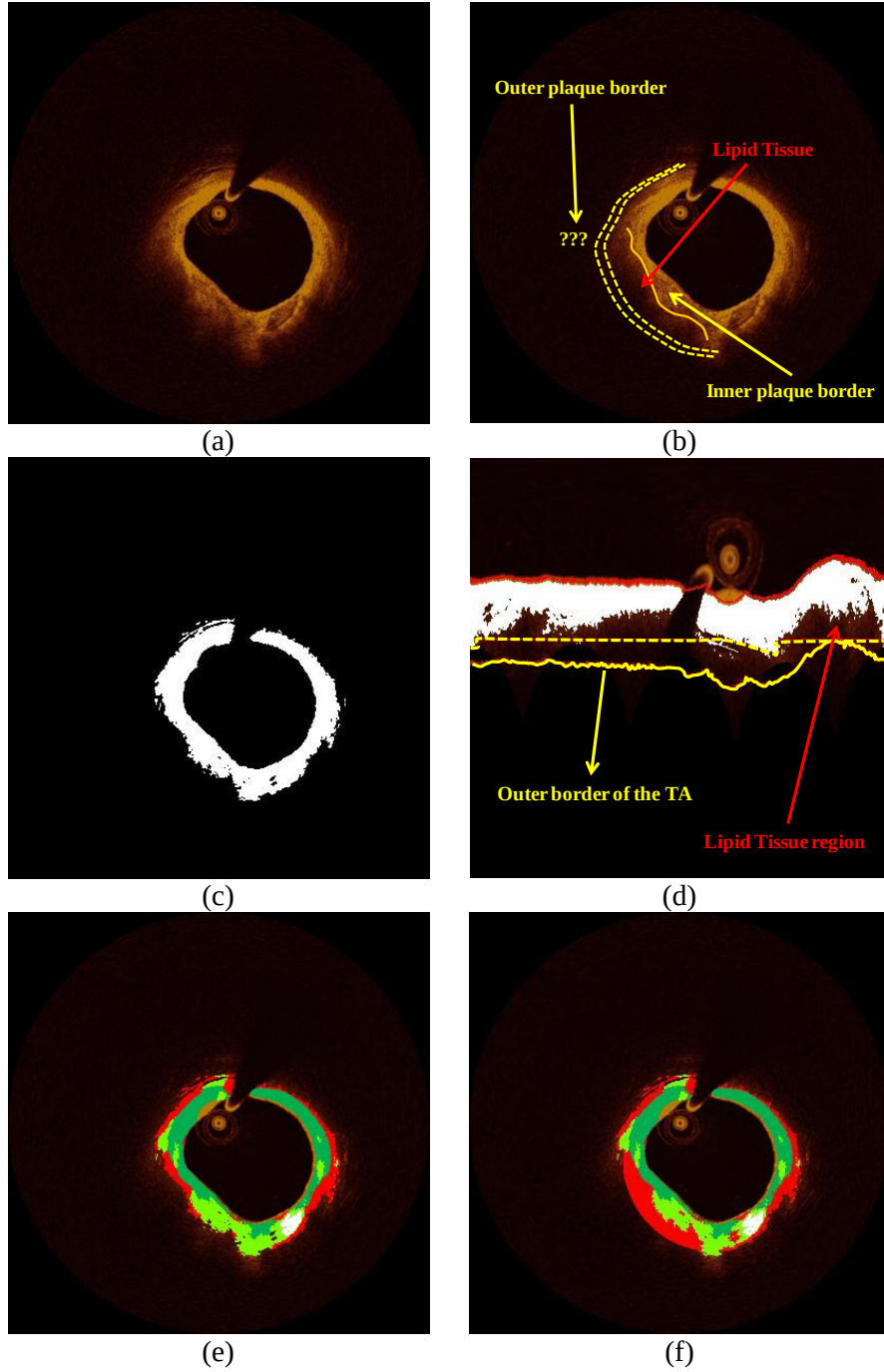


Figure 4.5: The post processing step of the proposed methodology: (a) initial image, (c) expert limitation on detecting the outer border of the LT plaque, (c) the initial image with the segmented region marked with white color, (d) the expanded lumen border transformed to polar coordinates, the distal border of the TA (yellow line) is reduced to 0.75 mm and a new line is drawn (yellow dashed line) in order to detect the outer border of the LT, (e) the image characterized before applying the post processing step, and (f) The image characterized after applying the post processing step, (CA: white, LT: red, FT: dark green and MT: light green).

4.3 Dataset

20 seven OCT pullbacks from 22 patients, who underwent OCT investigation for clinical purposes after being consented, were used to train and validate the proposed methodology. The images were acquired by a C7XR Fourier Domain system (Lightlab Imaging, Westford, MA, USA) with the use of an automated pull-back device under continuous injection of contrast medium.

From our data set (27 OCT pullbacks) 556 OCT images were randomly selected and one expert annotated the lumen borders. Images portraying stented segments as well as frames with a minimal thrombus burden or a small amount of residual blood that did not affect image quality were included in the validation set. On the other hand frames portraying malapposed struts or having an increased thrombus burden or residual blood in the lumen were excluded from the analysis. We also excluded frames having motion artifacts [87]. To validate the performance of the proposed segmentation methodology we compared the expert's estimations with those derived by our approach.

Furthermore, two experts selected 200 images with no artifacts that portrayed diseased coronary segments identified the presence of tissues with a typical morphology and detected the CA, LT, FT and MT [51, 87, 148]. In this analysis MT was defined as the tissue that has features noted in CA and LT. Any disagreements in experts' annotations were resolved by consensus.

60 images were randomly selected and used to train the classification algorithm and the rest to validate the performance of the proposed methodology. From the training set of the 60 images a fully balanced dataset of 12000 pixels was created by randomly selecting 4000 pixels from each one of the annotated plaques LT, FT and MT. In the 140 images that were used for testing the methodology, 150 tissue regions with a typical morphology were annotated (34 CA, 36 LT, 47 FT and 33 MT) by the two experts and their mean estimation was used as gold standard to validate the proposed methodology.

4.4 Results

4.4.1 Validation Metrics

The mean estimation was extracted using the following methodology: we compute the mean perpendicular distances from the first to the second border estimation. For an image $I(i, j)$, let Eb_1 and Eb_2 be the border estimation of the first and the second observer, respectively and $PD(i, j)_{1,2}$ the perpendicular distance from a pixel $p_1 \in Eb_1$ to a pixel $p_2 \in Eb_2$, where $i, j \in I(i, j)$. The mean estimation of the two borders is defined as:

$$ME_{border} = \sum_{k=1}^{PL} \frac{PD_k(i, j)_{1,2}}{2}, \quad (4.8)$$

where PL is the perimeter of Eb_1 . In addition we computed the inter-observer variability for the two expert observers in the 150 tissue regions (CA: $-0.02 \pm 0.16 \text{ mm}^2$, LT: $-0.08 \pm 0.19 \text{ mm}^2$, FT: $-0.02 \pm 0.10 \text{ mm}^2$ and MT: $0.06 \pm 0.26 \text{ mm}^2$).

To validate the proposed methodology we computed Pearson's correlation coefficients, performed Bland-Altman analysis for the areas estimated by our methodology and the experts annotations and computed the positive predictive value (PPV). As true positive values (TP) we denote the common area detected by the methodology and annotated by the experts. As false positive (FP) values we denote the area detected by the methodology and not by the experts and as false negative (FN) the area annotated by the experts and not detected by the methodology. Additionally, the ratio of overlapping areas between the methodology and experts annotations was computed. The ratio of overlapping areas was defined as:

$$R_{over} = \frac{TP}{TP + FN}. \quad (4.9)$$

The ratio of overlapping areas is actual the sensitivity of the methodology in detecting the different tissue types. However, by computing the overlapping area (sensitivity) metric we are not able to understand if our methodology overestimates the detected areas ($FP > FN$). Thus, we computed an additional validation metric the ratio of non-overlapping areas defined as:

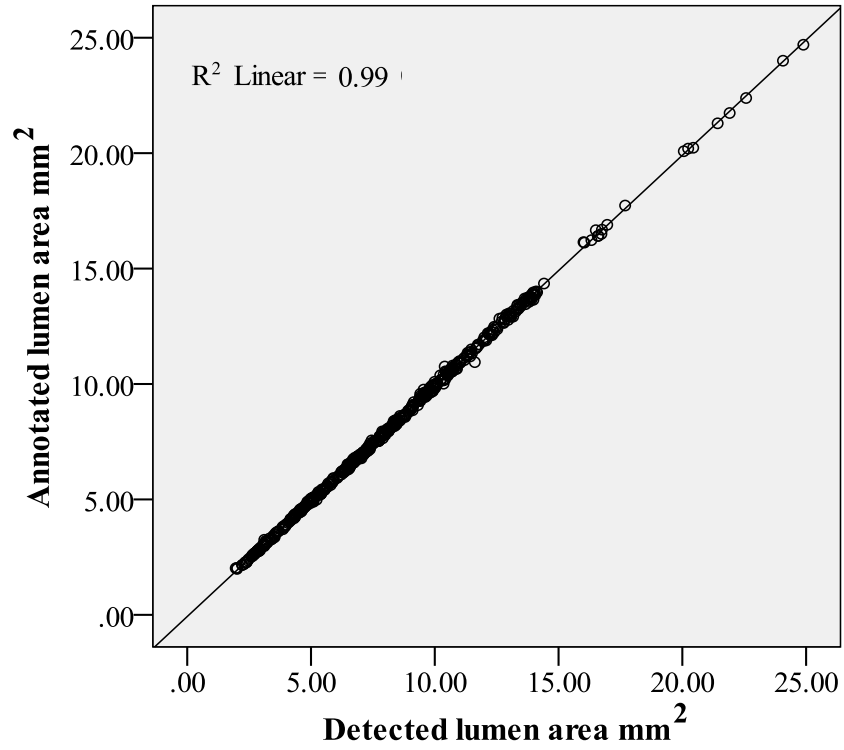
$$R_{non-over} = \frac{FN + FP}{TP + FN}. \quad (4.10)$$

4.4.2 Validation Results

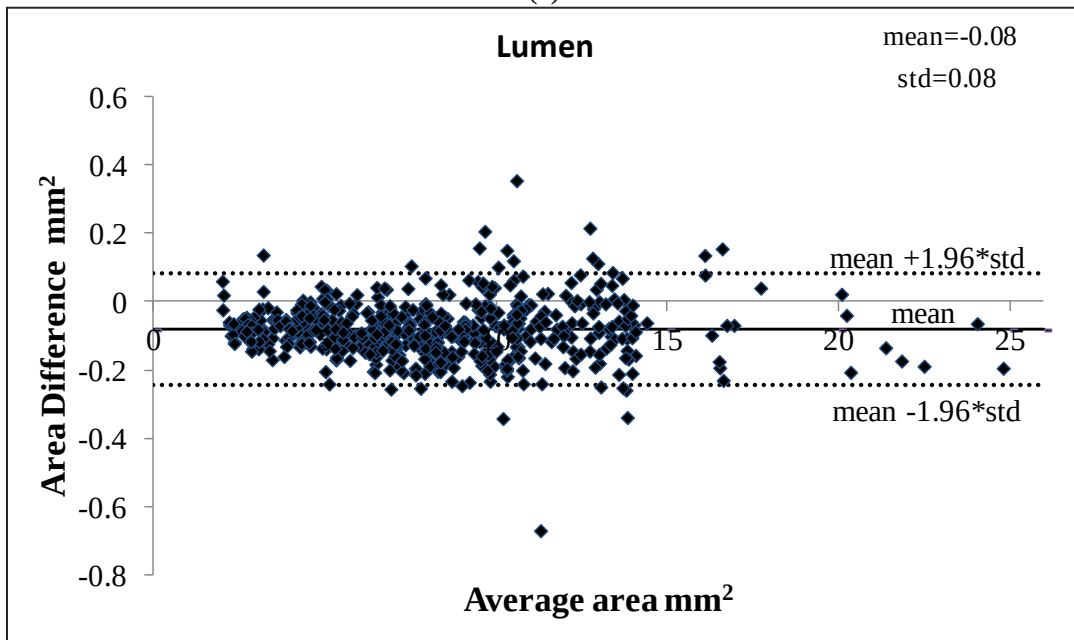
An excellent agreement was noted for the lumen, CA, FT, LT and MT areas estimated by our approach and the experts (Table 4.1). The correlation coefficient analysis and the Bland and Altman plots for the lumen areas are shown in Figure 4.6. Figure 4.7 shows the correlation plots between the areas estimated by our approach and the areas estimated by the two experts for the 4 tissue types (CA, LT, FT and MT) while Figure 4.8 shows the results of the Bland-Altman analysis.

Table 4.1: Results of the proposed methodology using different validation metrics.

Validation Metrics	Lumen	Plaque Type			
		CA	LT	FT	MT
Pearson's correlation coefficient	0.99	0.96	0.96	0.97	0.96
Bland-Altman limits of agreement	-0.080 ± 1.96x0.082 mm ²	0.008 ± 1.96x0.04 mm ²	0.06 ± 1.96x0.14 mm ²	-0.09± 1.96x0.13 mm ²	-0.04± 1.96x0.1 mm ²
Overlapping area (sensitivity)	0.99	0.81	0.71	0.87	0.81
Non-overlapping area	0.02	0.35	0.51	0.37	0.47
Positive predictive value	0.98	0.83	0.76	0.78	0.74



(a)



(b)

Figure 4.6: (a) Correlation plot and (b) Bland-Altman plots for the lumen areas estimated by our methodology and the expert.

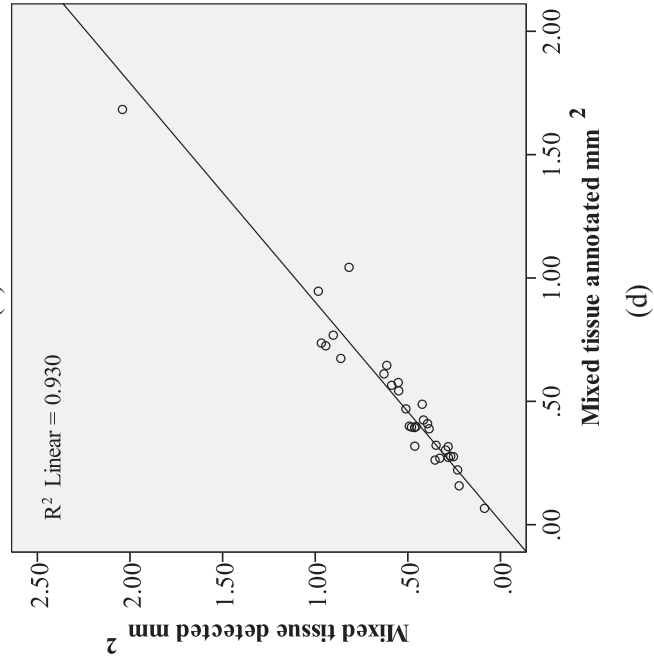
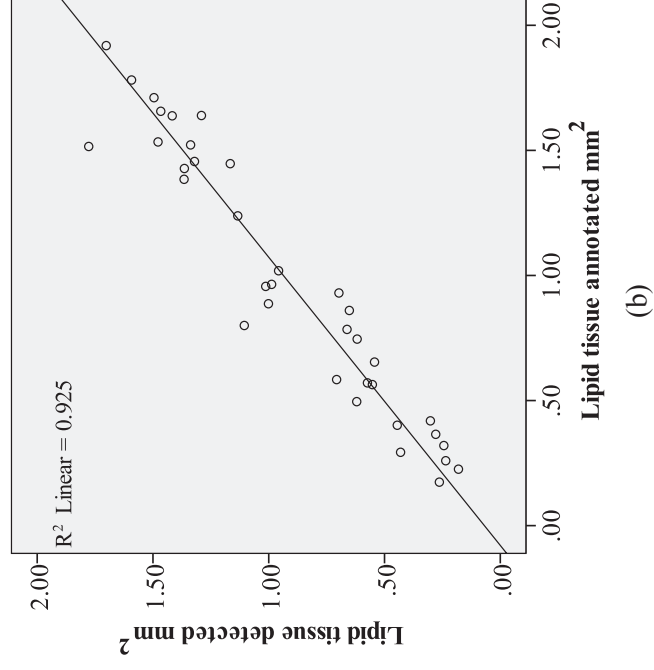
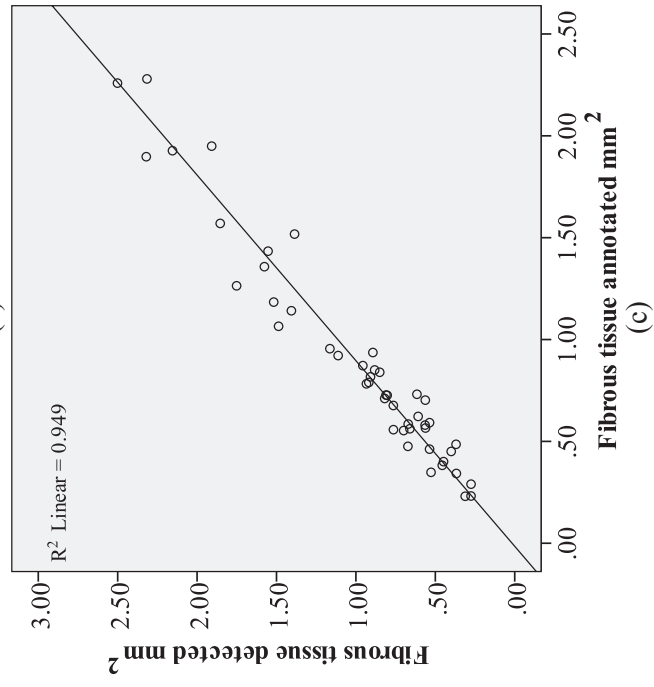
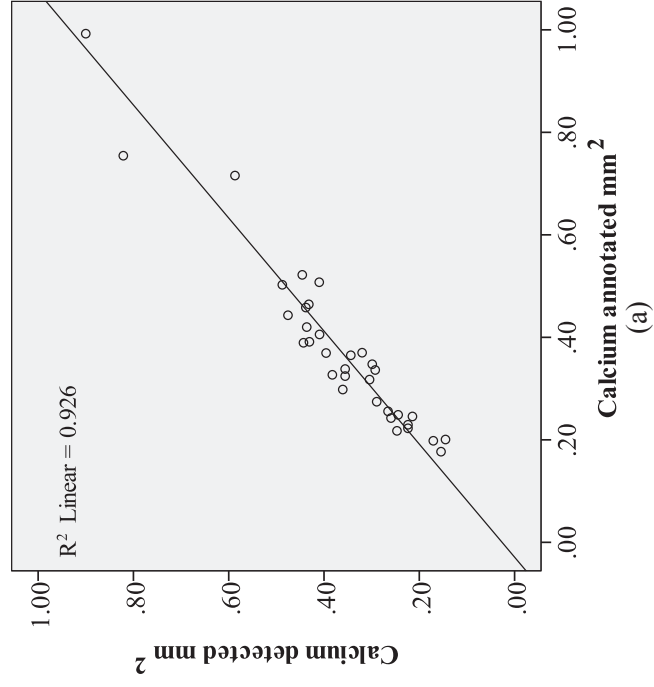


Figure 4.7: Correlation plots for the (a) CA, (b) LT, (c) FT and (d) MT areas estimated by our methodology and the two experts.

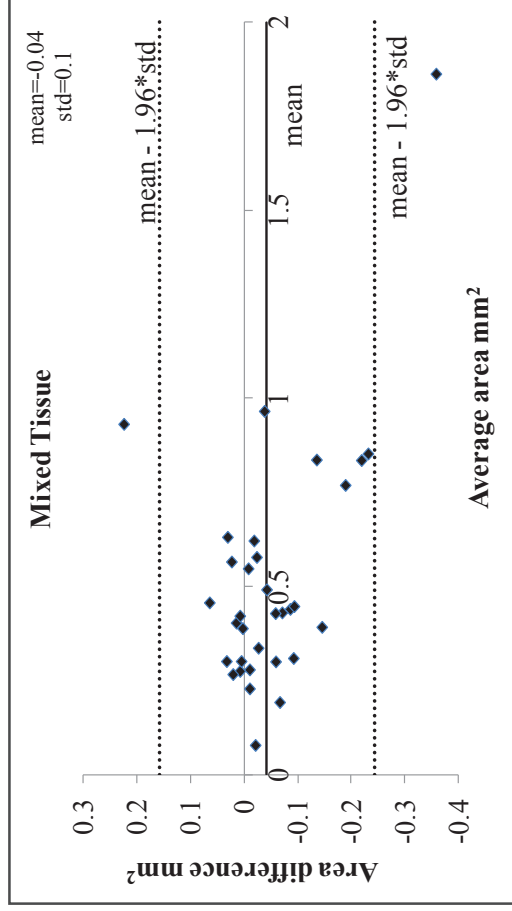
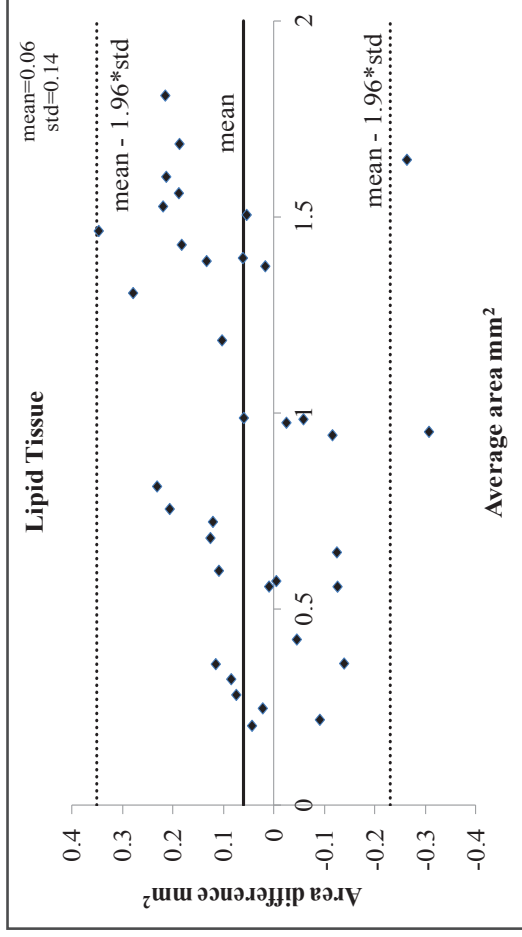
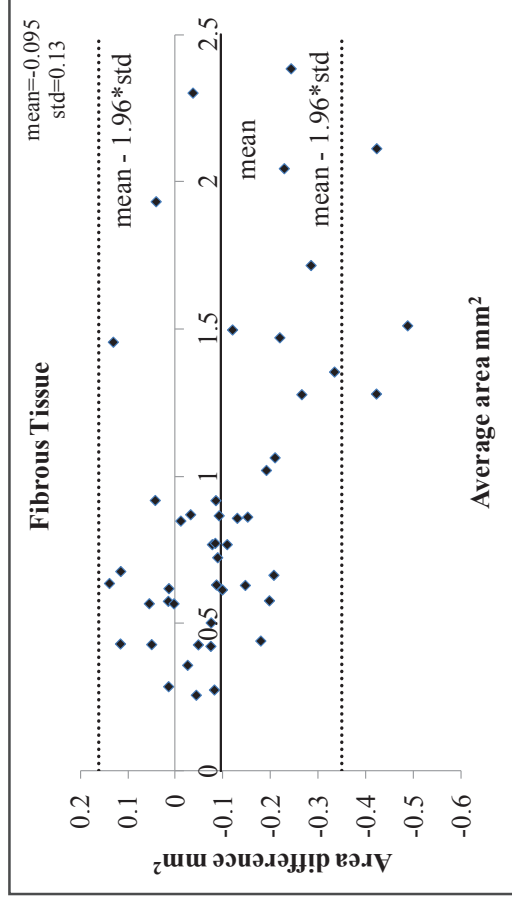
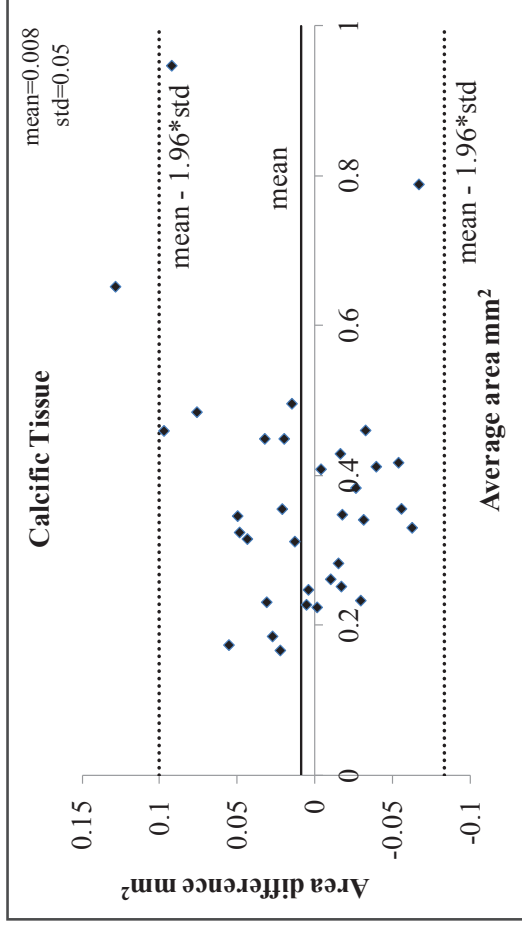


Figure 4.8: Bland-Altman plots for the (a) CA, (b) LT, (c) FT, and (d) MT areas estimated by our methodology and the two experts.

The qualitative validation metrics showed an excellent agreement between the proposed methodology and the estimations of the expert for the lumen areas (overlapping area ratio: 0.99, non-overlapping area ratio: 0.02). The overlapping area analysis for the tissue types demonstrated that the proposed approach classifies correctly most of the pixels detected by the two experts that portray tissues with a typical morphology (overlapping area ratio: 0.81 for the CA, 0.71 for the LT, 0.87 for the FT and 0.81 for the MT). The disagreement between the presented methodology and the mean estimations of the experts was low for the CA and FT tissue (non-overlapping area metric: 0.35 and 0.37 respectively). On the other hand for the LT and the MT the non-overlapping area ratio was 0.51 and 0.47 respectively, indicating that the position of the detected tissue within the vessel wall is not exactly the same. These discrepancies noted in the LT and MT are due to the limited ability of the proposed methodology to accurately detect their lateral extremities and to the fact that the distal border of both tissues is not visible in OCT. Examples of the proposed methodology in detecting the lumen borders are shown in Figure 4.9 while Figure 4.10 presents application examples of the proposed methodology in detecting the 4 tissue types.

4.4.3 Application

To assess and visualize the lumen borders and plaque composition in FD – OCT examinations an in - house tool was developed using Matlab r2011b. To speed up the algorithm C++ code was implemented and executed using Matlab's MEX libraries. The user can import FD – OCT examinations (raw data files extracted from the FD-OCT equipment) view the OCT images, view the longitudinal view of the pullback, characterize the different plaque types and detect the lumen borders of the pullback. The time complexity of the proposed methodology for detecting the lumen border and characterizing the plaque is approximately 40 seconds per frame using a core i7 desktop computer with 8 GB of RAM.

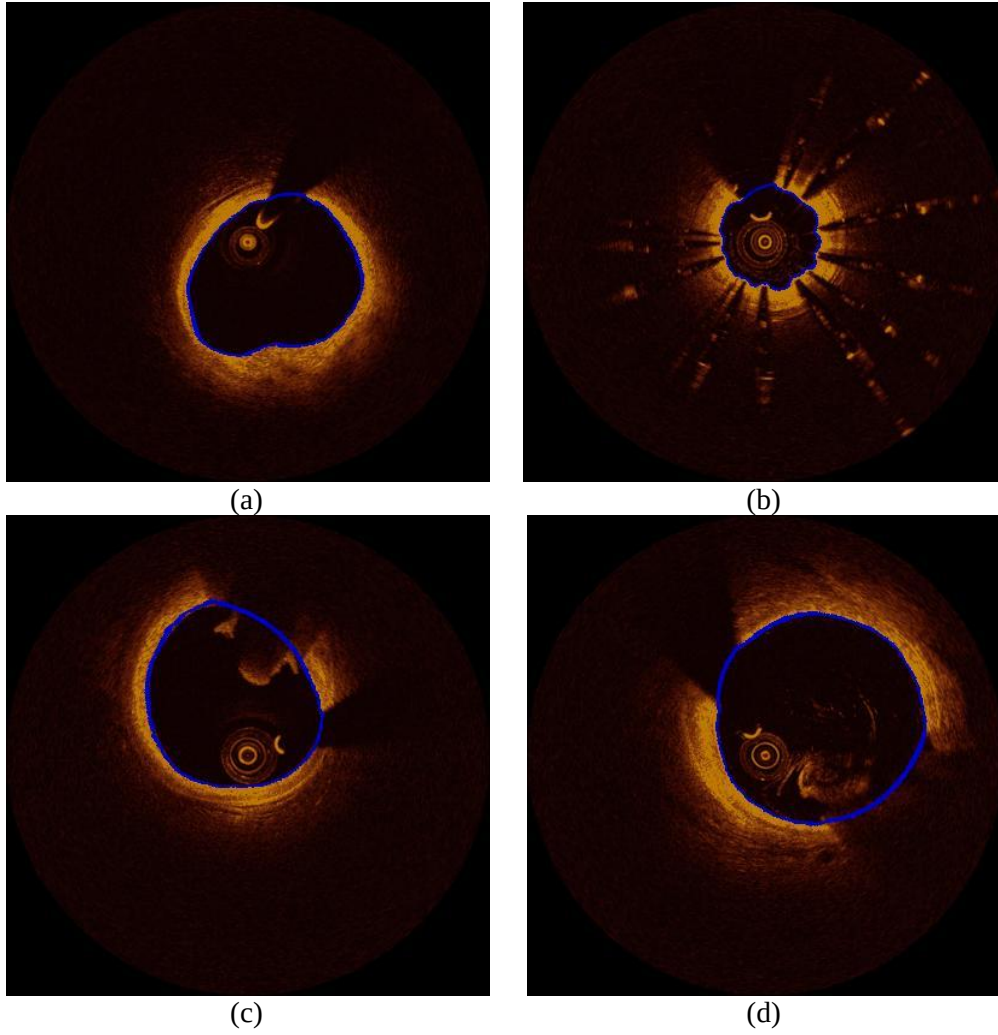


Figure 4.9: Results of the proposed lumen detection step in (a) a native segment, (b) in a stented segment, (c) in a segment having thrombus, and (d) in a segment having residual blood. The lumen border is marked with blue.

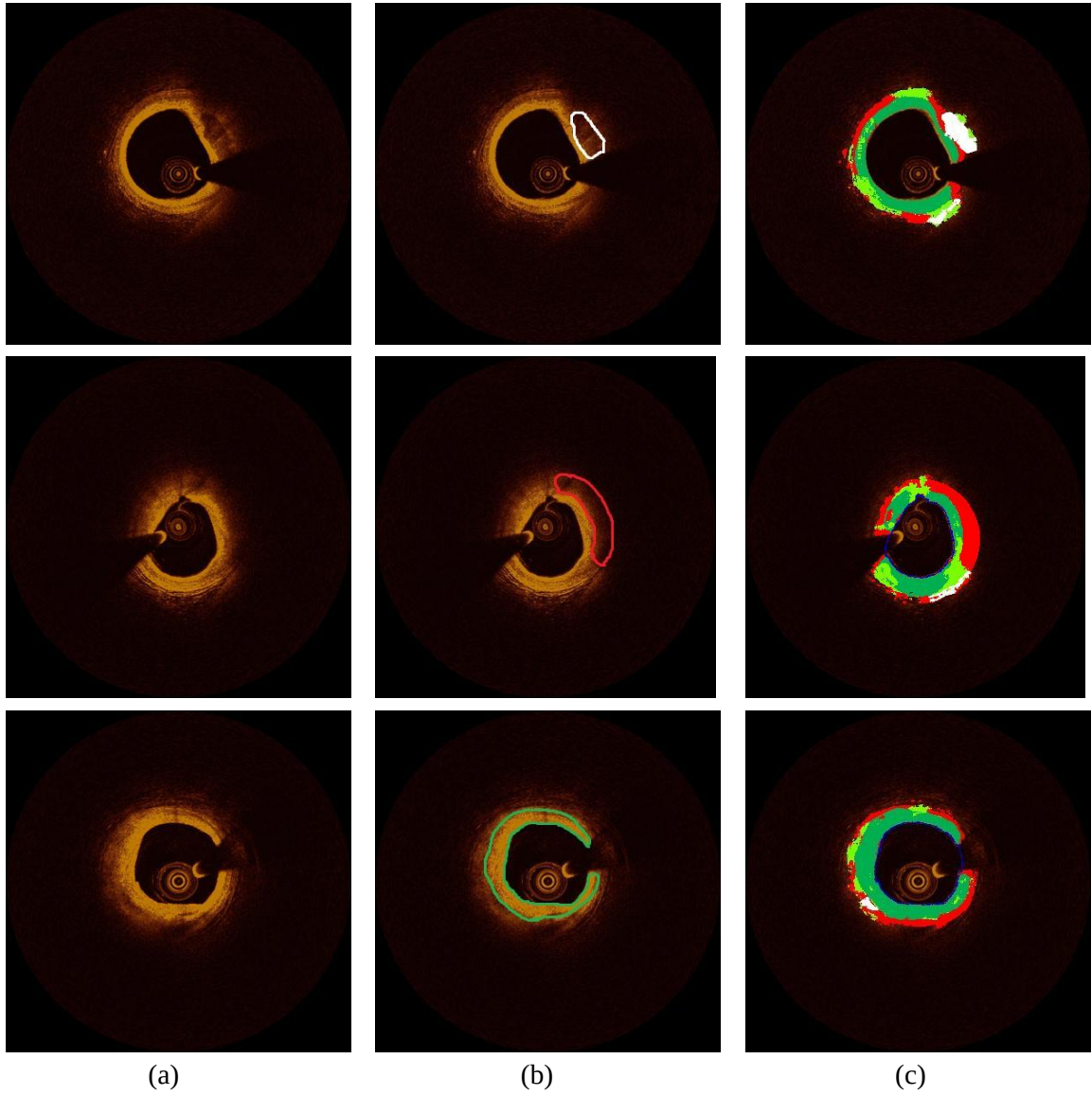


Figure 4.10: Application examples of the proposed methodology: (a) Initial images, (b) annotations over the initial images (white-CA, red-LT, green-FT and light green MT) over the initial image, (c) color coded images produced by our methodology using the initial images (white-CA, red-LT, green-FT and light green MT).

4.5 Discussion

A fully automated luminal border detection and plaque characterization methodology that allows expedite analysis of the FD – OCT data is presented. In the proposed methodology the luminal borders are extracted and used to define the tissue area (TA). On the extracted

TA, segmentation techniques are applied, initially differentiating the extravascular tissue from the plaque and then four tissues types are detected namely: CA, LT, FT and MT.

The two methodologies that have been presented in the past for characterizing the type of the plaque in FD – OCT data relied [88, 89] on the analysis of the reflected light and attempted to correlate the attenuation coefficients with various plaque formations. They both focused on the separate processing each A – line and used the attenuation coefficient of the light to correlate each individual line with a specific plaque type. However, these techniques do not combine information provided by adjacent A – lines to classify more accurately plaque components and failed to define a cutoff value for each plaque type so as to develop a precise and complete plaque classification methodology.

On the contrary the methodology presented in this study is a fully automated approach that is capable to detect the luminal borders and identify and quantify the composition of the superficial plaque (depth: 1.5mm). To our knowledge this is the first approach that utilizes image processing, medical knowledge and machine learning techniques to characterize different tissue types and it is able to process without user interaction a sequence of good quality FD-OCT images that do not have artifacts. The validation of the lumen detection methodology showed that our approach can address some of the common artifacts seen in FD-OCT images (i.e., the catheter artifact, small thrombi or residual blood) and detect accurately the lumen borders (overlapping area ratio: 0.99 and non-overlapping area ratio: 0.02). We also found good agreement between the manual annotations and the estimations of the methodology for the measured areas in the 4 plaque components (CA: $0.008 \pm 1.96*0.04 \text{ mm}^2$, LT: $0.06 \pm 1.96*0.14 \text{ mm}^2$, FT: $-0.09 \pm 1.96*0.13 \text{ mm}^2$ and MT: $-0.04 \pm 1.96*0.1 \text{ mm}^2$). In addition, the qualitative validation of the proposed approach for the four tissues types demonstrated that the methodology characterize correctly most of the pixels portraying these tissues (overlapping area ratio >0.71 for all tissue types). However the non-overlapping area analysis showed an increased non-overlapping area ratio for the LT and MT (LT: 0.51 and MT: 0.47) indicating that the proposed methodology has a limited ability to detect the lateral extremities of these tissues. Additionally, there is a discrepancy between the approximation of the observers and the proposed methodology for the distal border of both tissues which is not visible in OCT (Figure 4.10).

To date FD - OCT images were analyzed manually by expert observers. This process however is time consuming, it is restricted to a fraction of the obtained data i.e. 1 frame at every 0.4 mm or 1 mm interval and the accuracy of the reported results relied on the expertise of the observers. The proposed methodology appears to overcome the abovementioned limitations as it is capable to process the FD - OCT data and provide reliable estimations for the lumen border and plaque components. However further improvements are required and a more robust validation using histological data is needed before this methodology have applications in clinical setting. A potential combination of the presented methodology with the information provided by the data derived from the analysis of the reflected OCT signal (i.e., the backscattering and attenuation coefficients) is likely to provide additional information (i.e., detection of microstructures such as the presence of macrophages and neo-vessels) and allow more accurate detection of different tissue types and especially of the LT and MT.

4.5.1 Limitations

A significant limitation of the proposed methodology is the fact that it is unable to detect the media which is necessary for the characterization of the phenotype of the plaque (i.e., normal vessel wall, pathological intimal thickening, fibroatheroma, thin cap fibroatheroma, etc) and the identification of the normal vessel wall [4]. Additionally the methodology is unable to detect microstructures such as macrophages and neo-vessels and measure the thickness of thin fibrous caps over fibroatheromas.

The estimations of expert observers were used as gold standard to validate the performance of the proposed methodology in tissues with a typical morphology. However, quite often the morphology of a tissue is not typical and the experts may provide unreliable estimations especially in the characterization of deeply embedded tissues [155]. Therefore further validation is required using histological data; these data will allow a more precise assessment of the methodology's performance and will provide the substrate and additional information which can be used to re-train and optimize the efficacy of the proposed methodology.

Finally, the presented methodology fails to provide accurate estimation about the composition of the plaque in stented segments and in frames having artifacts (i.e., non-uniform rotational distortion, proximity artifacts or tangential signal dropout artifacts). Moreover, although the methodology is able to detect the lumen border in images having a small amount of residual blood or small thrombi it is unable to provide reliable estimation about the composition of the plaque in these segments.

Chapter 5: Computed Tomography (CT)

5.1 Introduction

5.2 Proposed method

5.3 Dataset

5.4 Results

5.5 Discussion

5.1 Introduction

Accurate detection and quantification of coronary plaque burden and assessment of its characteristics appear to affect prognosis in patients with coronary artery disease and they are of outmost importance for assessing the effect of new pharmacological treatments [79, 156, 157]. Intravascular ultrasound (IVUS) is considered as the method of choice in assessing atherosclerotic plaque burden [67, 68, 158] and plaque characteristics [159]. However, IVUS is an invasive method which has risks, it cannot be used in the contemporary practice for the study of the entire arterial tree and it is not indicated for asymptomatic individuals.

Coronary Computed Tomography angiography (CTA) [160] has been introduced as a non-invasive imaging modality to visualize the coronary arteries. Based on the Hounsfield Units (HU) scale, the inner and outer wall of the vessels can be estimated and the atherosclerotic plaques can be classified to Calcified (CP) and Non-Calcified (NCP). Several methods have been presented in the literature that have been developed to assess plaque burden and characterize the composition of the plaque from the CTA [96, 101-107]. The outcome of the proposed approaches was compared with the estimations of IVUS which was treated as the reference gold standard.

However, all the above mentioned methods were semi-automated requiring increased manual interaction for the extraction of the lumen and outer vessel wall borders and did not present any classification or plaque reconstruction method. They only correlated the plaques using manual estimations [96, 102, 103, 106] or commercially available

softwares [104, 105, 107]. In addition they focuses only on volume comparison ignoring the spatial location of the volumes within the studied IVUS and CT segments.

A new-semi automated methodology for the 3D Reconstruction of coronary arteries and plaque morphology using CTA is presented. In order to validate the 3D plaques detected by the proposed method we used as gold standard the results of a recently presented IVUS plaque characterization method [158]. The detected 3D CP plaques were extensively validated and a significant correlation was observed between CTA and IVUS. Additionally, the reconstructed inner and outer vessel wall models derived by the proposed methodology were compared with those reconstructed using the IVUS borders. The comparison results demonstrated an excellent agreement. The innovative aspects of the proposed methodology are:

- it focuses on the reconstruction of both the lumen, outer vessel wall and the CP plaques,
- the methodology is semi-automated and the user intervenes only in the plaque parameters adaptation stage (third stage),
- it is able to reconstruct the lumen, outer vessel wall and plaque geometry taking into consideration the vessel curvature and thus it allows assessment of the distribution of the plaque in the 3D space,
- the validation is performed in 3D space by comparing the volumes of the lumen, outer vessel wall and CA, derived by CTA and by IVUS after a spatial co-registration of the IVUS onto the CTA data.

5.2 Proposed method

The proposed methodology includes 6 stages: in the first stage the CTA images are pre-processed to remove the artifacts and detect the vessel silhouette. In the second stage the artery borders are roughly defined and used to extract the initial centerline of the vessel. In the third stage, the plaque parameters are adapted in the CTA's HU scale to optimize a 4-component Gaussian Mixture Model (GMM) [126] to the HU histogram. In the fourth stage, radial images are produced perpendicular by to the initial centerline which is further processed in the fifth stage where the lumen and the outer wall border of the

vessel are detected and the plaque region is estimated. Finally, in the sixth stage the 3D surfaces of the lumen - outer vessel wall and CP plaques are constructed. The stages of the proposed methodology are shown schematically in Figure 5.1.

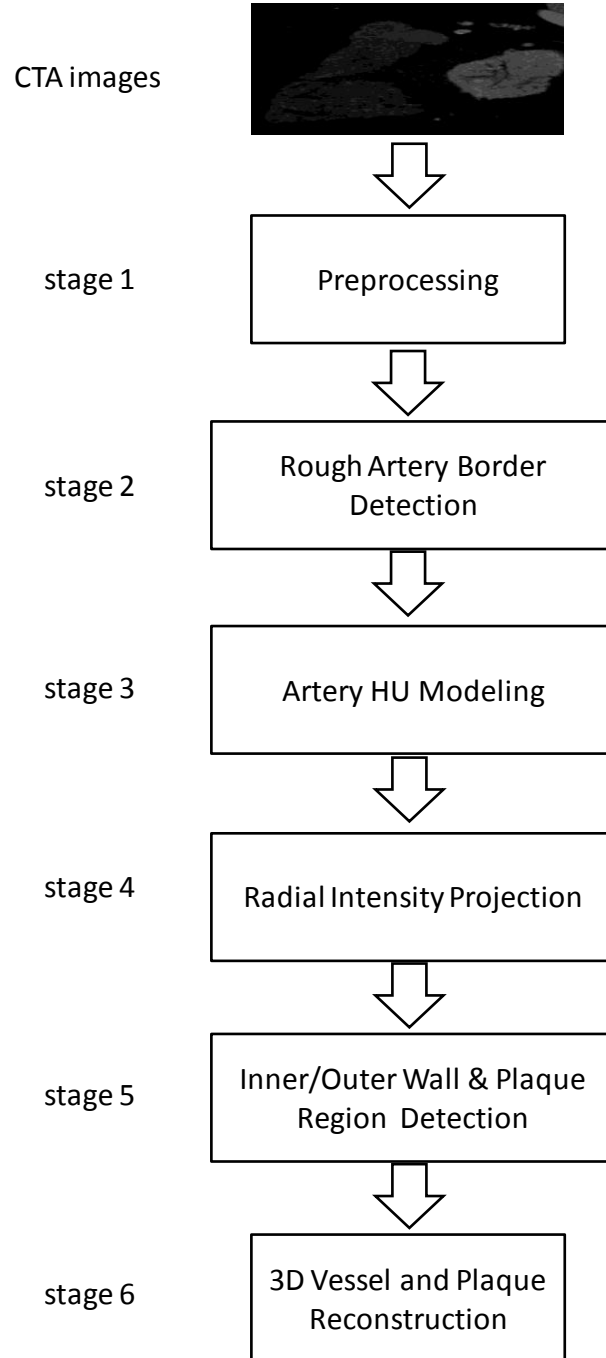


Figure 5.1: The stages of the proposed methodology starting from the initial CTA images and concluding with a full 3D model of inner/outer walls and plaque objects.

5.2.1 Preprocessing

A pre-processing step is applied on the acquired CTA images that includes:

- contrast enhancement [123],
- image thresholding [128] which allows identification of the vessel,
- and the application of a Frangi Vesselness filter [161] which permits detection of structures which correspond to coronary vessels.

5.2.2 Rough Artery Border Detection

The following process is implemented to extract the initial lumen borders and define a first approximation of the luminal centerline:

- The user defines a seed point in the main artery (the only user action in the whole methodology).
- A region growing method is applied using 26-connected pixels neighbor connectivity for lumen border detection.
- Edge Detection is applied to estimate the lumen borders.
- The lumen border are used to define an initial vessel centerline extracted using the center of gravity of each curve [162]. Then the centerline is smoothed (using moving average filter) in order to have a smooth first derivative of the centerline curve.

The initial centerline points are fitted with a B-Spline. The B-Spline is used in order to provide smooth first derivatives needed for producing a radial image perpendicular to a specific centerline point (Figure 5.2).

5.2.3 Image HU Modeling

The distribution of HU intensity over the artery is modeled by a Gaussian Mixture Model (GMM) [126]. A GMM model is a parametric probability density function which is represented as a weighted sum of M Gaussian component densities given as:

$$p(x | \lambda) = \sum_{i=1..M} w_i N(x | \mu_i, \sigma_i), \quad (5.1)$$

where x is a 1-dimensional continuous data (HU value), w_i the mixture weights, μ_i and σ_i are the mean vector and the standard deviation of the component Gaussian densities ($N(x|\mu_i, \sigma_i)$), respectively and λ is the set:

$$\lambda = \{w_i, \mu_i, \sigma_i\} \quad (5.2)$$

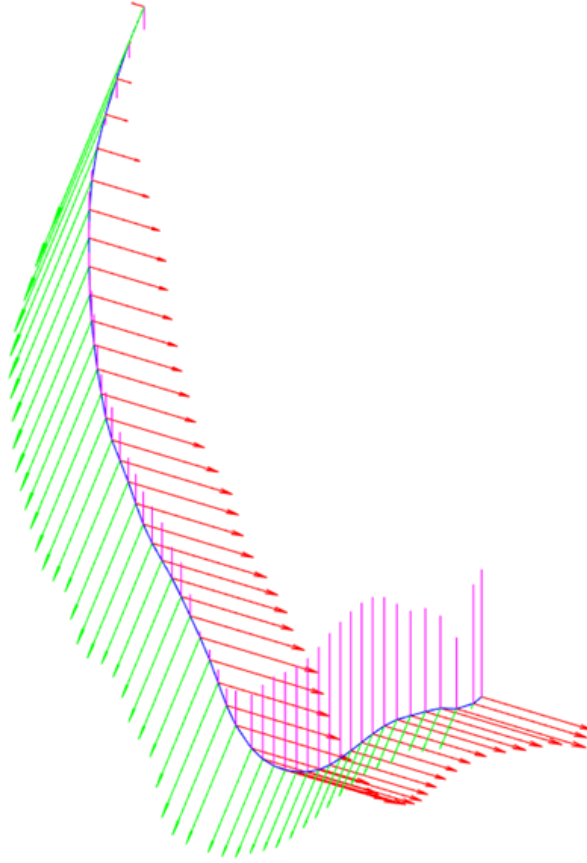


Figure 5.2: The centerline (blue) of an artery segment with the derivatives (red, purple and green) at each point.

The maximum posterior probability of the sample x belongs to a specific component k , and is given as:

$$P(M = k | x) = \frac{w_k N(x; \mu_k, \sigma_k)}{\sum_{i=1..M} w_i N(x; \mu_i, \sigma_i)} \quad (5.3)$$

In our approach each component of the GMM models corresponds to one of the following classes: i) lumen, ii) calcified plaque, iii) non-calcified plaque and iv)

background. In order to train the GMM model the user manually selects 4 points that correspond to the four classes ($X \in \{X_L, X_{CP}, X_{NCP}, X_B\}$) and then each pixel is classified to one of the four classes based on the class/component with the maximum posterior probability.

5.2.4 Radial Image Projection (RIP)

In the initially extracted centerline at every 0.2 mm a point is defined and a radial image is produced perpendicularly to the centerline point using the B-spline derivatives extracted on the specific point [123]. These images are called Radial Intensity Projection (RIP) images. For an accurate RIP image the estimated centerline point is required to lie in the middle of the lumen. However, as described in Section 5.2.2 the centerline is smoothed in order to avoid surface intersections as depicted in Figure 5.3. This smoothing results in an offset of the estimated centerline from lumen's center. In order to overcome this problem an iterative correction process is applied. The iterative process includes the following steps:

- The radial image is produced for the given centerline and the current offset (initially offset is set to zero).
- The center of mass of the radial image is calculated and a new offset is computed.

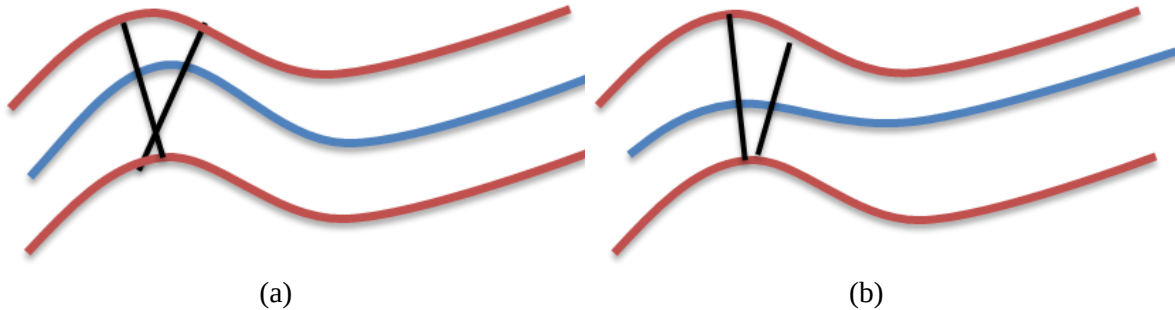


Figure 5.3: Centerline smoothing: (a) rather accurate centerline and the intersection imposed on perpendicular surfaces and (b) smoothed centerline where no intersections of perpendicular surfaces occurs.

The process is repeated five times (the cases with 2,3,4,5 and 6 repetitions were also tested), number which is sufficient to minimize the offset. An example of the above

procedure is depicted in Figure 5.4 for a synthetic image with a binary circle with an initial large offset from the center Figure 5.4-a.

The initial center of the circle should lie in the center of the image. It can be observed that after a few iterations the radial image is corrected (Figure 5.5-d). After the correction step the final RIP image is used for the object extraction process. An example of the RIP image is given in Figure 5.5-b.

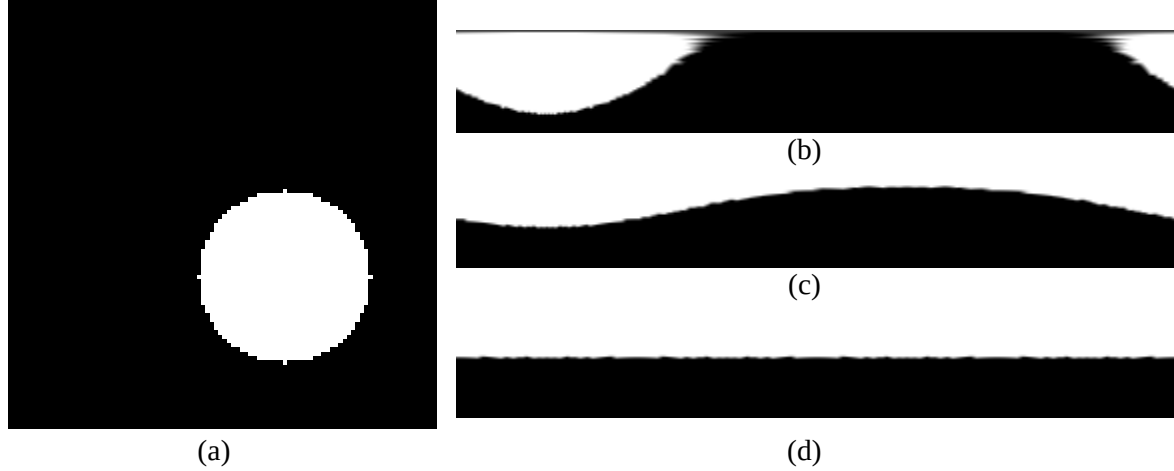


Figure 5.4: The radial image correction process on a synthetic image (a) with large offset from the center, (b) in the first iteration, (c) in the third iteration and (d) in the last (fifth) iteration.

5.2.5 Inner - Outer vessel wall and Plaque Region Detection

From the RIP of each slice we extract the Region of Interest (ROI) of the artery. The ROI is extracted based on the detection of the zero crosses ($HU < -130$) (Figure 5.5-b). The areas below the zero crossing are removed (Figure 5.5-c). After defining the ROI, the HU profile of the RIP image is calculated for each radius interval (Figure 5.5-d). Based on the profile, we classify the ROI using the GMM model (Figure 5.5-e).

Based on the ROI classification we define profile regions. The lumen wall is set to the first non-lumen point. The outer vessel wall is set to the last non-background point. Therefore, two curves are created one for the lumen and one for the outer vessel wall. After smoothing, the curves are transformed back to the Cartesian coordinates.

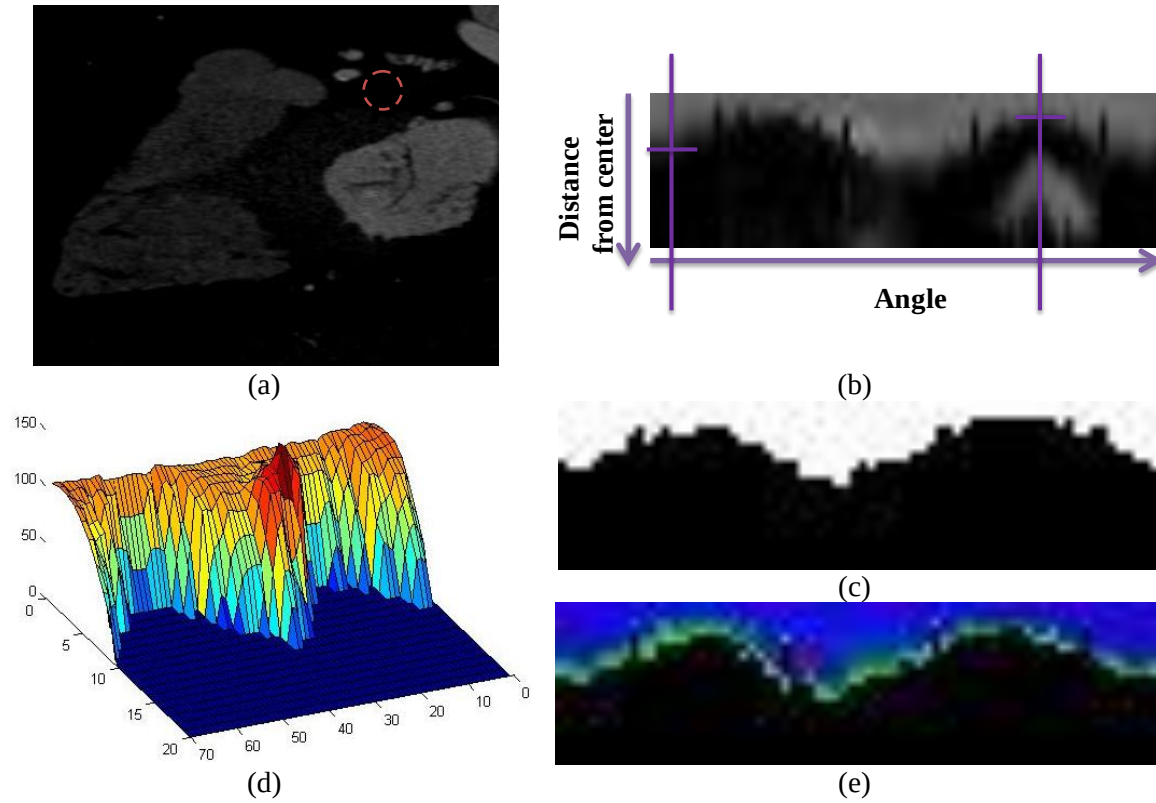


Figure 5.5: (a) The original DICOM image. In the red circle is the vessel region detected based on the initial stages of our methodology (stages 1, 2), (b) The radial image produced taking the RIP image corresponding to the centerline point of the specific vessel region and zero crosses of the RIP image, (c) The resulting ROI, (d) The HU profile from the RIP image and the extracted ROI, and (e) The classified ROI.

Using the original radial image, the area between the inner and outer vessel wall defines the plaque region. The classified ROI (Figure 5.6-a) of the image is thresholded, keeping only pixels belonging to the specific plaque type (Figure 5.6-b). resulting to binary objects. Then these binary objects are identified and the boundary of those objects is extracted. Smoothing is performed on the extracted boundary curves. Based on the physical size of each pixel the area of the plaque can be determined.

5.2.6 3D Surface Construction

N points for each of the inner/outer vessel wall and plaque lesions are extracted clock wisely for each image of the centerline. Then the triangulation approach is implemented to construct the mesh faces. The border points (N) (inner/outer wall or plaque) of two

sequential images, frame i and frame $i+1$ are connected constructing a triangle mesh. Thus $2 \times N$ surfaces are constructed for each pair of sequential images.

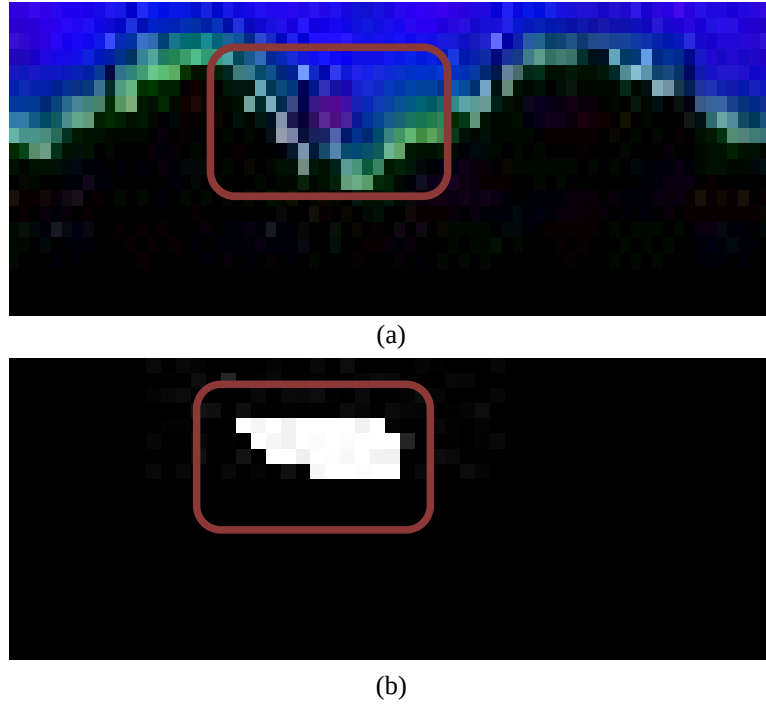


Figure 5.6: (a) region with pixels classified as calcific plaque, and (b) the corresponding thresholded image where calcified objects are detected.

5.3 Dataset

The data acquired from 20 patients, that underwent coronary angiography, IVUS imaging and CTA for clinical purposes, were used to validate the proposed methodology. Data collection were provided by the Invasive Cardiology Laboratory of the IFC-CNR, Pisa and by Heart Institute, University of São Paulo, São Paulo, Brazil. The study protocols were approved by the local Ethics Committees. Coronary CTA was performed using a 64-slice scanner (Light Speed, General Electric Health Care).

Patients with a significant ($>50\%$) luminal stenosis on CTA had further invasive investigations. Coronary angiography was performed with the use of a flat panel system (Innova 2100, GEHC). To further assess the severity of the detected lesions on X-ray angiography, IVUS imaging was performed with the use of 64-crystal electronic ultrasound probe (Eagle-Eye, Volcano corporation) that was pulled-back by an automated pull back device at a speed 0.5mm/sec. The acquired IVUS and CTA data were stored in DICOM format and transferred to a workstation for off-line analysis.

All CTA data were processed using the proposed methodology and the IVUS data using a previously described plaque characterization method [158]. CP was detected in 20 IVUS examinations and were used to validate the 3D CP detected by the proposed CTA methodology

5.4 Results

To validate the 3D reconstructed arteries and the detected 3D CP detected by the proposed methodology, IVUS plaque characterization [158] and 3D IVUS reconstruction was performed [163] in corresponding segments and the non-invasive-based (CTA) and invasive imaging (IVUS) based models were compared.

5.4.1 CT-IVUS segments correlation

Experts selected manually, segments of arteries which were assessed with both CTA and IVUS imaging. Side branches were used as fiducial points to define correspondence between CTA and IVUS.

5.4.2 Artery and CP reconstruction using IVUS

IVUS based reconstruction was performed using the methodology introduced in [163]. The method combines IVUS and X-ray angiography and places the lumen and media adventitia borders detected in IVUS frames [21] onto the 3D luminal centerline derived from two angiographic projections. In the present work instead of using the 3D centerline derived from angiography, we used the 3D center line derived from CTA. To detect the CP in the selected IVUS segments a recently developed plaque characterization method [[158] was employed. Hence, the lumen-outer vessel wall morphology and the CP of the arteries are reconstructed by combining IVUS and CTA.

5.4.3 Sensitivity analysis of GMM initialization

To examine the sensitivity of the GMM model for each set of HU units (selected by the user): $X \in \{X_L, X_{CP}, X_{NCP}, X_B\}$ a new set is calculated: $X' \in \{X'_L, X'_{CP}, X'_{NCP}, X'_B\}$, where $X' = X \pm \Delta T$ (ΔT was randomly added or subtracted from X according to

probability 0,5), $\Delta T = (20, 40, 60, 80, 100, 120, 140, 160, 180, 200)$. Therefore, for each X' the GMM parameters are estimated and produce the inner and outer volume of three arteries that were used for testing the sensitivity of GMM initialization. To estimate the percentage of volume change($Volume^{\Delta T}$) for each ΔT (or X') we computed the percentage change as:

$$C = 100 - \frac{|Volume^{\Delta T} - Volume^0|}{Volume^0}, \quad (5.4)$$

where $Volume^0$ is the initial volume. However, the computed volumes in all three arteries which were used to test the sensitivity of GMM initialization, did not change significantly. In all three arteries, both inner and outer volumes C ranged from 99,4 - 99,9 %.

5.4.4 Validation

The inner and outer walls reconstructed by the two modalities were compared using their volume and surface area. The 3D CP objects detected by the two modalities were compared using the following metrics: volume, surface area (the total area of the object's faces), maximum length, inner angle and the overlapping volume (volume detected in both CTA and IVUS models) between two objects. As maximum length of an object we denote the distance between the first and the last voxel of the object along the centerline and as inner angle θ_{in} the mean circumferential extend of the n 2D slices, with angle θ , that were used to reconstruct the object (Figure 5.7):

$$\theta_{in} = \frac{\sum_{i=1}^n \theta_i}{n}. \quad (5.5)$$

Using the previously described metrics, Pearson Correlation Coefficient (Table 5.1) and Bland–Altman plots were computed to measure the correlation between CTA and IVUS CP, inner and outer wall. The Bland-Altman and correlation plots results for detecting CP and inner outer wall are shown in Figure 5.8 and Figure 5.9 respectively. Figure 5.10 presents the Bland-Altman and correlation plot results for the overlapping volume of CP objects.

The volume validation results showed that 3D CP plaques and the inner and outer vessel wall volumes of CTA correlated well with those obtained from IVUS, having Pearson's correlation (r) 0.79, 0.87 and 0.83, respectively. The correlation of CP objects is higher when their length ($r = 0.86$), inner angle ($r = 0.95$) and surface area ($r = 0.88$) is compared; meaning that although the methodology detects the CP objects in the same location within the arterial wall as they have the same maximum inner angle length, their volume is increased in the CTA probably because IVUS is unable to assess then entire CP since there is an acoustic shadow behind calcium deposits [164, 165]. Another explanation of this finding is that in CTA high-attenuation structures, such as calcified plaques, appear enlarged (bloomed) [166]. These combined effects result in a moderate volume correlation ($r = 0.79$). The differences in the estimations of CTA and IVUS for the calcium component are shown in Figure 5.11.

Table 5.1: CTA and IVUS comparison of volume, surface area, length and maximum inner angle.

Metrics	CP objects		Inner wall		Outer wall	
	Pearson's correlation r	Degree of correlation R^2	Pearson's correlation r	Degree of correlation R^2	Pearson's correlation r	Degree of correlation R^2
Volume	0.79	0.64	0.87	0.76	0.83	0.69
Volume Intersection vs IVUS	0.96	0.94	-	-	-	-
Volume Intersection vs CT	0.82	0.71	-	-	-	-
Surface area	0.86	0.75	0.94	0.89	0.94	0.89
Length	0.95	0.91	-	-	-	-
Angle	0.88	0.78	-	-	-	-

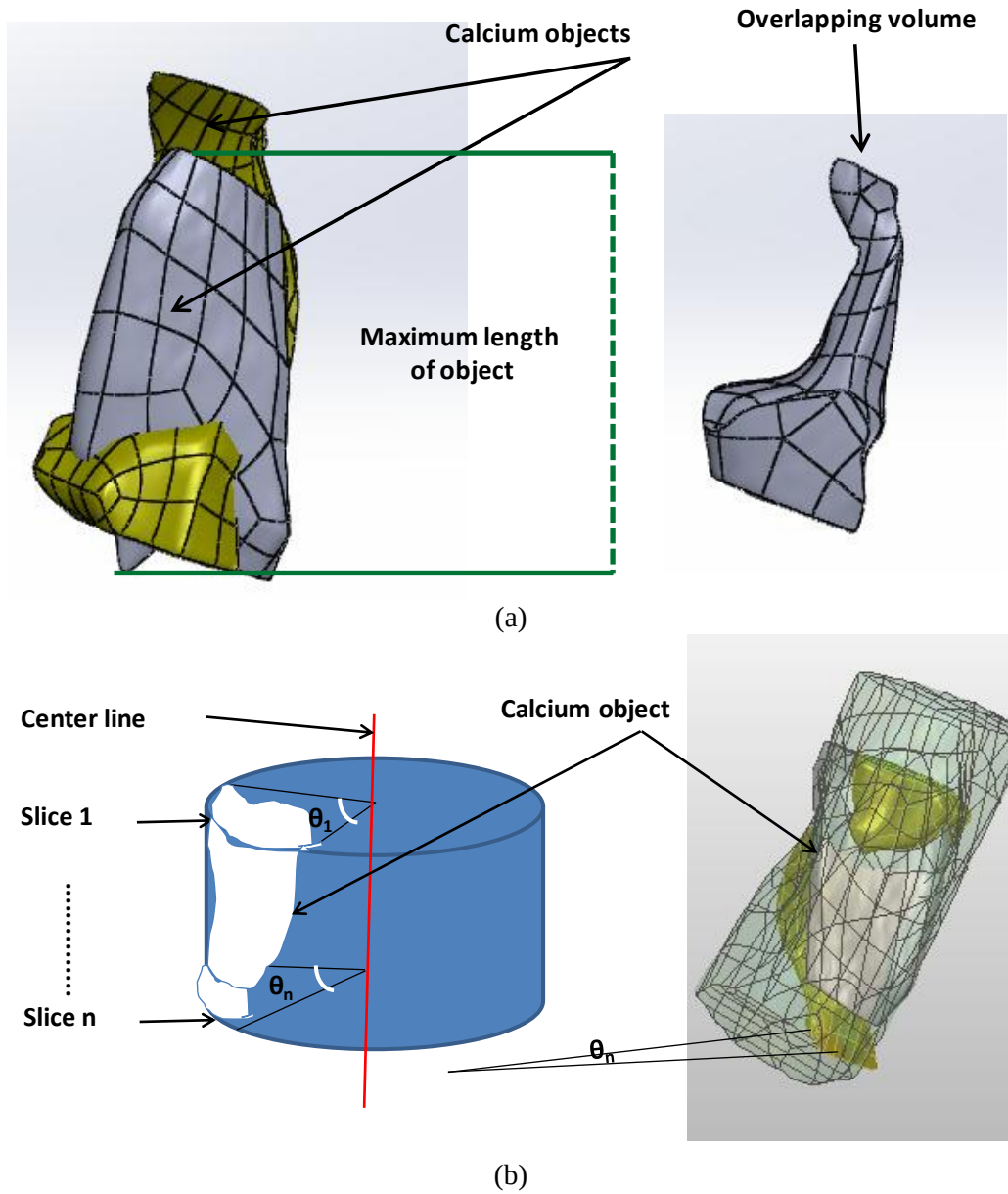
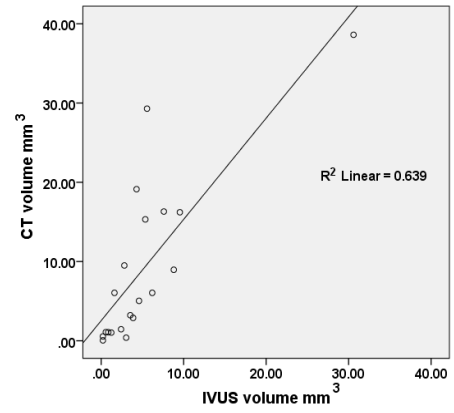
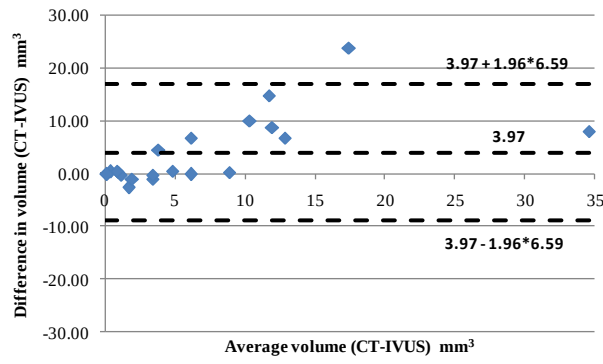
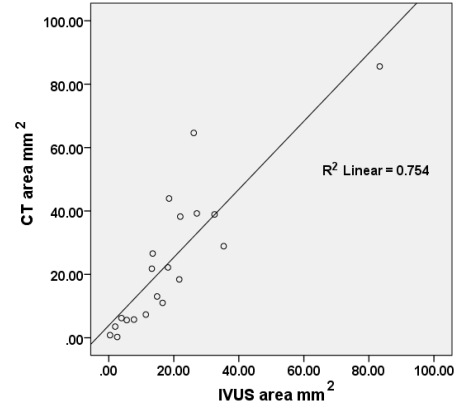
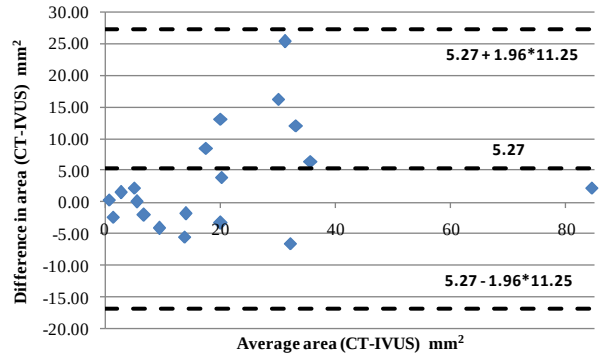


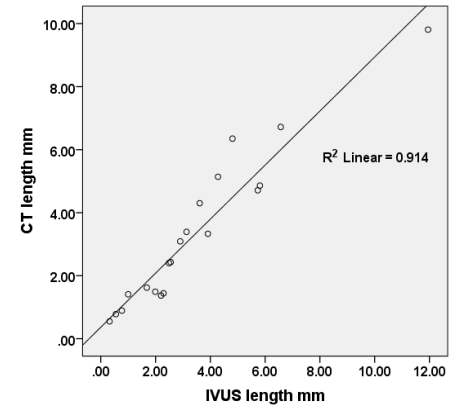
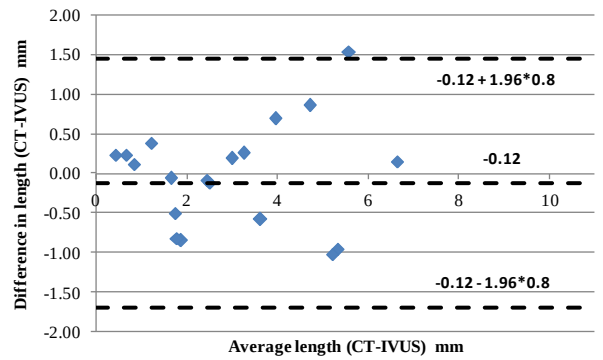
Figure 5.7: (a) The maximum length of a CTA object and the overlapping volume area with the IVUS object, and (b) schematic presentation of the inner angle metric used to compare the two objects



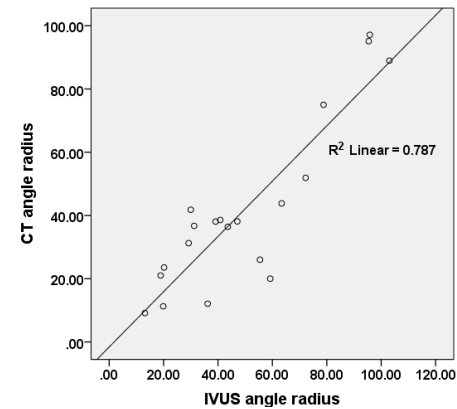
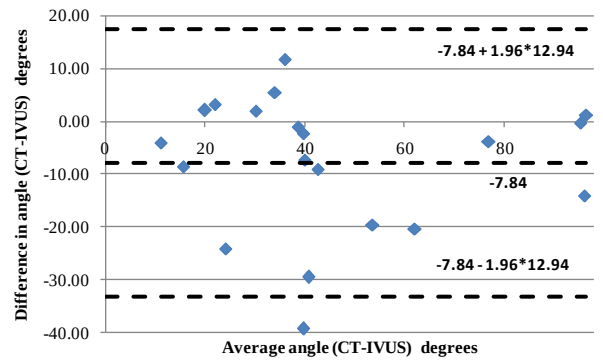
(a)



(b)



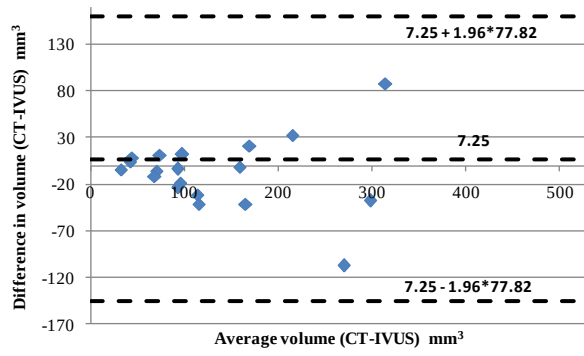
(c)



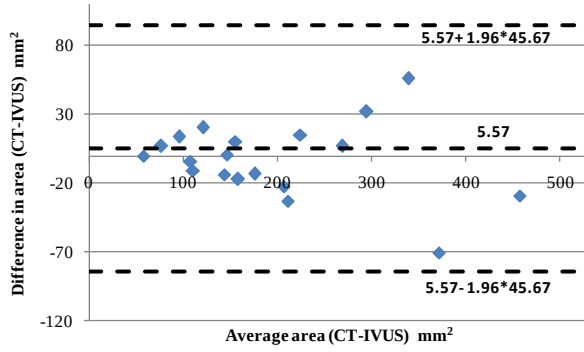
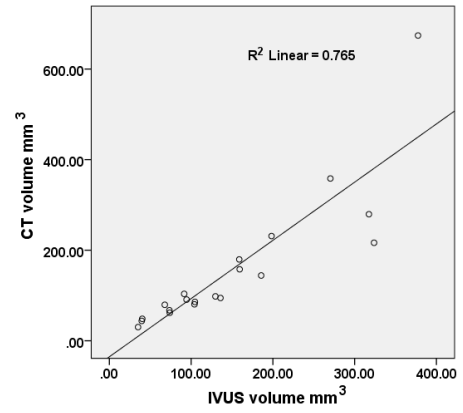
(d)

Figure 5.8: Bland-Altman (left) and correlation plots (right) for CTA-IVUS 3D objects comparison: (a) Volume comparison, (b) Surface area comparison, (c) Maximum length comparison, and (d) Inner angle comparison.

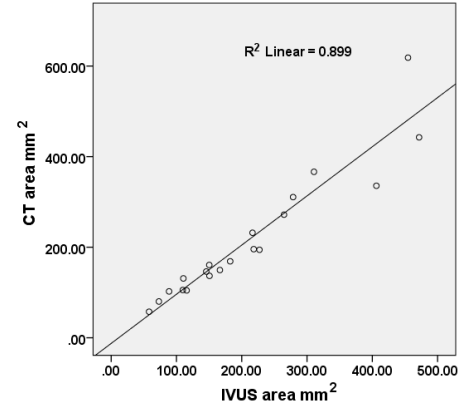
Inner Wall



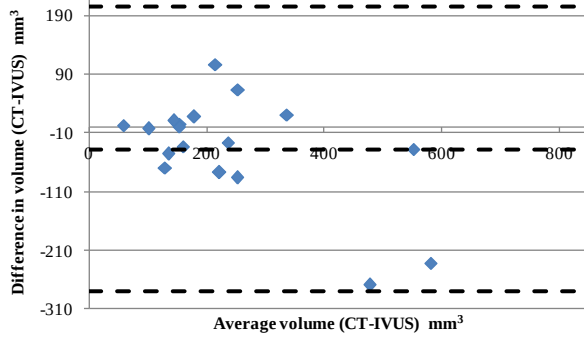
(a)



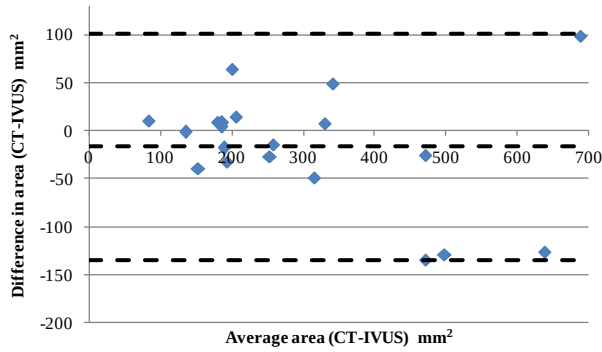
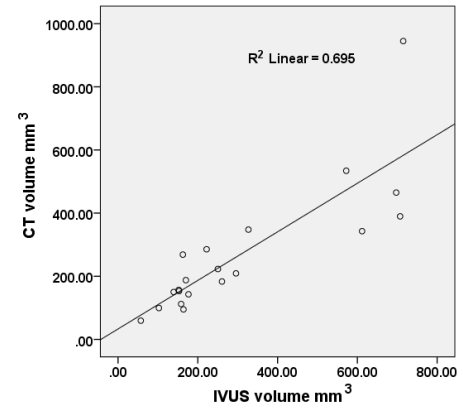
(b)



Outer Wall



(c)



(d)

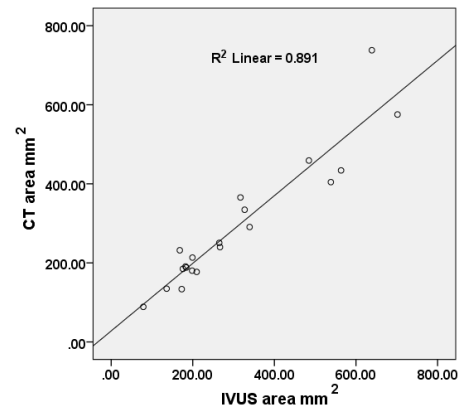


Figure 5.9: Bland-Altman (left) and correlation plots (right) for CTA and IVUS for the: (a) lumen volume, (b) Lumen surface area, (c) Outer vessel wall volume, (d) Outer vessel surface area

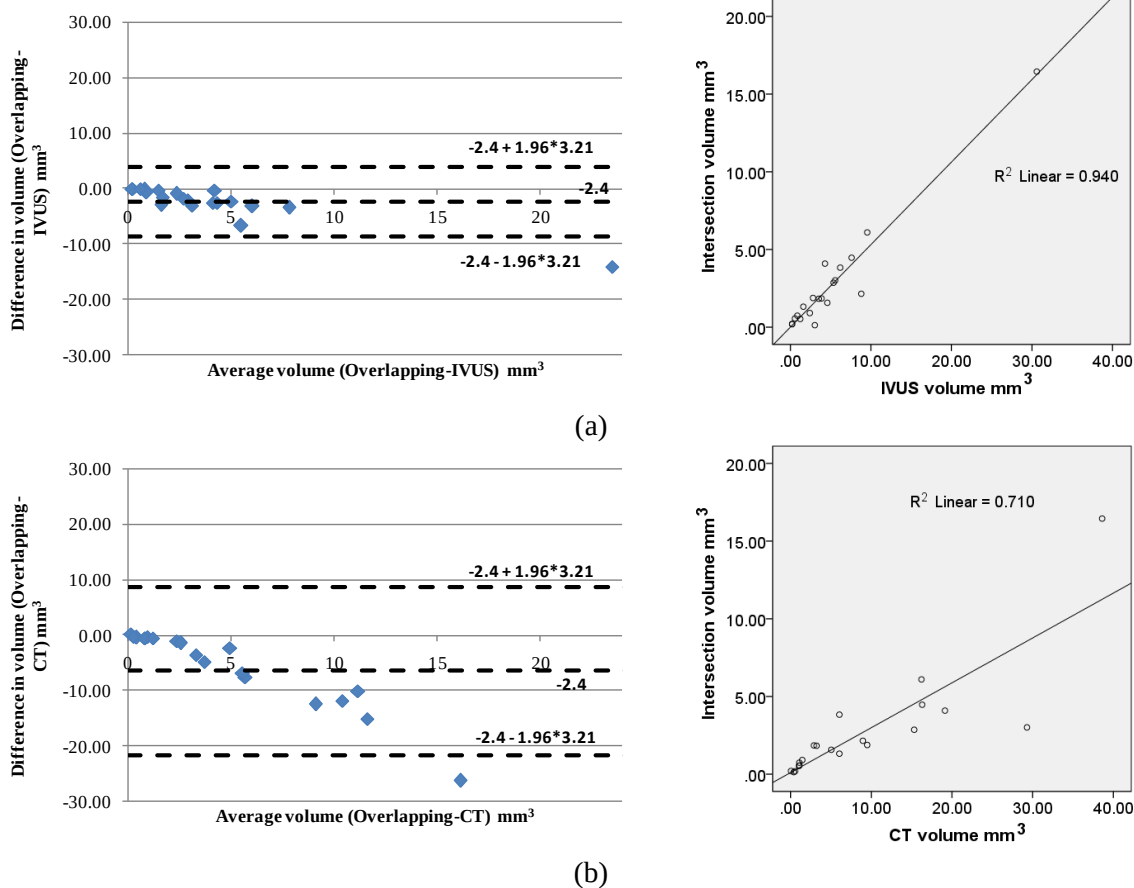


Figure 5.10: Bland-Altman (left) and correlation plots (right) for Volume intersection 3D objects comparison: (a) Intersection vs IVUS volume comparison, and (b) Intersection vs CT volume comparison.

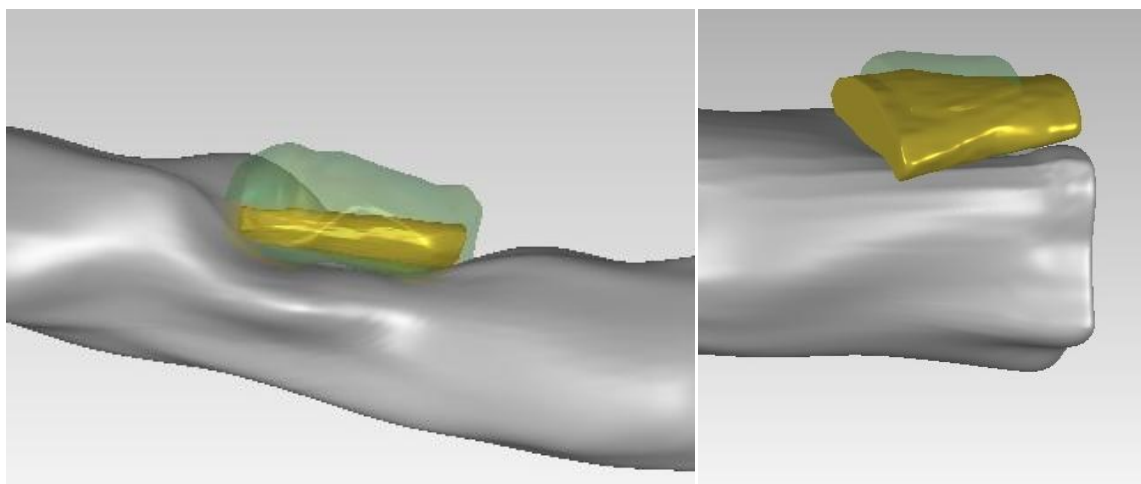


Figure 5.11: Lumen and 3D CP objects detected by CTA and IVUS: the gray object corresponds to lumen, the gold object to CPs detected by IVUS and the transparent green object correspond to CP detected by CTA. The volume mismatching between the objects is increased as the distance from the lumen to the plaque voxels is increased.

Table 5.2: Comparison of the proposed method with the Literature.

Comparison with the Literature: CP detecting			
Method	Degree of correlation R^2	Pearson's correlation r	Note
Brodoefel et al. [104]	0.07	0.1	-
Hur et al. [106]	-	0.7	Only area comparison
Papadopoulou et al. [96]	-	0.91	Calcium is merged with Mixed plaque
Utsunomiya et al. [107]	-	0.40	Only area comparison
Voros et al. [103]	-	0.84	-
de Graaf et al. [105]	-	0.73	-
Proposed methodology	0.64	0.79	-

5.5 Discussion

In this chapter, a semi-automated methodology for reconstructing the lumen, outer vessel wall and CP plaque in arteries using CTA images was presented. The methodology is based on a 3-component GMM [167] to detect the lumen, outer vessel wall borders and the CP and NCP plaque components and it is the only semi-automated methodology available in the literature that enables 3D reconstruction of coronary anatomy and plaque characterization. The validation of the proposed approach demonstrated that our methodology provides geometrically correct 3D models and permits reliable characterization of the composition of the plaque.

Over the last years several methodologies have been designed for the segmentation of the CTA cross sectional images and the characterization of plaque components [96, 102-104, 106, 107]. However, these methodologies are time consuming since they often require corrections of the detected borders and none of them is capable to reconstruct the coronary anatomy in an automated fashion allowing comprehensive visualization of luminal and outer vessel wall morphology, and assessment of the distribution of different

plaque components. The proposed approach overcomes the abovementioned limitations as it permits fast semi-automated segmentation and tissue characterization, reliable coronary reconstruction and 3D representation of coronary anatomy and pathology.

In contrast to previous works which focus on a simplistic comparison of the volumes and areas of the regions of interest in CTA and IVUS, a more complex validation methodology that allows comparison of the 3D reconstructed models was implemented. A high correlation between IVUS and CTA models for the lumen, and outer vessel wall surfaces and volumes was reported. The presented methodology demonstrated that the CP have the same circumferential and longitudinal extent, however, there was a relative small difference in the computed volumes ($r = 0.79$); fact that should be attributed to the fact that IVUS cannot portray the entire CP (acoustic shadow) [164, 165] and to that in CTA large CP plaques appear enlarged (blooming effect) [166].

Table 5.2 presents a comparison between the results of the proposed methodology and the methodologies proposed in the literature. Although, other studies [96, 103] demonstrated a higher volume correlation (Table 5.2) for the CP between the two imaging modalities, they have not compared the exact location of the CP objects (overlapping volume, Figure 5.10). A high correlation was noted ($r = 0.94$) when comparing the CP overlapping volume versus IVUS volumes, while a moderate correlation ($r = 0.71$) was noted when comparing the CP overlapping volume versus CT volumes. This additional validation step demonstrates that the CP objects derived by the two imaging modalities are located in the same position.

The proposed methodology is anticipated to enhance the applications of CTA in the study of atherosclerosis as it allows expedite coronary reconstruction (the required time is less than 15 seconds for an artery of 20 mm using a desktop computer with an Intel core i7 processor with 16 GB of RAM) and comprehensive representation of coronary anatomy and plaque distribution. In addition the obtained 3D models are expected to allow blood flow simulation and evaluation of the shear stress distribution and assessment of the impact of flow dynamics on atherosclerotic disease progression.

A limitation of the proposed methodology is the fact that it is not a fully automated approach. In addition the CTA reconstruction can be performed only in non-bifurcated

arteries as the reconstruction of coronary bifurcations is a more complex process. Finally, the methodology cannot be applied to stented segments as erroneous CP objects are detected due to stent's blooming effect.

Chapter 6: Error propagation in atherosclerotic plaque characterization

6.1 Introduction

6.2 Proposed method

6.3 Dataset

6.4 Results

6.5 Discussion

6.1 Introduction

Diagnostic imaging plays an important role in medicine today. Delineation of vessels solves practical applications such as diagnosis of coronary artery disease, assessment of degree of stenosis in carotid artery pathology, etc [168]. Advantages in imaging technology led to a wide variety of imaging modalities for depicting the silhouette and the interior of blood vessels. These modalities can be either invasive [169, 170], i.e. intravascular imaging (IVUS), optical coherence tomography (OCT) and near-infrared spectroscopic imaging (NIRS) or non-invasive [171, 172], i.e. x-ray angiography (XRA), magnetic resonance imaging (MRI) and computed tomography (CT), each one having its advantages and disadvantages [173].

Imaging systems perform measurements and all measurements have uncertainties due to the equipment imperfection, human imprecision, instrumental calibration, etc. These uncertainties [174] describe how accurately a mathematical model can represent the true physics, and are determined after system tests and by taking into account the measurement procedure to estimate its reliability. To estimate the error of a system (group of instruments), the accuracy of a specified output approximated by a given numerical method is measured. Imaging systems [175] transmit and acquire signals using a detector in order to construct the image. However, all imaging systems are subject to error sources and the detected signals that construct the image include the variations of the detector or possible calibration errors [176]. Those artifacts can affect image quality

[177, 178], even though some of them are corrected by medical equipment software, resulting sometimes in false image representation. These artifacts, along with the image quality can affect automated or semi-automated computer-aided image processing methods. Spatial resolution [179] is a major determinant of image quality and describes the level of detail that can be distinguished on an image. Spatial resolution relates to how small an object can be in order to be detected by a particular imaging system. Imaging resolution of a digital image can be described in many different ways, including spatial resolution. Therefore, spatial resolution of an imaging system affects its resolution and the ability to resolve vessel microstructures; consequently, producing an error in the final image. Image formation errors include the limitations produced both by the pixel resolution (spatial resolution) of the image acquisition system and by any other possible systematic errors. Spatial resolution, usually is reported in line pairs per centimeter (lp/cm) and provides an adequate metric of the uncertainty implied by imaging system's formation errors.

The latest advances in computer technology and the need for automation of the diagnosis of vascular or carotid artery disease have made possible the development of automated methods for processing the vessel images derived from the various imaging modalities. These methods can be mainly grouped to methods segmenting the vessels [60, 85, 86] and methods characterizing the atherosclerotic plaque [67, 158, 180-182] of the segmented vessels, regardless of the imaging modalities used. Segmentation methods vary depending on the imaging modality or method being automatic or semi-automatic. Vessel segmentation methods are the key components of the automated plaque characterization methods; in order for the plaque characterization method to be applied, the results of a segmentation method are used as input. However, all the plaque characterization methods have a serious drawback: plaque characterization and segmentation steps are validated independently. This leads to overestimated results as the segmentation error computed in the validation step and the acquired system image formation errors are not propagated in the final result.

A methodology to study the error propagation of both segmentation step and image formation is presented. The proposed error propagation methodology is validated using two different plaque characterization methods [158, 180]. The first method [158]

processes IVUS images and segments the artery using a semi-automated border detection method [60], based on the use of deformable models [183]. The pixels of the segmented area are then classified to dense calcium (DC), necrotic core (NC), fibrous tissue (FT), fibro-fatty tissue (FFT) and normal tissue-media (M), using a classification algorithm. The second method [180] segments and characterizes the artery automatically by classifying the plaque in four different plaque types: calcified plaque (CA), lipid tissue (LT), fibrous tissue (FT) and mixed tissue (MT).

Although the methods [158, 180] have been validated thoroughly neither included the segmentation step error and the image formation error to the final plaque characterization results. Currently, the proposed methodology is applied to present a more thorough and realistic validation. Specifically, for the IVUS method the influence of segmentation error and the image formation error to the detection of DC, NC, FT, FFT, M and the entire plaque area are validated. Similarly to IVUS, for OCT the influence of both segmentation image formation error to the detection of CA, LT, FT, and MT are validated.

Although the plaque characterization results are affected by the propagated errors, still the results of the methods [158, 180] are accurate enough (Pearson's coefficient > 0.86 in all plaque types) to be used for research or clinical applications.

6.2 Proposed method

The vessel plaque characterization methods are based on information derived from plaque segmentation algorithms, which detect lumen and media adventitia borders of a vessel. A block diagram showing the steps that these methods follow and the error that is propagated from one step to another is presented in Figure 6.1.

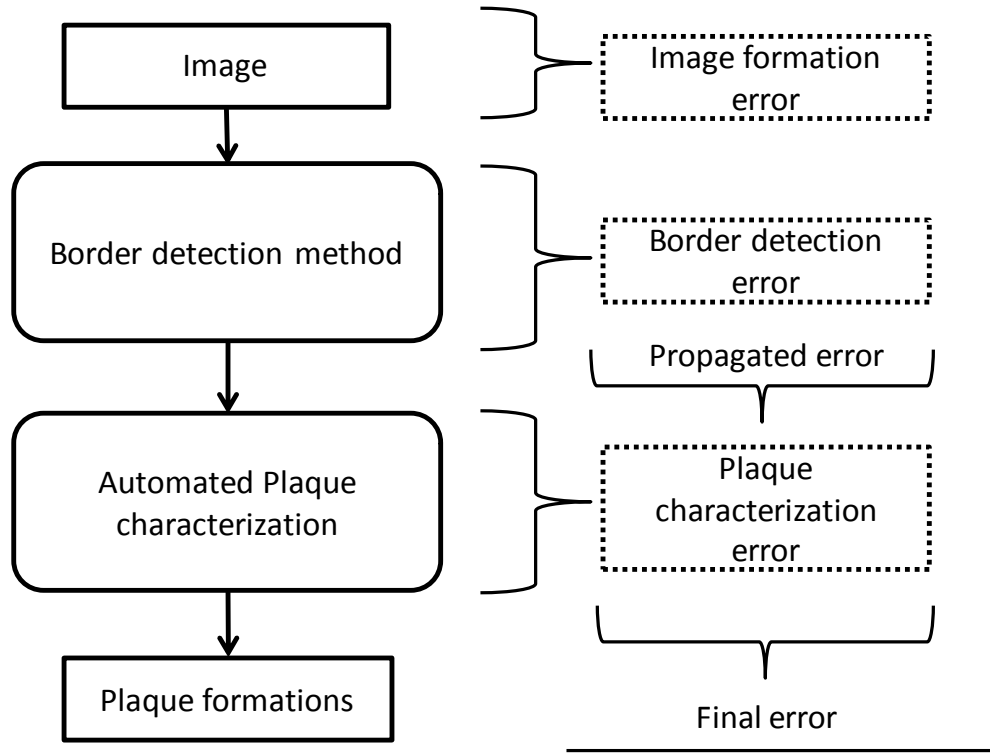


Figure 6.1 Block diagram of the steps that plaque characterization methods follow and the propagation of the error from one step to another.

6.2.1 Image formation error computation

The image formation error is calculated for the different modalities and plaque types based on simulation experiments. For the estimation of the maximum image formation error (MIFR) the shape S_i of each plaque based on expert annotation, was used. A typical plaque shape based on expert annotation is shown schematically in (Figure 6.2- a). For each S_i , with area A_p the following simulation approach was used :

1. Two grids: one with low resolution equal to the spatial resolution of the examined imaging modality and one with higher resolution ($10 \times \text{spatial resolution}$, Appendix E) (Figure 6.2- a, b). were constructed,
2. The following procedure was repeated 100 times:

- a. the shape S_i was randomly⁶ translated over the center of the grid,
- b. the cells of the high-resolution grid which are entirely inside the translated S_i (Figure 6.2- c) were computed. Let B be the number of the cells that are both inside the translated S_i and the low resolution cell. The low-resolution cell is considered as activated (C_{act}) if $B/10 > thr$ ($thr = 0.01$, Appendix E) (Figure 6.2- d),
- c. the area of activated cells were computed as: $AC = \sum C_{act} \times C_{area}$ where C_{area} the area of the low resolution cell, while the MIFR for the specific S_i and repetition was computed as:

$$MIFR(\%) = \frac{100 \times |(AC - A_p)|}{A_p}. \quad (6.1)$$

3. The mean and std of the $MIFR(\%)$ corresponding to each shape S_i , were calculated and presented in Table 6.1

6.2.2 Incorporation of the Image Formation Error in the Segmentation Error

The error which is propagated to the plaque characterization method is composed of the segmentation error of the method as well as the image formation error. The segmentation error is estimated for each modality by comparing the area of the ground truth and the method's detected area.

The error on the segmentation algorithm is estimated in terms of percentage to the surface of each case. The mean errors (E) is estimated as:

$$E = E_b \pm E_s, \quad (6.2)$$

where E_b is the bias and E_s is the standard deviation.

Based on the bias and the standard deviation, an upper (overestimation) and a lower limit (underestimation) are considered. Those two cases statistically cover 68.27% of all [184]

⁶ based on a uniform distribution $\mathcal{U}(0.2 \times \text{spatial resolution})$ for both horizontal and vertical directions.

cases and are considered for error propagation in geometry reconstruction. Therefore, for an area A , the overestimated area, A_o , is calculated as:

$$A_o = A + (E_b + E_s) \times A, \quad (6.3)$$

and the underestimated area A_u as :

$$A_u = A + (E_b - E_s) \times A. \quad (6.4)$$

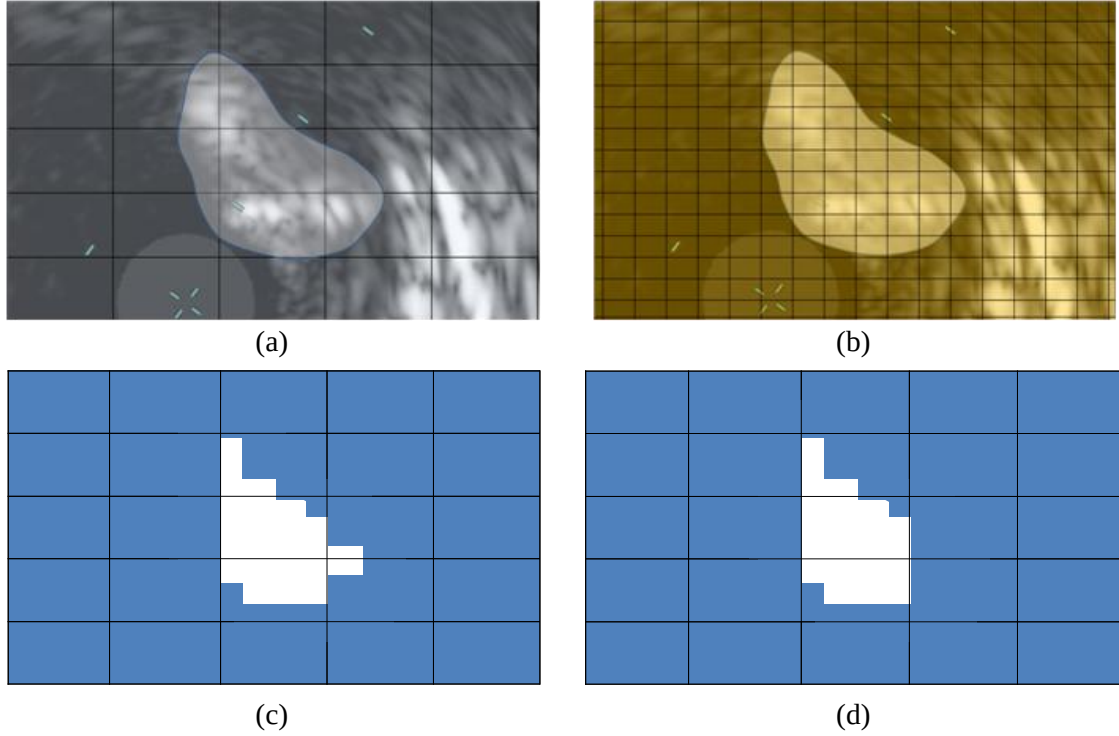


Figure 6.2: Schematic presentation of the grids construction: a) the typical plaque shape S_i , based on expert annotation (light blue) corresponding to a typical lesion and the grid (blue) representing the resolution equal to the spatial resolution (low resolution), b) the high resolution grid (yellow), c) the low resolution grid corresponding to pixels of the high resolution grid which are entirely inside the object and d) the low resolution grid corresponding to pixels activated for the object.

To incorporate the image formation error, two cases were assumed, where the segmentation error is already taken into account, let A_o and A_u for overestimated and underestimated areas, respectively. In those cases, the image formation error estimated using Eq. 6.1, was incorporated.

For A_o , an additional overestimation is considered due to the formation error, which is given by:

$$A_o' = A_o + MIFR \times A_o. \quad (6.5)$$

Similarly for A_u an additional underestimation is considered due to the formation error, which is given by:

$$A_u' = A_u - MIFR \times A_u. \quad (6.6)$$

6.2.2 Development of Error Propagation Models

Plaque segmentation algorithms provide for each image frame two border lines: the lumen and the outer border. The region of interest (ROI) of the plaque detection algorithms is defined as the area between these two borders and is used as input to the plaque characterization methods. In order to estimate the error propagated in each plaque type, the ROI areas were modulated, until the desired area to be achieved (Eq. 6.5 or Eq. 6.6), and were characterized.

The propagation of image segmentation errors in the plaque characterization algorithm is a random process, without any specific constraints, therefore to modulate the ROI area (A_{ROI}) in each frame, we have implemented the following procedure:

1. select either the lumen or the outer border of the ROI,
2. divide the lumen (or the outer border) to four quadrants and choose one of the four,
3. increase the lumen quadrant (or decrease the outer quadrant) when the goal is to overestimate the ROI ($A_{ROI} = A_o'$) and decrease the lumen quadrant (or increase the outer quadrant) when the goal is to underestimate the ROI ($A_{ROI} = A_u'$),
4. if $A_{ROI} < A_o'$ (or $A_{ROI} < A_u'$) return to step 1.

A schematic presentation of above described procedure is shown in Figure 6.3. The rationale, for using four quadrants was based on the assumption that an increment/decrement in the whole region of the lumen or outer border produces erroneous

results (positive predictive value=1 or the sensitivity of the method=1); the area calculated by the method minus the common area between the two measurements and the golden standard area minus the common area between the two measurements is always zero. To overcome this limitation, the center of gravity of each lumen/outer border is detected, four quadrants are drawn and the lumen border can be increased or decreased uniformly (dividing the lumen to eight parts was also tested, and no significant change in the results was reported).

6.3 Dataset

To validate the proposed error propagation methodology, IVUS, and OCT images were used. All participants provided informed consent, while the study was approved by the institution's Ethical Committee. To characterize the atherosclerotic plaque in IVUS images a plaque characterization method [158] which detects four tissue types: DC, NC, FT, FFT and Media (normal tissue - M), was used. For OCT images, a method [180] that detects four tissue types: Ca, LT, FT and MT was used.

6.3.1 IVUS

The IVUS data were provided from the Department of Cardiology, Medical School of the University of Ioannina, using an electronic probe (Eagle eye Gold, Volcano Therapeutics, Inc.) of the following characteristics: synthetic aperture 3.5F and frequency 20MHz. IVUS images (size 648x648 pixels) and their corresponding virtual histology (VH-IVUS) [77] frame was also acquired. From these dataset 35 IVUS frames were used as containing all four plaque types, and characterized [158] in order to study the error propagation. The color-coded VH-IVUS frames were used as gold standard.

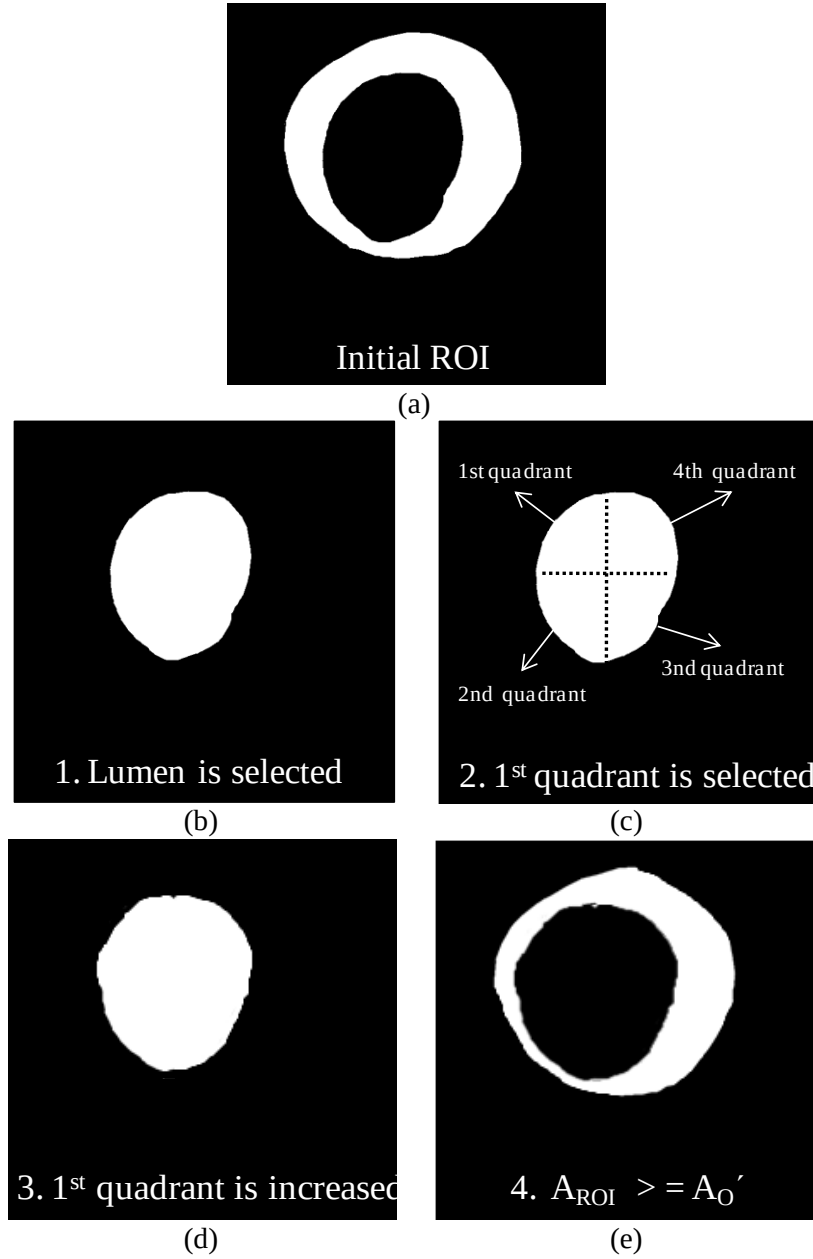


Figure 6.3: Schematic presentation of the ROI underestimation in a frame: (a) Initial ROI, (b) Lumen randomly selection, (c) Lumen division in four quadrants and randomly selection of one, (d) Increased Lumen, (e) Modulated ROI area.

6.3.2 OCT

OCT data were acquired from 22 patients, who underwent OCT investigation for clinical purposes using a C7XR Fourier Domain system (Lightlab Imaging, Westford, MA, USA) equipment. 35 OCT frames were randomly selected and characterized [180]. The data

were provided by the San Giovanni Hospital, Rome, Italy. Annotations of two experts were used as gold standard for validating the proposed error propagation methodology. Any disagreements in experts' annotations were resolved by consensus and the inter-observer variability for the two expert observers in the 35 tissue regions was $-0.02 \pm 0.16 \text{ mm}^2$, $-0.08 \pm 0.19 \text{ mm}^2$, $-0.02 \pm 0.10 \text{ mm}^2$ and $-0.06 \pm 0.26 \text{ mm}^2$, for CA, LT, FT and MT, respectively.

6.4 Results

In the 35 IVUS frames the error propagation study was performed on the detection of: all plaque components: DC, NC, FT, FFT and M. In the 35 OCT frames error propagation study was performed on the detection of: CA, LT, FT, and MT.

6.4.1 MIFR computation

For the acquisition of IVUS images, a Volcano system that generates intravascular images from an array of 64 microcrystals and has a spatial resolution of 0.15 mm (150 microns) [185] was used. Finally, for the acquisition of OCT images, a C7XR Fourier Domain system generating images having a spatial resolution 0.015 mm (15 microns) [26, 27] was used. The MIFR values computed for each plaque area calculated by IVUS and OCT are summarized in Table 6.1.

6.4.2 Validation

To validate the performance of the proposed approach, regression analysis was performed and the agreement between the plaque areas calculated by the algorithm and the plaque areas obtained by the gold standard was studied. Regression analysis and the Bland and Altman plots were constructed. Additionally, the positive predictive value (PPV) and ratio of overlapping/ non-overlapping ($R_{over} / R_{non-over}$) areas were computed as:

$$PPV = \frac{TP}{TP + FP}, \quad (6.7)$$

$$R_{over} = \frac{TP}{TP + FN}, \quad (6.8)$$

$$R_{non-over} = \frac{FN + FP}{TP + FN}. \quad (6.9)$$

As true positive values (TP) the common area between the two measurements was defined, as false positive (FP) values the area calculated by the algorithm minus the common area and as false negative (FN) the golden standard area minus the common area.

Table 6.1: IVUS and OCT image formation error.

Imaging modality	Area	Mean value (mm ²)	Max. Image formation error (MIFR) (%)
IVUS	ROI (wall)	6.84	8.99 ± 0.60
	Dense calcium	0.59	32.40± 6.67
	Necrotic core	0.99	23.18± 4.64
	Fibrous tissue	1.97	16.72± 1.59
	Fibro-Fatty tissue	1.49	18.54± 2.38
	Media	2.54	14.18± 1.47
OCT	Calcium	0.30	4.30 ± 0.13
	Lipid tissue	0.98	2.30 ± 0.06
	Fibrous tissue	0.76	2.61 ± 0.06
	Mixed tissue	0.46	3.36 ± 0.20

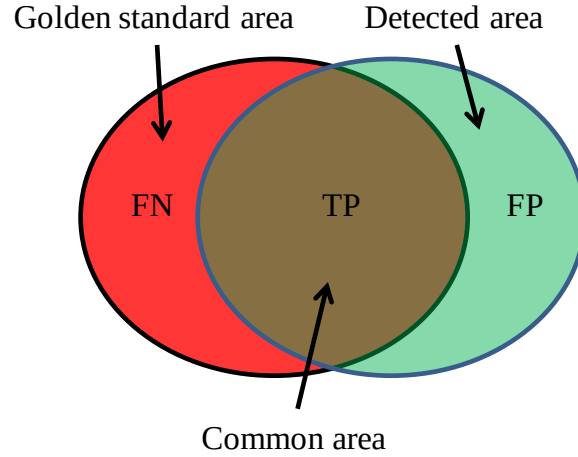


Figure 6.4: Schematic presentation of the true positive (TP - brown area), false negative (FN - red area) and false positive (FP - green area) areas defined for computing the positive predictive (PPV) value and ratio of overlapping/ non-overlapping ($R_{over} / R_{non-over}$) areas validation metrics.

Before propagating the error from the border detection algorithm to the plaque characterization algorithm in the 35 IVUS frames, an initial validation was made, using the initial ROI (with area A). When using the initial ROI (initial artery borders) of IVUS, the ROI area is not affected and consequently the plaque area remains the same. Thus, no validation results are presented for detecting all the plaque types (Figure 6.7). Figure 6.5 presents the comparison of DC, NC, FT and FFT areas extracted from VH and characterized by the algorithm [158] using the initial, the overestimated, and underestimated lumen and external border areas while their corresponding Bland and Altman plots are shown in Figure 6.6. The comparison of plaque and normal tissue (M) using the initial, the overestimated and underestimated lumen and external border areas and their corresponding Bland and Altman plots are presented in Figure 6.7.

Similarly to IVUS, in OCT an initial validation was performed using the initial ROI. As the OCT method detects only the lumen border and expands the border up to 1.5 mm in order to define the ROI we overestimated and underestimated the lumen borders and performed an error propagation analysis in: Ca, LT, FT, and MT. The methodology results were compared with areas detected by the experts (Figure 6.8) and their corresponding Bland and Altman plots are presented in Figure 6.9.

Additionally to the error propagation of both the border detection and image formation (MIFR), the propagated error only from the border detection algorithm was calculated. The results are summarized in Table 6.2, Table 6.3 and for IVUS and OCT, respectively. Finally, the PPV value and $R_{over} / R_{non-over}$ areas using the initial, overestimated and underestimated ROI areas for IVUS and OCT, are presented in Table 6.4

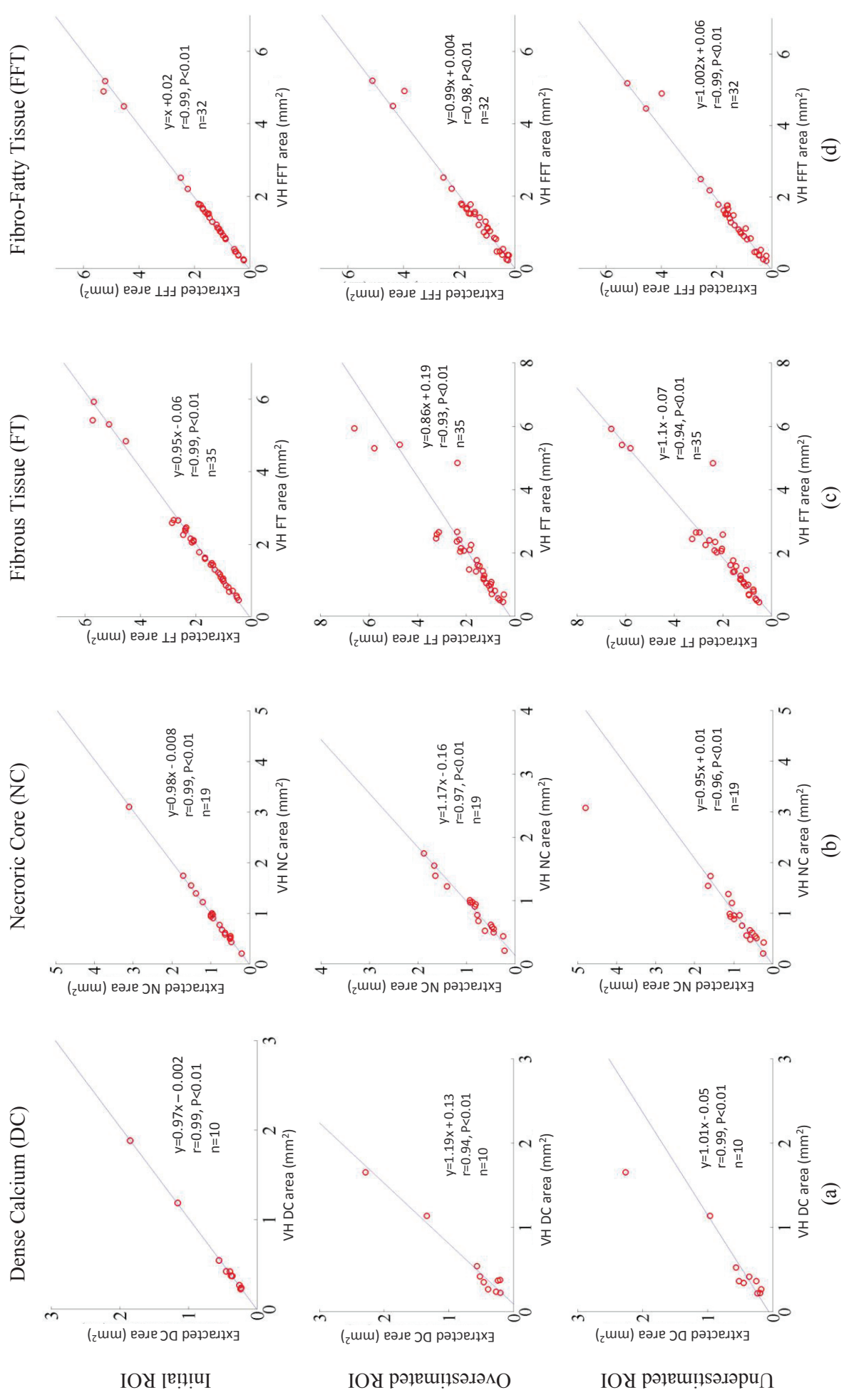


Figure 6.5: Comparison of: (a) Dense Calcium, (b) Necrotic Core, (c) Fibrous Tissue and (d) Fibro-Fatty Tissue areas extracted from VH and characterized by the method [158] using the initial, the overestimated and underestimated ROI areas.

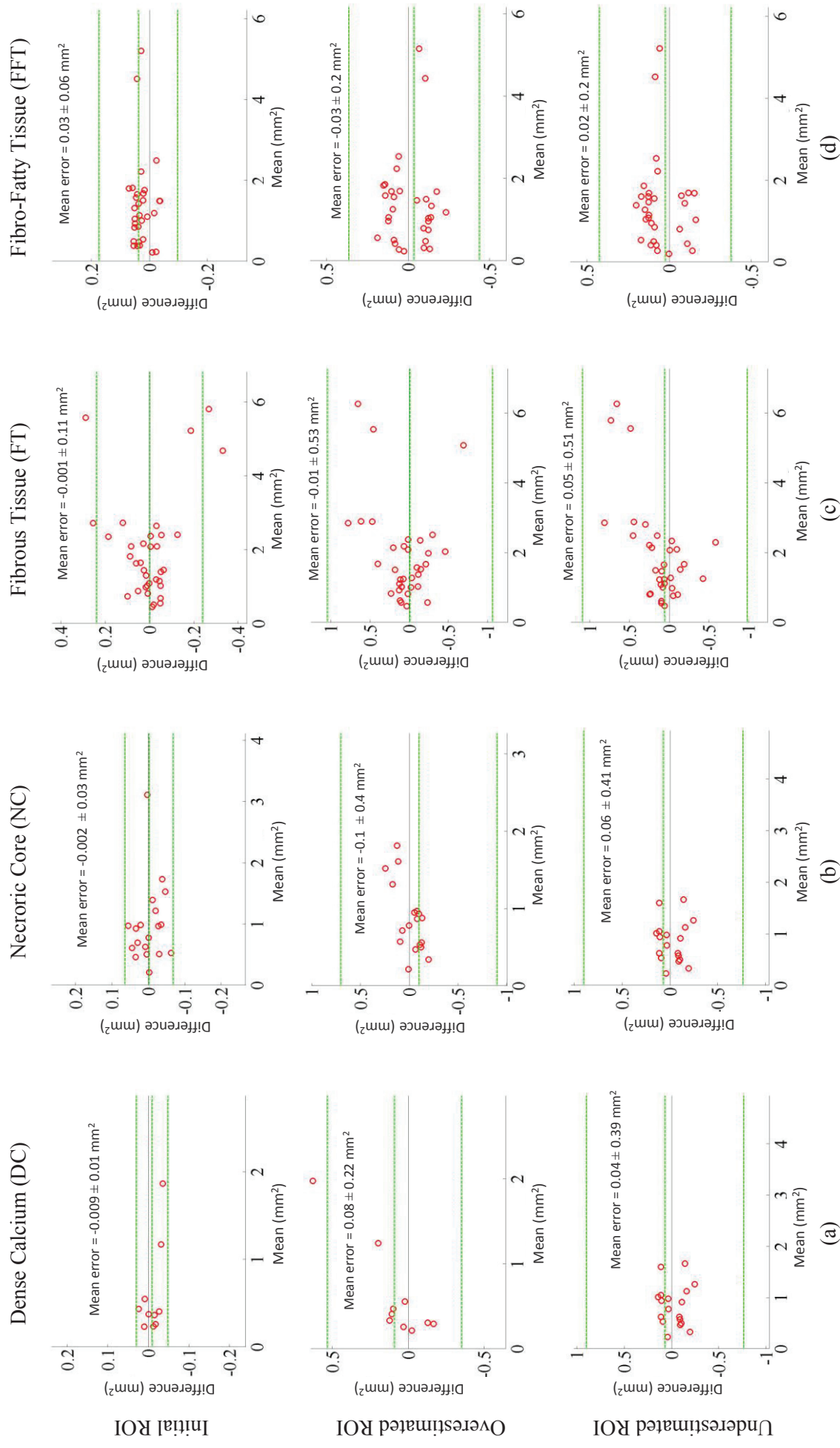


Figure 6.6: Bland and Altman plots for: (a) Dense Calcium, (b) Necrotic Core, (c) Fibrous Tissue and (d) Fibro-Fatty Tissue areas extracted from VH and characterized by the method [158] using the initial, the overestimated and underestimated ROI areas.

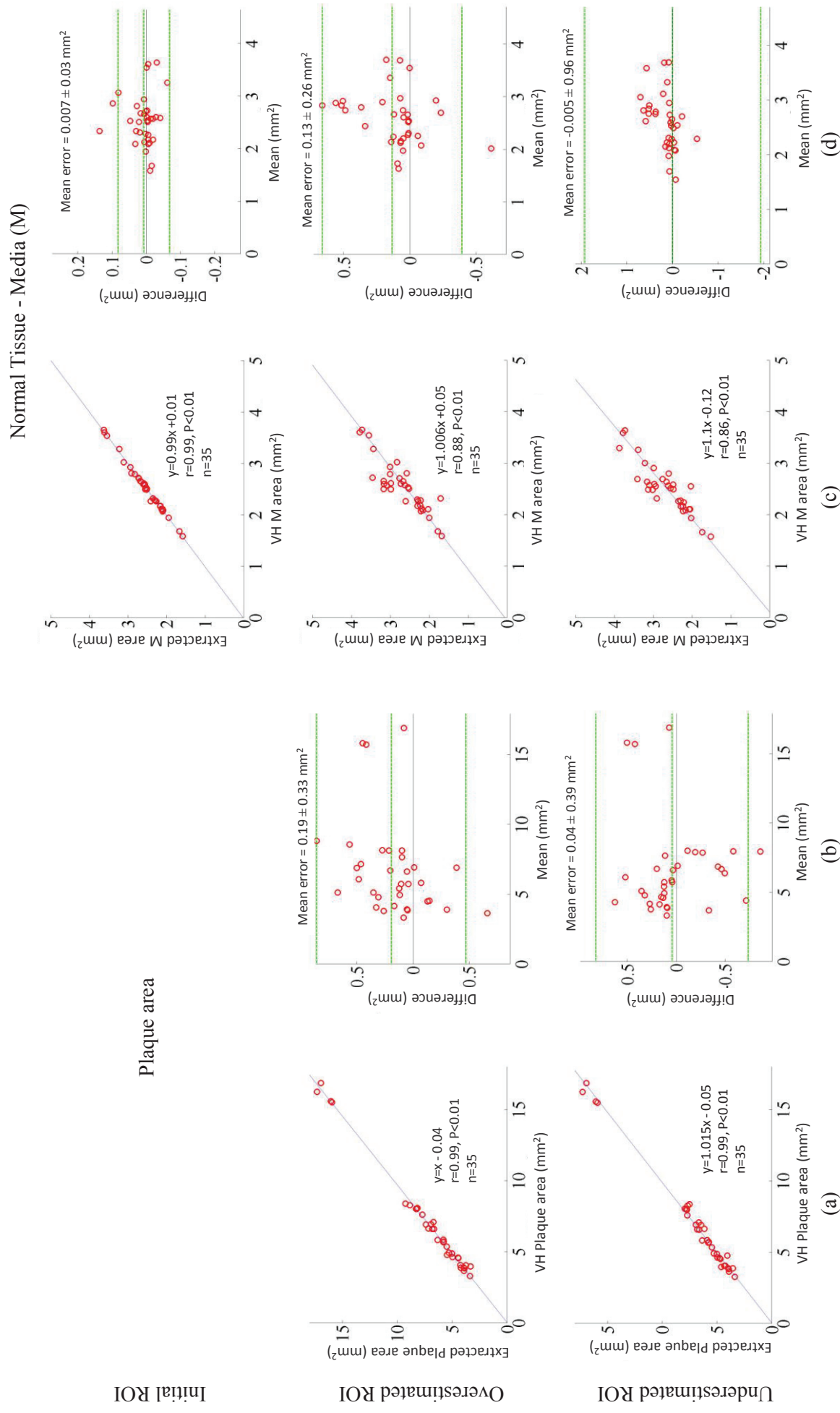


Figure 6.7: Comparison of: (a) plaque and (c) normal tissue - Media, and Bland and Altman plots for (b) plaque and (d) normal tissue - Media areas extracted from VH and characterized by the method [158] using the initial, the overestimated and underestimated ROI areas.

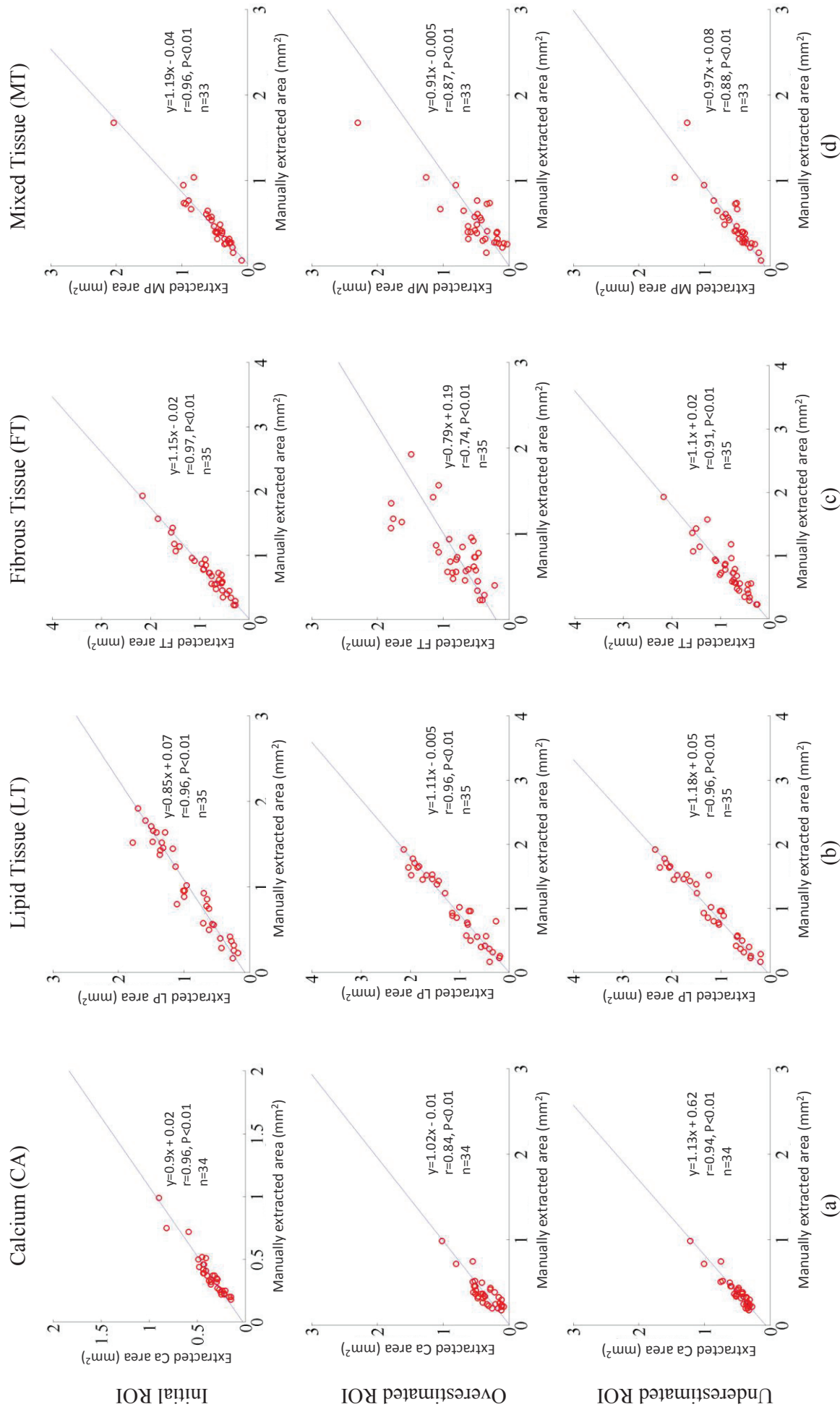


Figure 6.8: Comparison of: (a) Calcium, (b) Lipid tissue, (c) Fibrous tissue and (d) mixed tissue areas annotated by experts and characterized by the method [180] using the initial, the overestimated and underestimated ROI areas.

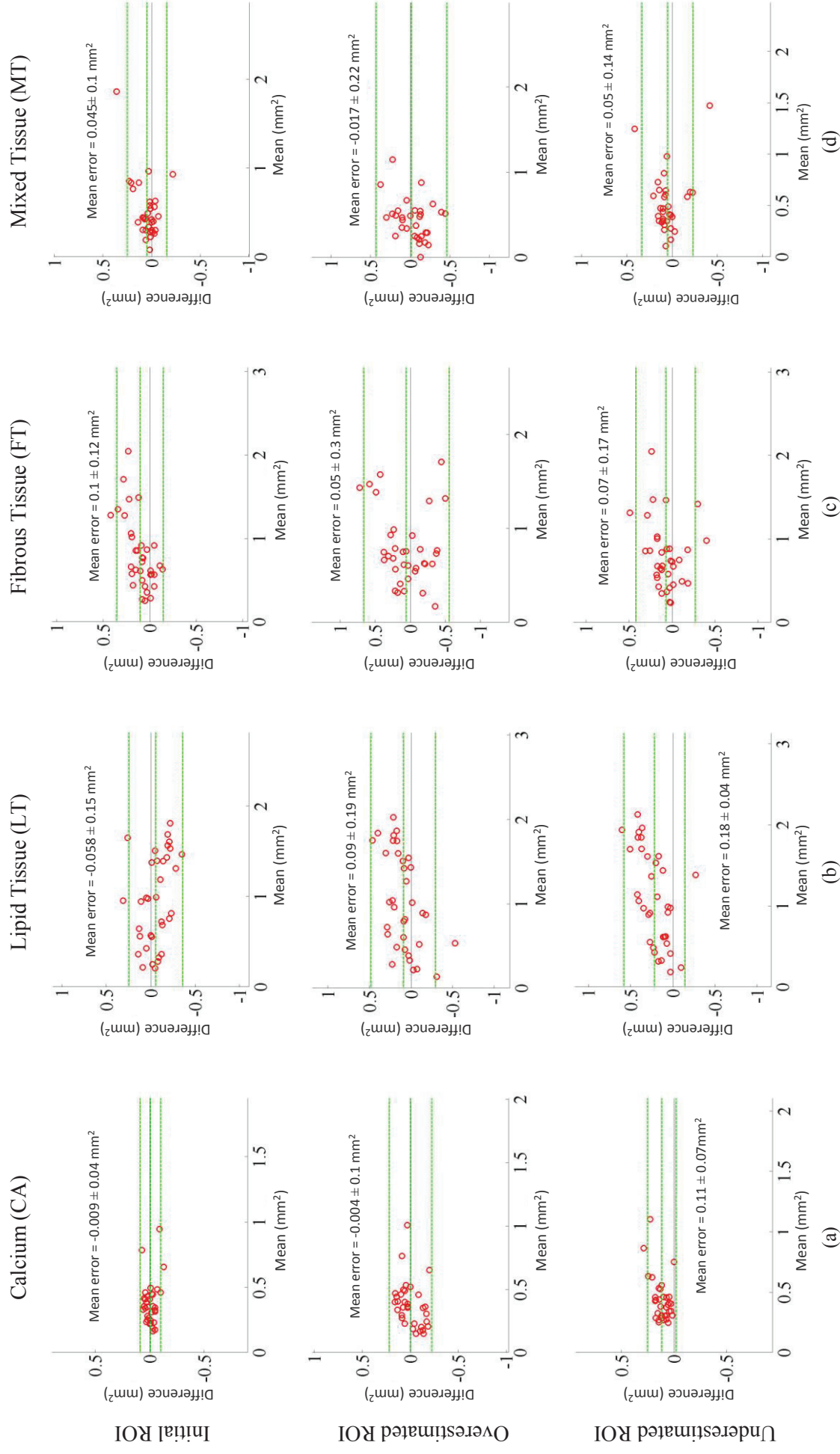


Figure 6.9: Bland and Altman plots: for (a) Calcium, (b) Lipid tissue, (c) Fibrous tissue and (d) mixed tissue areas annotated by experts and characterized by the method [180] using the initial, the overestimated and underestimated ROI areas.

Table 6.3: Summary of error propagation results for OCT when propagating the error from the border detection algorithm and the error from both the border detection algorithm and image formation (MIFR).

Plaque formations	Validation using the originally produced artery borders		Lumen area overestimated		Lumen area underestimated	
	Mean bias (mm ²)	r	Mean bias (mm ²)	r	Mean bias (mm ²)	p
Border error						
Border + MIFR error						
Calcium	-0.009 ± 0.047	0.96	0.060 ± 0.047	0.96	-0.23± 0.047	0.96 <0.05
			-0.004 ± 0.11	0.84	0.11± 0.070	0.94 <0.05
Border error						
Border + MIFR error						
Lipid tissue	-0.005 ± 0.150	0.96	0.010± 0.15	0.96	-0.28± 0.150	0.96 <0.05
			0.090 ± 0.19	0.96	0.18 ± 0.040	0.96 <0.05
Border error						
Border + MIFR error						
Fibrous tissue	0.100 ± 0.120	0.97	0.170 ± 0.12	0.97	-0.12± 0.120	0.97 <0.05
			0.052 ± 0.30	0.73	0.07 ± 0.170	0.91 <0.05
Border error						
Border + MIFR error						
Mixed tissue	0.045 ± 0.100	0.96	0.11± 0.10	0.96	-0.18± 0.100	0.96 <0.05
			-0.017 ± 0.22	0.86	0.05 ± 0.140	0.88 <0.05

Table 6.4: Positive predictive (PPV) value and ratio of overlapping/ non-overlapping ($R_{over} / R_{non-over}$) areas computed using the initial, overestimated and underestimated ROI areas.

Imaging modality	Plaque type		Validation using the initial ROI areas	Validation using the overestimated ROI areas	Validation using the underestimated ROI areas
IVUS	Dense calcium (DC)	R_{over}	0.96	0.74	0.82
		PPV	0.98	0.95	0.87
		$R_{non-over}$	0.05	0.29	0.29
	Necrotic core (NC)	R_{over}	0.97	0.88	0.87
		PPV	0.98	0.91	0.91
		$R_{non-over}$	0.04	0.20	0.20
	Fibrous tissue (FT)	R_{over}	0.96	0.84	0.88
		PPV	0.97	0.92	0.92
		$R_{non-over}$	0.06	0.21	0.18
	Fibro-Fatty tissue (FFT)	R_{over}	0.98	0.90	0.93
		PPV	0.98	0.95	0.95
		$R_{non-over}$	0.03	0.13	0.11
	Media (M)	R_{over}	0.99	0.93	0.94
		PPV	0.99	0.95	0.96
		$R_{non-over}$	0.01	0.10	0.09
OCT	Calcium (CA)	R_{over}	0.92	0.79	0.83
		PPV	0.95	0.94	0.95
		$R_{non-over}$	0.12	0.25	0.20
	Lipid tissue (LT)	R_{over}	0.87	0.88	0.86
		PPV	0.92	0.96	0.95
		$R_{non-over}$	0.20	0.16	0.18
	Fibrous tissue (FT)	R_{over}	0.91	0.74	0.85
		PPV	0.92	0.94	0.97
		$R_{non-over}$	0.16	0.30	0.17
	Mixed tissue (MT)	R_{over}	0.90	0.73	0.83
		PPV	0.93	0.95	0.97
		$R_{non-over}$	0.16	0.29	0.18

6.5 Discussion

A methodology to evaluate the influence of the border detection methods and image formation errors to the overall accuracy of plaque characterization methods in arterial images is presented. The presented methodology is validated using recently presented plaque characterization methods [158, 180] applied in real datasets using IVUS, and OCT imaging systems. The methods utilized [158, 180] detect the vessel borders and

characterize the atherosclerotic plaque. By calculating the maximum error that can be propagated to the plaque characterization result a more robust validation is presented. The proposed methodology shows that the final plaque characterization results can be affected by the propagated errors: ROI segmentation and spatial resolution errors.

The introduction of the proposed error propagation methodology in the IVUS plaque characterization method shows that the outcome of the method is affected by a significant factor. In order to study the propagated error in each of the sequential steps of the IVUS method, the propagated error from the border detection algorithm was also computed. As presented in Table 6.2, when propagating the border detection error, there is not a significant change in the results: the mean bias is not much affected and the Pearson's correlation coefficients of the plaques remain the same (0.99). However, when the border detection error and the image formation error is propagated, the final results do change. Although, there is no major change in the mean bias, the Pearson's coefficients of the detected plaque types change for both overestimation and underestimation of the ROI (lumen and external border) area: reduced to 0.93 from the initial 0.99. Normal Tissue-Media is the tissue type affected mostly by the error propagation: the Pearson's coefficient is decreased from 0.99, which was initially reported to 0.86 and 0.88 overestimated and underestimated ROI area, respectively. However, this is a prospective result as the Normal Tissue-Media detection is affected mostly by the Euclidian distance of the lumen to the external border [158]. Still, all mean biases of the different types of plaque are very close to zero, proving that the final plaque characterization results after propagating the border detection error and image formation error are still accurate enough for inferring clinical decisions.

The proposed error propagation methodology when applied to the OCT plaque characterization method the final results are affected more than in case of IVUS method. In both underestimated and overestimated ROI area, the mean bias is increased and the Pearson's coefficient is decreased (maximum decrement 0.73 from 0.97 in fibrous tissue), meaning that the plaque detection accuracy is reduced. Although OCT (0.015 μm) has better spatial resolution compared to IVUS (0.15 μm), the propagation of the lumen and image formation errors to the final results decreases the plaque detection accuracy. However, the OCT method is limited in detecting the normal vessel wall (outer border of

the ROI) and the external border of the ROI is the expanded lumen up to 1.5 mm. In addition, plaque detection is not only based on the image intensity and texture as IVUS, but also on medical knowledge; i.e. a plaque region in order to be characterized needs to be fully bordered (CA) or its outer border is estimated based (LT) on the plaque curvatures. Thus, the method is sensitive to lumen border detection as the ROI increment or decrement affects the final results. Still, the final results can be considered accurate enough.

The validation of both two imaging methods that were tested (IVUS, OCT) using the proposed error propagation methodology presented more realistic results and revealed that the accuracy of the detected plaques may be reduced in worst case scenarios. In addition, in plaque detection methods which are sensitive to border detection, as OCT, an erroneous lumen detection can lead to erroneous results. Therefore, the proposed methodology could be used as an additional validation step in methods characterizing the atherosclerotic plaque. However, such methods should be two stage methods: in the first stage the lumen and outer wall are detected in order to determine the ROI and in the second stage the plaques are characterized. Furthermore, the methods need to be validated using the same validation metrics as the tested ones (IVUS, OCT). Finally, the proposed error propagation methodology when applied to the imaging methods (IVUS, OCT) does not affect the spatial location of the different plaque types when compared to the gold standard. As presented in Table 6.4, *PPV* value and $R_{over} / R_{non-over}$ validation metrics are not significantly affected in both overestimating and underestimating the ROI areas.

One limitation of the proposed error propagation methodology is that the error coming from the golden standard (VH-IVUS or experts) cannot be propagated. In order to incorporate such an error in the present study, validation of VH-IVUS plaque detection and experts annotations variability on OCT images must be performed using the same validation metrics. The inter observer variability for experts annotations, compared to VH-IVUS has been computed and validated using histology [77, 113] and different metrics. However, in order to match histological cross sections with IVUS frames, experts stain with haematoxylin the histology (producing Masson's trichrome images) and annotate the stained histological images. Therefore, there is an extra error (both from Masson's

trichrome images procedure and annotations) in the histology which is propagated in the VH-IVUS results. The propagation of such errors in the final characterization is a real challenge and requires both the presence of histological segments and the validation of the errors using the same metrics.

Chapter 7: Micro-CT and histological data inflation

7.1 Introduction

7.2 Proposed method

7.3 Dataset

7.4 Results

7.5 Discussion

7.1 Introduction

Acute thrombus formations results to life threatening consequences such as stroke or myocardial infarction. The mechanisms of coronary artery disease (CAD) initiation and progression [80] have been studied for many years [5]. It is well known that plaques at high risk for rupture and thrombus formation are not necessarily those that cause severe stenosis [186, 187]. In order to investigate plaque features of vulnerability and changes in their composition an accurate visualization of the vessel wall is needed. Several invasive and non-invasive imaging modalities [168, 173] are nowadays available which are major determinant of the clinical outcome [188], and provide the appropriate interventions. Imaging methods, allow assessment of luminal pathology, quantification of plaque burden and characterization of the type of the plaque.

Intravascular ultrasound (IVUS) [48] is currently the most widely used invasive imaging modality which provides high resolution cross-sectional images of the coronary arteries. In IVUS images an expert observer can permit detailed evaluation of the lumen, media-adventitia border and evaluate the plaque composition with a moderate accuracy [26]. To increase the accuracy of IVUS in characterizing the atherosclerotic plaque, several methodologies have been proposed in the literature allowing automated processing of the IVUS frames [67, 68, 70, 158, 189]. However, these methods were based on experts annotations and none of them was further validated using histological cross sections, which is considered as the current gold standard in plaque characterization. Therefore, the Virtual histology intravascular ultrasound (VH-IVUS) [77] plaque characterization

method was developed and validated based on histology [113]. VH-IVUS has become the method of choice in IVUS plaque characterization and is widely used in the clinical and research arena nowadays [190, 191].

Although histology is considered as the gold standard for atherosclerotic plaque characterization an accurate registration between the histology and IVUS images is needed [192]. Therefore, experts detect anatomical landmarks such as side branches, surrounding tissue layout, etc, in both IVUS and histological images. Once the artery histological segments are matched with the corresponding IVUS segments, the histological images are produced. However, histological image extraction is a time consuming procedure having several limitations. The harvested segments are mounted in a paraffin tray, then the pathologist cut the segments in cross sections and stains them with haematoxylin to produce Masson's trichrome images (histological images). Then the histological images are matched with the IVUS images and experts annotate the various plaque types in the stained histological images. Anatomically, the lumen of the produced histological images is narrowed eccentrically or concentrically. Tissue fixation at unphysiological intraluminal pressure as well as dehydration from paraffin embedding leads to artery wall shrinkage and coarctation, which is evident as gross deformations in cross-sectional histological images [193]. Therefore, the entire artery wall of the produced histological images cannot be matched with the artery wall in IVUS images and only a small area plaque can be matched and used for validation. This procedure was followed to validate the Virtual Histology (VH-IVUS) plaque characterization method [113]. However, the lack of histological data, the time-consuming and lab intensive procedure of histological image extraction procedure [194], makes the validation of IVUS methods extremely difficult.

Micro-CT is a powerful imaging technique providing high resolution images of small specimens. The feasibility of micro-CT for the analysis of the coronary artery wall has already been evaluated [195]. It provides high quality images able to visualize the 3D shape and size of the plaque precisely, even though it cannot be used in large animals *in-vivo*. Due to its excellent spatial resolution, micro-CT has been shown to be able to detect small early lesions at submillimetric scale and to be well correlated with histology [195]. Although, a detailed plaque characterization is an advantage of histology considered as

the current gold standard for visualizing plaque components, micro-CT could be also used as gold standard in plaque characterization due to its excellent resolution. However, the images acquired from both micro-CT and histology are deformed, due to the lack of blood pressure. Hence, both techniques require expert's intervention in order to be used for plaque characterization.

Traditionally, medical image-registration can be classified to rigid (images simply need to be rotated and translated to achieve correspondence) or non-rigid (correspondence between two images cannot be achieved without some stretching or deformation of the images) [196]. Experts can perform rigid image registration between IVUS and deformed images; deformed images can only be rotated to achieve correspondence without any morphological transformation. The images that are co-registered cannot have a point-by-point correspondence between the reference and target images as the micro-CT/histological images are deformed. Therefore an automated image registration method cannot be applied in order to transform the co-registered images into one coordinate system. To overcome this limitation in deformed images derived from both histology and micro-CT a non-rigid registration method, based on artery inflation is needed.

In this chapter, an inflation methodology for non-rigid registration of micro-CT/histological and IVUS images is proposed. The methodology is based on the assumption that lumen is deformed and compressed due to the lack of blood pressure; however the translation of center of gravity is negligible. The methodology consists of three main steps: a) IVUS/deformed image contour co-registration, b) Deformed image and IVUS lumen detection, and c) Image Inflation. The proposed methodology was used to inflate histological and micro-CT images and was validated by comparing the plaque areas in the pre and post inflated images with the plaque areas in the IVUS images. The method increases the plaque area matching between the inflated and the IVUS images improving the reliability for a correct plaque labeling process as well as it enhances the accuracy of the produced training dataset from the histological images.

7.2 Proposed method

The methodology for the artery inflation includes the following steps:

- IVUS/deformed image contour co-registration. Medical experts manually select paired frames of deformed images and IVUS images along the imaged vessels.
- Deformed image and IVUS lumen detection. The lumen borders of both deformed images (S_1) and IVUS images (S_2) are automatically detected.
- Image Inflation. A distance map is created from the areas inside S_1 and S_2 and is used to create a transformation matrix T . The inflated image $I(i, j)$ is produced by 2D interpolation of the non-inflated image using the transformation matrix T .

The above steps are presented in Figure 7.1. The methodology for vessel inflation estimates the deformation of the inner wall and applies an inverse transformation to create an image as close as possible to the uncompressed vessel.

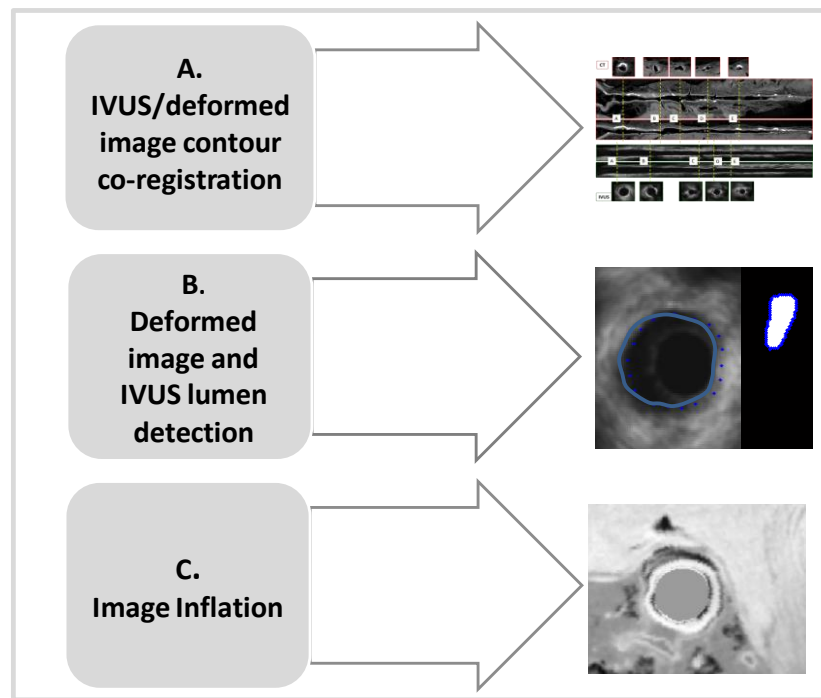


Figure 7.1: Schematic presentation of the proposed methodology.

7.2.1 IVUS/deformed image contour co-registration

As medical experts can only perform rigid image registration [196] between IVUS and deformed images, they selected manually paired frames of deformed images and IVUS on corresponding segments of the artery along the entire vessel. Fiducial points such as

side branches and calcified plaques are used as landmarks. By measuring the distance between branches the same artery segments are detected on both deformed images and IVUS. An example of deformed images and IVUS registration is shown in Figure 7.2 where five different landmarks were identified on both IVUS and micro-CT cross sections, by the experts.

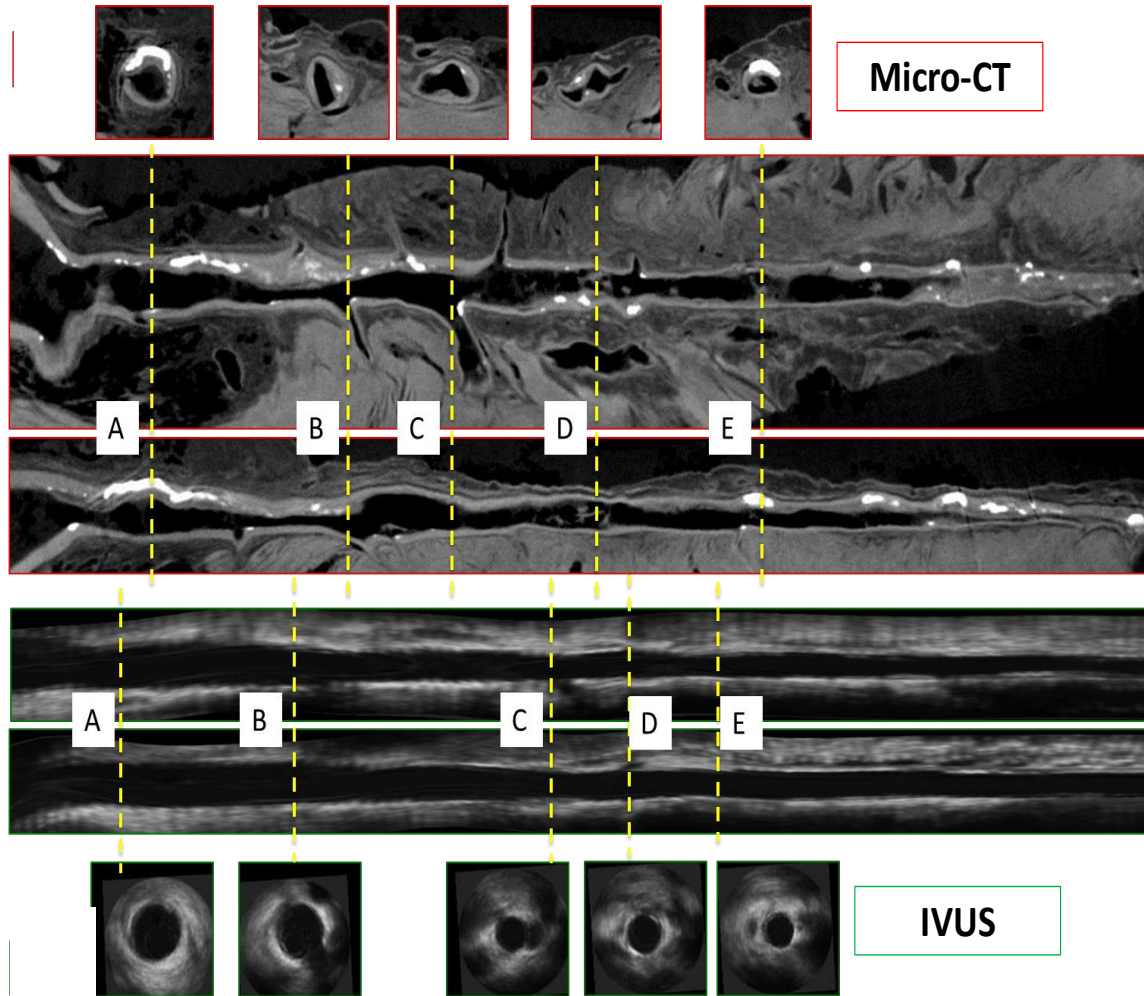


Figure 7.2: Micro-CT and IVUS registration. Five different landmarks were identified on both IVUS and micro-CT cross sections, by the experts. Bifurcations were detected on B, C, Bifurcations and Calcium were detected on D, E and Calcium was detected on A.

The landmarks identified in the two image sources (micro-CT/histological images and IVUS) are used for frame alignment, and border registration. The slice index of two landmark points is used for frame alignment, and the location of the landmark in the

image is used to rotate the IVUS borders accordingly in order to be aligned with the corresponding micro-CT/histological frames.

7.2.2 Deformed image and IVUS lumen detection

The second step of the methodology is the detection of both the deformed image lumen borders and the IVUS lumen borders in the co-registered images. The lumen border S_1 of the deformed image $I_D(i, j)$ is semi-automatically detected:

1. The user approximates manually the center of the lumen (C_i, C_j) of the I_D image.
2. Image I_D is segmented using an intensity based threshold T [24] ($T = 400$, sensitivity analysis was performed in the range of 100-1000).
3. The binary objects of the segmented image are labeled. As lumen area is considered the binary object that includes the center of the lumen (C_i, C_j) .

The procedure followed for detecting the lumen border on the deformed images is shown schematically in Figure 7.3.

To detect the lumen border S_2 in IVUS, we have used the method proposed by Plissiti *et al.* [60], which employs deformable models. The approach entails an initial contour of the lumen border to be provided at the first frame of the IVUS series.

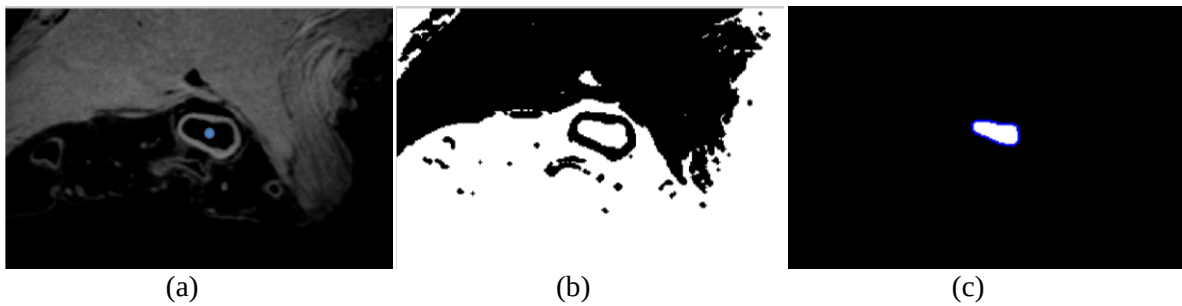


Figure 7.3: Procedure followed for detecting the lumen border on the deformed images: (a) Manual selection of the center of the lumen on the initial image, (b) the initial image is segmented using a threshold T , and (c) the detected lumen area.

7.2.3 Image Inflation

In order to inflate the image a transformation of each pixel on vertical and horizontal directions must be estimated. An assumption of our methodology is that the wall is not compressed therefore the translation of pixels corresponding to vessel wall is equal to the translation of the nearest lumen point. Therefore, for the estimation of the image transformation a mapping of contour S_1 (pre-inflated micro-CT/histology lumen) to S_2 (IVUS lumen) is required.

7.2.3.1 Active contours

An active contour ($e(t)=[i(t), j(t)]$, $t \in [0,1]$) is a method for delineating an object outline curve and has the ability to deform according to specific constraints. We minimize the energy function E_{active} , in order to obtain the final contour as:

$$E_{active} = \int_0^1 (E_{internal}(e(t)) + E_{external}(e(t))) dt, \quad (7.1)$$

where: $E_{internal} = k(s) \left| \frac{\partial e(t)}{\partial t} \right|^2 + \lambda(s) \left| \frac{\partial^2 e(t)}{\partial t^2} \right|^2$ and $k(s)$, $\lambda(s)$ are the regulation parameters. As an external energy $E_{external}$, we use the Gradient Vector Flow method [123].

7.2.3.2 Contour mapping

In order to calculate the image transformation map the active contour approach, described above, is followed [123]. Initially the following distance image $D(i, j)$ is created

$$D(i, j) = \begin{cases} D_{S_1}(i, j) + D_{S_2}(i, j) & i, j \text{ included in } S_2 \\ D_{S_2}(i, j) & \text{otherwise} \end{cases}, \quad (7.2)$$

where $D_{S_1}(i, j)$ and $D_{S_2}(i, j)$ is the signed distance of i, j from S_1 and S_2 , respectively.

The active contour [123] method is applied to the distance image $D(i, j)$, starting from S_1 , iterating until convergence. Using the distance image $D(i, j)$ the active contour is

forced to approximate the S_2 , let S_1' , as depicted in Figure 7.4-a. Therefore the active contour points of the initial model, approximating S_1 , and the corresponding points on the active contour result (after convergence), approximating S_2 , provide a mapping function from S_1 to S_2 as shown in Figure 7.4-b.

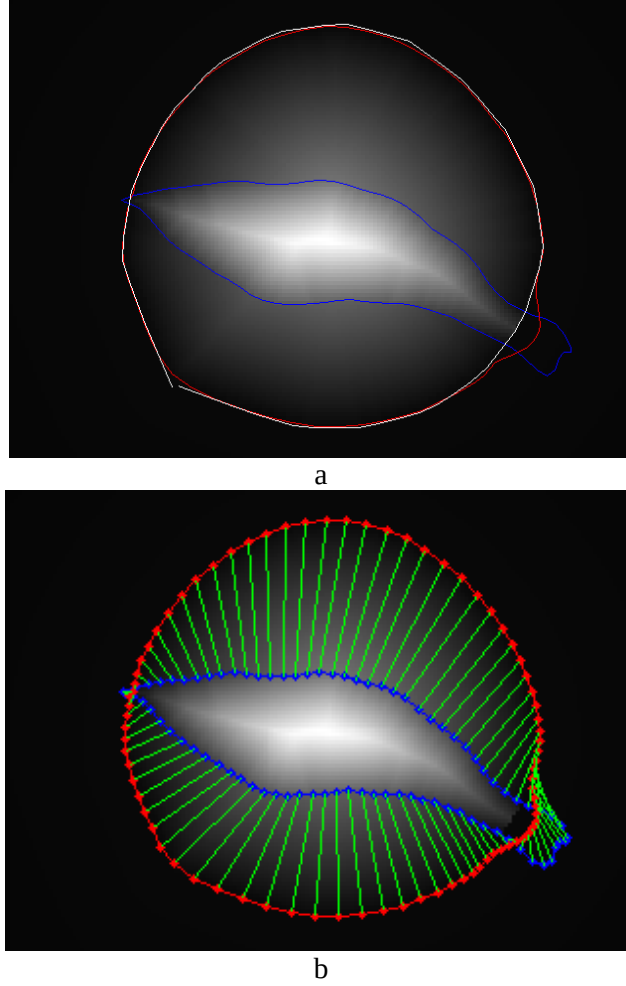


Figure 7.4: Distance image $D(i, j)$ with a) overlaid the contour S_1 (blue), the contour S_2 (white) and the convergence of the active contour model S_1' (red). b) Distance image $D(i, j)$ with overlaid the contour S_1 , the convergence of the active contour model S_1' (red) and their point-wise correspondence (green).

7.2.3.3 Transformation matrix

The contour mapping from S_1 to S_2 is used in order to create the transformation matrix $T(i, j)$ in vertical ($T_x(i, j)$) and horizontal directions ($T_y(i, j)$):

$$Tx(i, j) = \begin{cases} i + P_x^{S_1}(i, j) & i, j \text{ exterior to } S_1 \\ i & \text{otherwise} \end{cases}, \quad (7.3)$$

$$Ty(i, j) = \begin{cases} j + P_y^{S_1}(i, j) & i, j \text{ exterior to } S_1 \\ j & \text{otherwise} \end{cases}, \quad (7.4)$$

where $P_x^{S_1}(i, j)$, $P_y^{S_1}(i, j)$ are the translations on vertical and horizontal directions of the point on S_1 (translation to the corresponding point on S_1') closest to (i, j) , respectively. A schematic presentation of the translations on vertical and horizontal directions is shown in Figure 7.5.

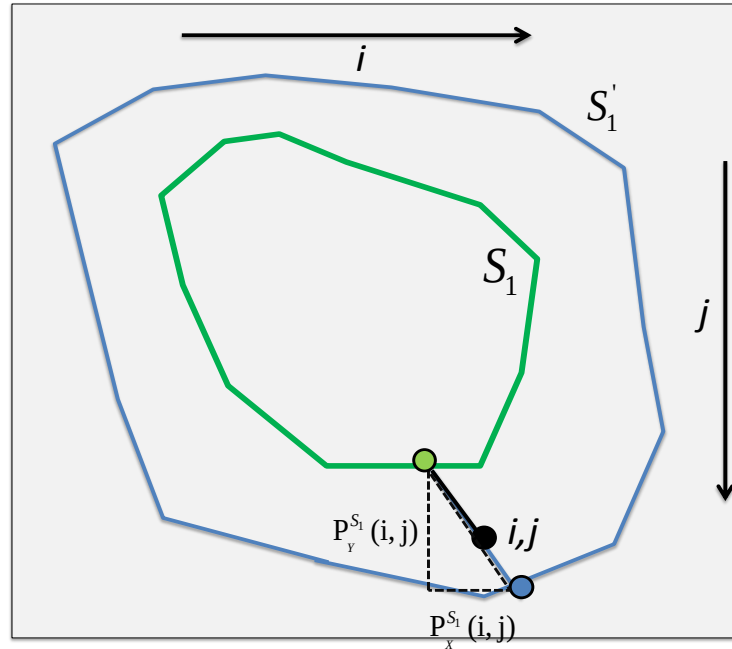


Figure 7.5: Schematic presentation of the translations on vertical and horizontal directions of the point on S_1 (translation to the corresponding point on S_1') closest to (i, j) .

The inflated image $I(i, j)$ is produced by the 2D interpolation of the non-inflated image using the Cartesian translation maps for $T_x(i, j)$ and $T_y(i, j)$ (Figure 7.6).

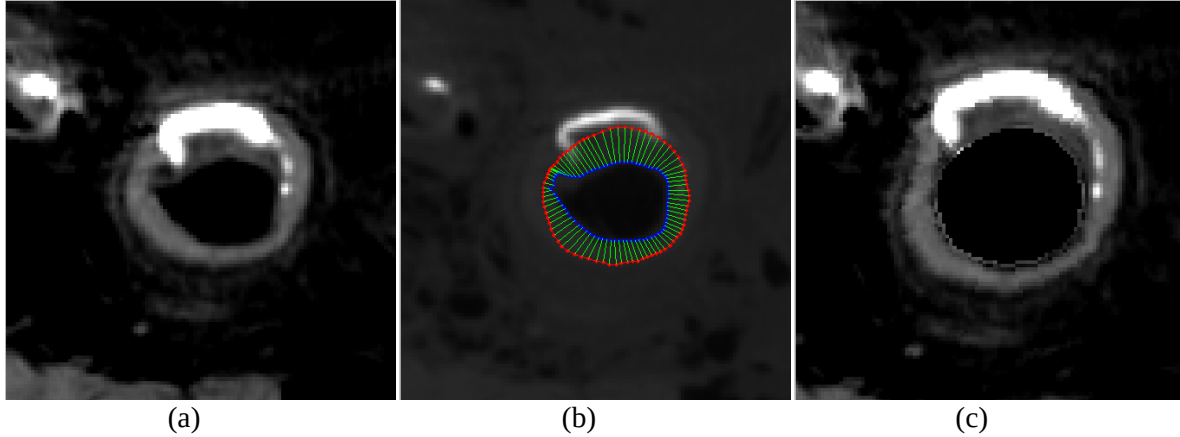


Figure 7.6: (a) Micro-CT pre-inflated image, (b) Mapping (green lines) of post-inflated lumen (red curve) to the pre-inflated lumen (blue curve) and (c) Micro-CT post-inflated image.

7.3 Dataset

7.3.1 Animal experiments

Following 4 months of high-fat, cholesterol (4%) supplemented diet, 3 pigs underwent left coronary angiography and IVUS examinations and then sacrificed by KCl i.v. bolus injection under anesthesia. The entire heart was harvested and the coronary arteries were processed for micro-CT acquisition and histopathological examination. Animal instrumentation and experimental protocols were approved by the Animal Care Committee 5 of the Italian Ministry of Health (Protocol number: 06/2009-B-2009/01/26). The approval was according to the Italian Law (DL-116, Jan. 27, 1992) and the National Institute of Health Guide for the Care and Use of Laboratory Animals.

7.3.2 IVUS

The dimensions of each IVUS frame were 384x384 pixels and the system used was the Volcano Therapeutics, Rancho Cordova, CA. The catheter was the Eagle Eye Gold, Volcano Therapeutics with 2.9-F and 20-MHz diameter and frequency, respectively.

7.3.3 Histopathology

The animals which were selected for histomorphometric validation of micro-CT coronary measurements, featured mild (preatherosclerotic changes), moderate (<50% of segments

with atheromas) and severe ($> 50\%$ of segments displaying atheromas) intima changes of the left coronary artery (LCA). After micro-CT scan, en bloc embedded LCA specimens were rewarmed, removed from the Falcon test tube, cross cut in segments of 5 mm length and sectioned (Microm HM 300, Bio-optica). Ten to twelve cross sections from each artery were obtained and stained with haematoxylin and eosin (H&E) and Masson's trichrome stains. All sections were examined under a light microscope (Olympus BX43) at 4x to 20x original magnification and digitized by a video system (Olympus D70 camera) interfaced to Olympus Cell Sens Dimension software for image acquisition and morphometric analysis. H&E and Masson's stained sections, under 2x microscope magnification, were digitized at 1600x1600 pixel resolution and compared to corresponding micro-CT and IVUS slices. Artery wall cross-sectional thickness and area, as well as maximal intimal thickness and cross-sectional area of each section were calculated.

7.3.4 micro-CT

An in-house developed micro-CT scanner installed at IFC-CNR, Pisa, was used [28]. All the tomographic acquisitions were made with 720 projections over 360 degrees, for a total scan time of 54 min and a total exposure of 2268 mAs. For each sample, we have obtained images of 512x512 dimensions.

7.4 Results

7.4.1 Validation metrics

The proposed image inflation methodology was validated using expert's annotations. A medical expert selected manually paired frames of micro-CT/IVUS images and histological/IVUS images. From the 3 arteries 22 micro-CT/IVUS and histological/IVUS pairs were selected. Plaque was visible in 16 micro-CT/IVUS segments and in 18 histological/IVUS segments. The same expert annotated the plaque in these 16 and 18 micro-CT/IVUS and histological/IVUS segments, respectively. The images were inflated using the proposed inflation methodology and plaque areas were calculated in the pre and post inflated images and were compared with the plaque areas of the IVUS images. In

order to find the best match for comparing the plaque area between the pre-inflated images with IVUS images the centers of gravity in the two areas were detected. The center of gravity $C(C_x, C_y)$ of each area for n pixels of the area perimeter is defined as:

$$C_i = \frac{1}{6A} \sum_{q=1}^{n-1} (i_q + i_{q+1})(i_q j_{q+1} - i_{q+1} j_q), \quad (7.5)$$

$$C_j = \frac{1}{6A} \sum_{q=1}^{n-1} (j_q + j_{q+1})(i_q j_{q+1} - i_{q+1} j_q) \quad (7.6)$$

where A is the plaque area.

To validate the proposed methodology we computed the Pearson's correlation coefficients between the plaque areas measured in the pre and post inflated images and the plaque areas measured in the IVUS images, we have performed Bland-Altman analysis and we have computed the positive predictive value:

$$PPV = \frac{TP}{TP + FP}, \quad (7.7)$$

As true positive values (TP) we define the common area between the two measurements, as false positive (FP) values we define the plaque area of the micro-CT or histological image minus the common area and as false negative (FN) the IVUS plaque area minus the common area. Finally, the ratio of overlapping/non-overlapping areas and the Jaccard/Sørensen–Dice similarity coefficients were computed. The ratio of overlapping/non-overlapping areas were defined as:

$$R_{over} = \frac{TP}{TP + FN}, \quad (7.8)$$

$$R_{non-over} = \frac{FN + FP}{TP + FN}, \quad (7.9)$$

and the Jaccard/ Sørensen–Dice similarity coefficients were defined as:

$$J = \frac{TP}{FP + FN}, \quad (7.10)$$

$$SD = \frac{2 \times TP}{2 \times TP + FP + FN}. \quad (7.11)$$

7.4.2 Validation

The results of the proposed methodology using the above validation metrics are presented in Table 7.1. Specifically R_{over} , $R_{non-over}$, J , SD and PPV validation metrics are calculated for comparison of IVUS and micro-CT/histological images at pre and post inflation. As presented in Table 7.1 the overlapping plaque area and Jaccard/Sørensen–Dice similarity coefficients are increased in post-inflated images while non-overlapping is decreased, meaning that the proposed methodology can provide a more realistic matching between the IVUS and micro-CT/histological images. The correlation and Bland-Altman plots for plaque areas between the areas measured in the pre and post inflated images and the plaque measured in the IVUS images are presented in Figure 7.7 and Figure 7.8, respectively. As shown (Figure 7.7 and Figure 7.8) plaque areas either change (micro-CT) or not (histological images) in pre and post inflation, a good agreement is achieved in both cases. Finally, Figure 7.9 and Figure 7.10 present application examples of the proposed image inflation method using micro-CT and histological images, respectively.

Table 7.1: The validation results of the proposed image inflation methodology. R_{over} , $R_{non-over}$, PPV , J and SD validation metrics are calculated for the comparison of IVUS and micro-CT/histological images pre and post inflation.

Validation metrics	Histological images		Micro-CT images	
	Pre-inflated	Post-inflated	Pre-inflated	Post-inflated
Sensitivity/ R_{over}	0.67	0.74	0.41	0.63
PPV	0.58	0.59	0.54	0.69
$R_{non-over}$	0.81	0.76	0.93	0.66
J	0.83	0.97	0.43	0.94
SD	0.62	0.66	0.46	0.95

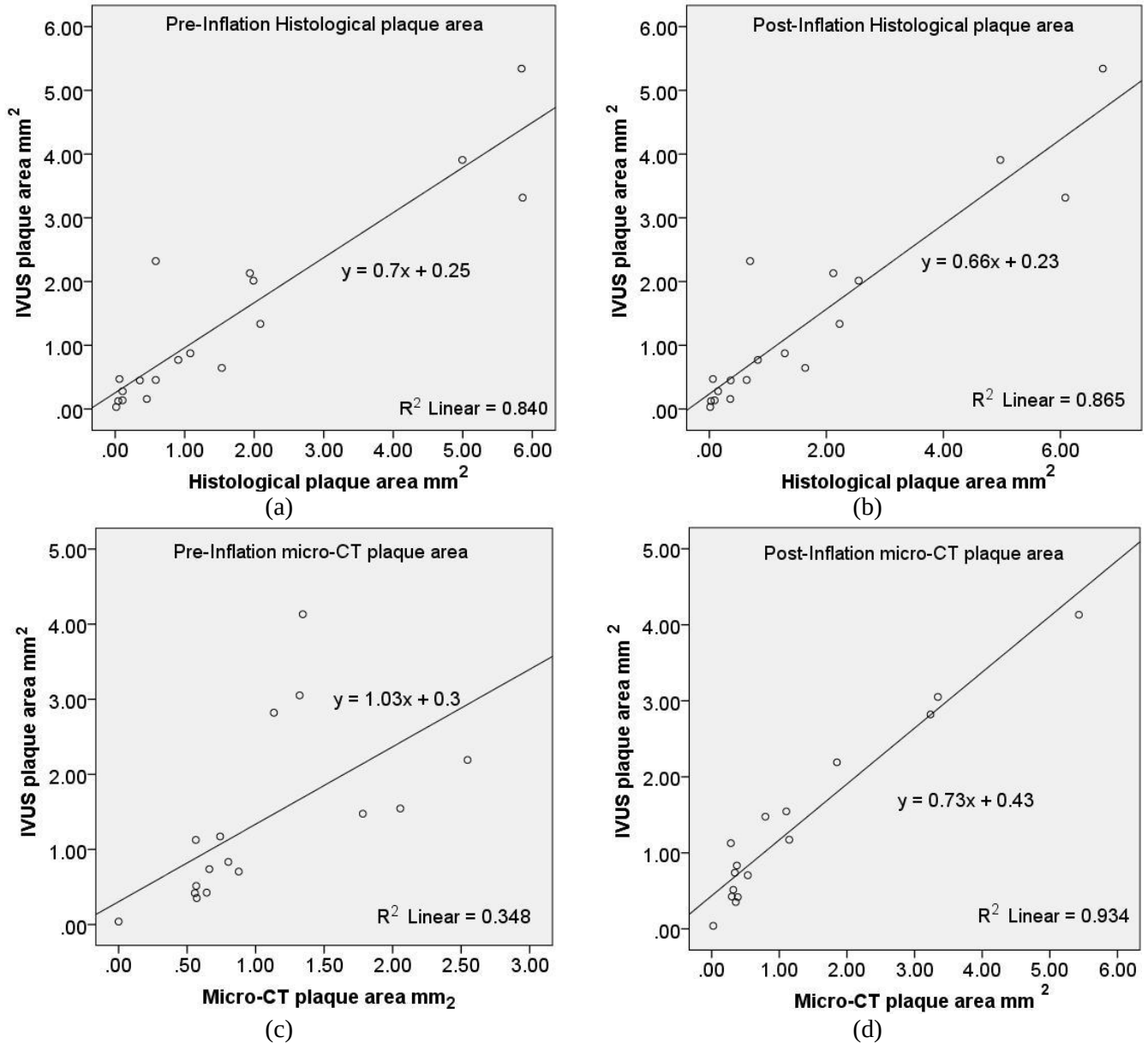
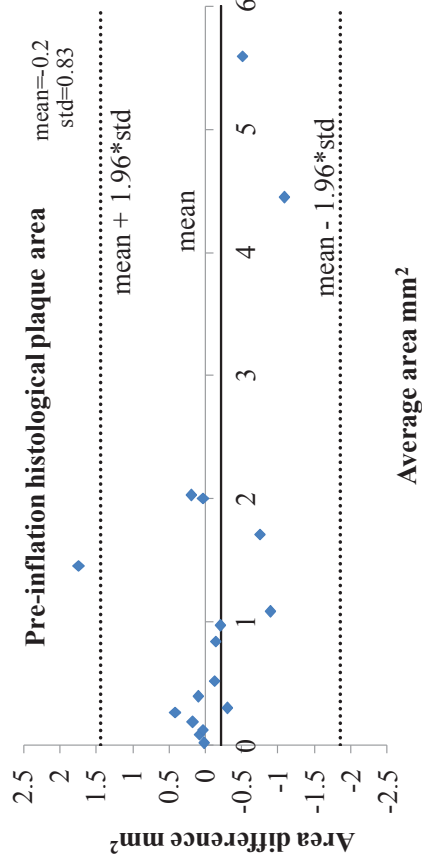
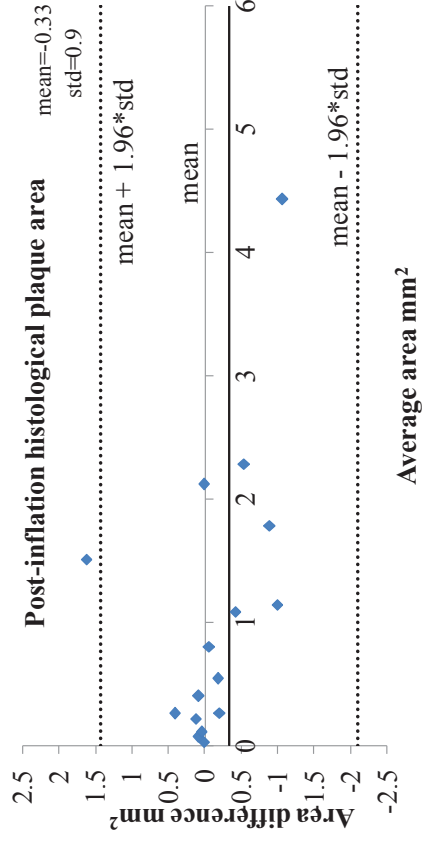


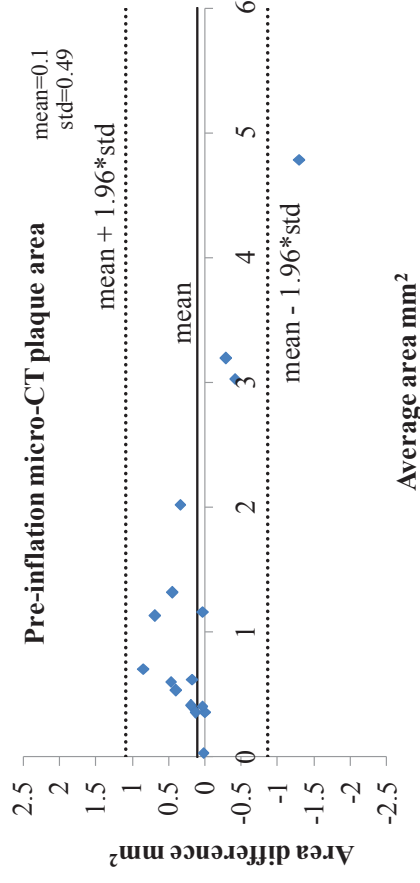
Figure 7.7: Correlation plots for plaque areas for the areas measured in the pre and post inflated images and the areas measured in the IVUS images: (a) pre- inflated vs IVUS images histological plaque areas comparison, (b) post- inflated vs IVUS images histological plaque areas comparison, (c) pre- inflated vs IVUS images micro-CT plaque areas comparison and (d) post- inflated vs IVUS images micro-CT plaque areas comparison.



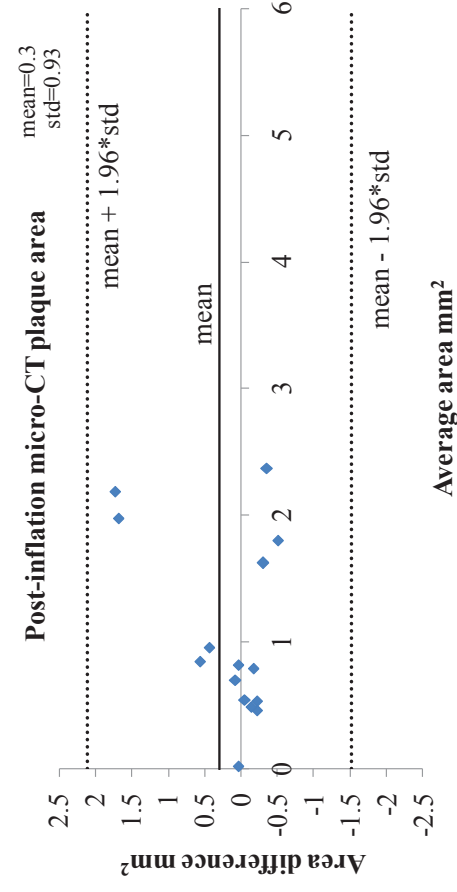
(a)



(b)



(c)



(d)

Figure 7.8: Bland-Altman plots for plaque areas for the areas measured in the pre and post inflated images and the areas measured in the IVUS images: (a) pre-inflated vs IVUS images histological plaque areas comparison, (b) post-inflated vs IVUS images histological plaque areas comparison, (c) pre-inflated vs IVUS images micro-CT plaque areas comparison and (d) post-inflated vs IVUS images micro-CT plaque areas comparison.

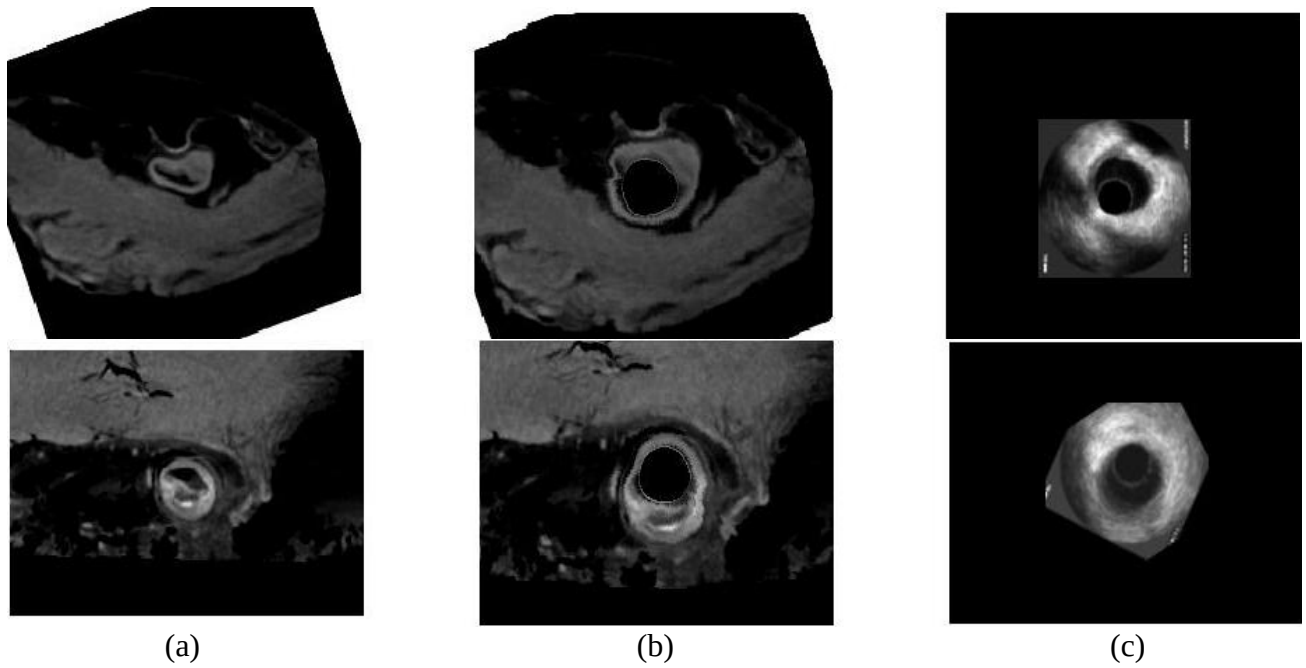


Figure 7.9: a) Micro-CT pre-inflated images, b) micro-CT inflated images and c) corresponding IVUS.

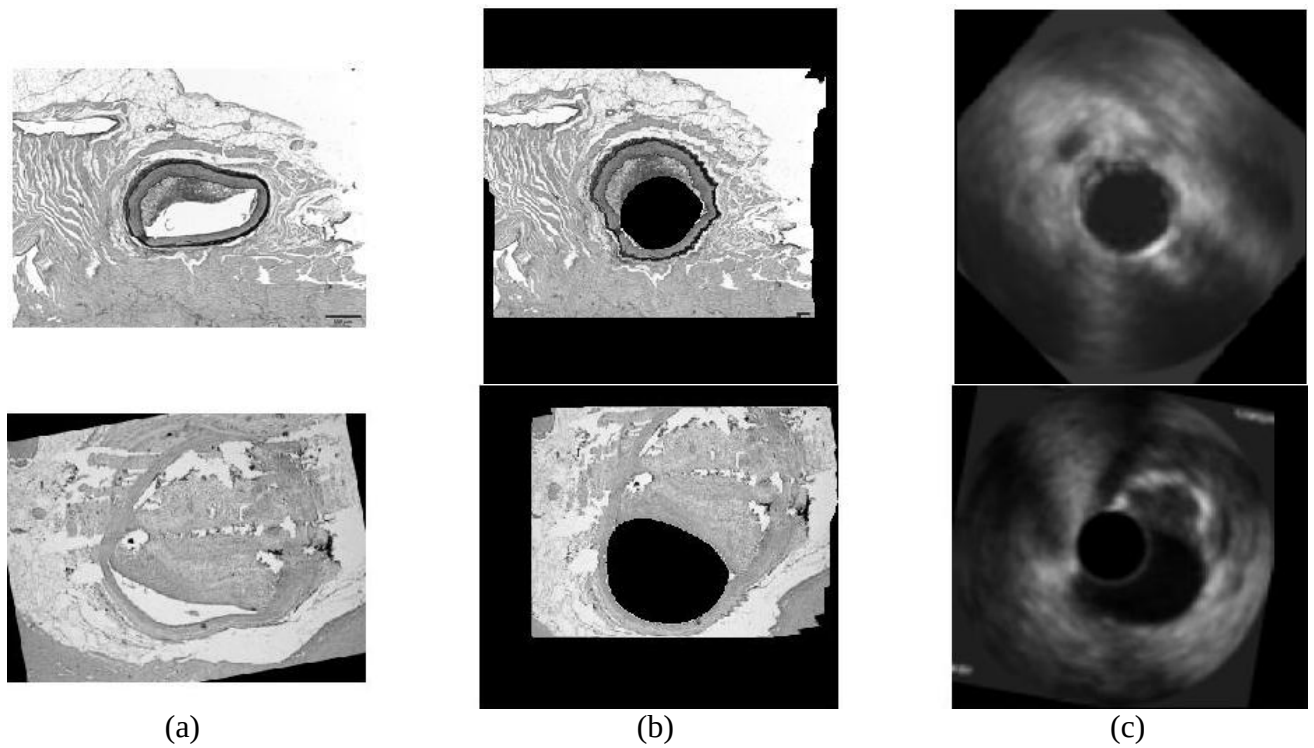


Figure 7.10: a) Histological pre-inflated images, b) histological inflated images and (c) corresponding IVUS images.

7.5 Discussion

In this work, a methodology for image inflation using micro-CT and histological images is presented. The methodology uses the lumen area of IVUS frames to inflate micro-CT/histological images which are deformed and compressed due the lack of intraluminal blood pressure. The methodology was validated by comparing the plaque areas in the pre and post inflated images with the plaque areas in the IVUS images.

Although several IVUS plaque characterization methods have already presented in the literature [67, 68, 158] there were not validated using histological cross sections, which is considered as the current gold standard in plaque characterization. Micro-CT could also be used as gold standard as the analysis of the coronary artery is similar to histology. However, both micro-CT and histological data acquisition produce deformed images due to the lack of intraluminal blood pressure. As a result the plaque areas of the produced micro-CT/histological images are also deformed and a realistic area comparison of the plaque between IVUS and histology cannot be performed. By applying the proposed image inflation methodology, the plaque comparison area is significantly increased. In histological images the increment of the overlapping area is 7% which reaches to 21% in the micro-CT images (Table 7.1). Additionally the Jaccard-Sørensen–Dice similarity coefficients are also increased in both histological (pre-inflated increment: 14% and post-inflated increment: 4%) and micro-CT images (pre-inflated increment: 51% and post-inflated increment: 49%). However, by computing the overlapping area (sensitivity) and the Jaccard-Sørensen–Dice coefficients we are not able to understand if the methodology overestimates the plaque areas (FP>FN). Thus, an additional metric: the non-overlapping areas was computed. The non-overlapping areas are considerably decreased (5% and 27% decrement in histological and micro-CT images, respectively). Plaque areas in pre and post inflation of histological images do not significantly change, however, there is a good agreement in both pre and post inflation comparison (Figure 7.7-a,b and Figure 7.8-a,b). On the contrary plaque areas change in micro-CT images resulting to a better agreement in post inflation comparison (Figure 7.7-c,d and Figure 7.8-c,d).

The proposed methodology was applied to porcine coronary arteries (18 histological and 16 micro-CT images) at three different stages and extent of diet-induced coronary

atherosclerosis development. For the first time a methodology for inflating images acquired from harvested arteries is presented. The methodology uses IVUS lumen borders as reference in order to produce the inflated histological/micro-CT images. By applying the proposed methodology in plaque characterization methods validated with histology, the reliability of the validation procedure is enhanced. The methodology could also be applied in plaque characterization methods based on other imaging modalities (i.e. optical coherence tomography), as long as they can sufficiently depict the lumen border.

Although by applying the proposed artery inflation methodology we overcome the experts' intervention step over the micro-CT/histological images in order to achieve the best matching in plaque areas, manual process is needed in order to identify fiducial markers. The corresponding starting and ending frames should be detected in both micro-CT/histological and IVUS images. Finally, due to standard histological tissue processing the plaque areas are increased in contrary to the plaque areas of the *in vivo* arteries. This limitation cannot be addressed by the proposed methodology.

Chapter 8: Conclusions and Future Work

8.1 Conclusions

8.2 Future Work

8.1 Conclusions

A study for characterizing the atherosclerotic plaque using invasive (IVUS and OCT) and non-invasive (CTA) imaging modalities is presented. Going beyond the state of the art a novel hybrid methodology able to identify the plaque composition in grayscale IVUS has been developed. The hybrid IVUS approach combines automatic thresholding, active contours and a RF classifier in order to detect accurately the plaque type and overcome the limitations of manual tracing. To examine the performance of the method its estimations are compared with the annotations of experts and the obtained results provided high overall accuracy. Thus, the proposed approach can be useful in research and can be applied to process grayscale IVUS data, quantify changes in the plaque composition and assess the effectiveness of new treatments. On the other hand VH-IVUS today constitutes one of the preferred methods for the evaluation of the type of the plaque as it provides comprehensive images and quantitative measurements which have allowed its broad use in the study of plaque development and of the effect of different treatments in plaque evolution. Going beyond the limits of VH-IVUS (large amount of data lost, VH software not always available, previously acquired data cannot be used) a second methodology was developed, able to characterize the composition of the plaque in all grayscale frames of an IVUS sequence. This is the only approach that permits identification of all plaque types and utilizes geometric features for more reliable plaque characterization. Although the initial validation results appear promising, further application of the method in real clinical practice is needed in order to evaluate its feasibility and efficacy.

For the first time in the literature a fully automated methodology that is able to detect the lumen borders and characterize the composition of the superficial plaque in OCT images, is presented. The methodology is based on the analysis of the OCT images and was

validated using the estimations of expert observers as gold standard. The results demonstrated that the methodology allows accurate border detection and reliable characterization of the different tissue types with a typical morphology. However, further validation of the proposed approach in tissues with typical morphology using histology data is needed before this methodology can be used in the clinical and research arena.

As non invasive methods are widely used in treating coronary artery disease nowadays, an automated methodology for the segmentation of the regions of interest and characterization of the composition of the atheroma in CT imaging data has been developed. The proposed approach allows coronary reconstruction in near real time and a holistic assessment of plaque morphology and distribution. The validation of the proposed approach demonstrated that the method is reliable and allows accurate representation of coronary anatomy. Thus, it is anticipated to be proven a useful tool in research arena.

Furthermore, the error propagation in plaque characterization methodologies combined with a vessel segmentation method has been investigated. For the first time in the literature, a propagation schema for calculating the error resulting from the application of sequential image processing methods towards plaque characterization in arterial segments was presented. Two application examples have been presented for plaque characterization in IVUS and OCT imaging modalities. All the methods presented in the literature, having sequential steps for characterizing the plaque do not take into consideration the error propagated from one step to another or the error resulting from the image formation. By applying the presented error propagation schema the maximum resulted propagated error is higher than the initial one and the expected propagated error lies in acceptable limits, not affecting clinical decision. Thus, the proposed schema could be used as an extra validation step in plaque characterization methods preceded by vessel segmentation.

Finally, both micro-CT and histology produce images corresponding to deformed vessels. This deformation is due to the lack of blood pressure. Currently, by using the proposed image inflation methodology this limitation can overcome and produce realistic micro-

CT/histological images. The proposed methodology could be used for validating several plaque detection methods with histological/micro-CT images.

8.2 Future Work

In atherosclerotic plaque characterization, there are several future directions. One of them is the validation of the presented plaque characterization methodologies using histological images which is the current gold standard in atherosclerotic plaque characterization. The application of the image inflation methodology to the validation data can be used in order to overcome the histology limitation of the deformed images. By validating the presented plaque characterization methodologies using histology, they could be used in the clinical arena. Another future direction is the development of plaque characterization methodologies for recently developed imaging technologies: Near-infrared spectroscopy IVUS and hybrid imaging technologies: IVUS-OCT imaging and Positron Emission Tomography–Computed Tomography (PET-CT). However these imaging technologies are currently under development and are not widely used as they are not commercially available or they do not have any certification mark (CE marking or FDA approval).

Further to the 3D plaque characterization of the presented CT methodology, the 3D reconstructed models with the plaque objects can be used for performing blood flow analysis and low-density lipoprotein (LDL) transport for calculating the distribution of wall shear stresses (WSS) and LDL accumulation in the artery, respectively. LDL accumulation in the sub-endothelial layer is one of the hallmarks of atherosclerosis onset and ignites plaque development and progression. WSS is causally related to the heterogenic distribution of atherosclerotic lesions and critically affects LDL deposition in the vessel wall.

Among the presented potential directions, future research plans include the development of plaque characterization methodologies for detecting the atherosclerotic plaque in new developed hybrid imaging systems: PET-CT and IVUS-OCT images. Additionally, future research will focus on the investigation of time-dependent changes in atherosclerotic plaque composition and the development of a methodology for predicting plaque

composition changes. This will allow risk stratification of atherosclerotic lesions and early identification of their subsequent natural history, and may consequently enable the application of novel therapeutic strategies.

Appendix

A: Cluster parameter selection (Chapter 4)

In order to find the best number of clusters we tested the performance of the *K-means* algorithm on the 34 CA images using $K=2, 3$ and 4 clusters. The results are presented in Table A.1. When using $K=2$ the CA could not be detected (Overlapping area for the CA=0.008) while when using $K=4$ the results of the overlapping area analysis for the CA was 0.27. On the other hand $K=3$ allowed accurate detection of the CA (overlapping area ratio: 0.81) and permitted detection of the outer tissue border.

Table A.1: Overlapping area, non-overlapping and positive predictive value for detecting calcium (CA) using different number of clusters.

Validation Metrics	# Clusters		
	K=2	K=3	K=4
Overlapping area (sensitivity)	0.008	0.81	0.27
Non-overlapping area	0.99	0.35	0.85
PPV	1	0.83	0.68

B: Pixels neighborhood size selection (Chapter 4)

To find the best pixels neighborhood ($n \times n$) size for extracting the feature set we performed a sensitivity analysis in the training set (60 images). Fifteen different neighborhood values ($n=5 \dots 15$) were used that provided 15 feature sets ($FS_5, FS_6, \dots, FS_{15}$). In the different feature sets a 10-fold cross validation methodology was applied to find the best set for the proposed methodology. RF classifier (110 trees and 8 features) was used and we found that FS_{11} outperformed the other feature sets; therefore we used a 11×11 pixels size neighborhood.

C: Feature selection and classifier parameter selection (Chapter 4)

Since WRP's input is a classification algorithm, we used RF (110 trees and 8 features) classifier to calculate the overall accuracy achieved for each subset which is created, and find the best WRP's features. From the 44 features ($f_{glcm}^1 - f_{glcm}^{32}$, $f_{lbp}^1 - f_{lbp}^{10}$, f_{en} , f_{mv}) of the feature set WRP selected 17 features: (f_{glcm}^1 , f_{glcm}^9 , $f_{glcm}^{11} - f_{glcm}^{13}$, f_{glcm}^{15} , $f_{lbp}^1 - f_{lbp}^{10}$ and f_{en}). To find the best input parameters for the RF classifier, several numbers of features and trees were tested. Using the 17 features selected by the WRP and a 10 fold cross validation methodology the best classification accuracy was achieved using 110 numbers of trees and 8 features and was used as input for the RF classifier.

D: Post processing threshold sensitivity analysis (Chapter 4)

To find the best threshold (Th_{post}) value we shifted Th_{post} from 0,5-0,875mm every 0,125 mm. Sensitivity-overlapping area, non-overlapping area and PPV of LT were computed for applying the post processing step using as Th_{post} 0,5 mm, 0,625 mm, 0,75 mm and 0,875 mm (Table 2). As it is shown in Table D.1 the best overlapping area was achieved when applying $Th_{post} = 0.625\text{mm}$, however the best PPV and the best non-overlapping area was achieved when applying $Th_{post} = 0.75\text{mm}$. Therefore Pearson's correlation coefficients were computed for these threshold values. $Th_{post} = 0.75\text{mm}$ was selected as threshold value for the post processing step as its Pearson's correlation coefficient (0.96) outperformed the $Th_{post} = 0.625\text{mm}$ correlation coefficient (0.77).

Table D.1: Sensitivity and PPV of LT when applying different post processing Th_{post} values.

	Threshold values Th_{post}			
	0.5 mm	0.625 mm	0.75 mm	0.875 mm
Overlapping area (sensitivity)	0.69	0.73	0.71	0.65
Non-overlapping area	1.09	0.57	0.51	0.57
PPV	0.47	0.70	0.76	0.74

E: MIFR threshold value computation (Chapter 6)

To find the best threshold, thr for the MIFR calculation, the area of Dense Calcium (Figure 6.2) was used and MIFR values for different thr in the range of $[0.01-0.99]$ were computed. As it is shown in Figure E.1, MIFR values are symmetric in the range of $0.01-0.99$ and the highest MIFR is achieved for $thr = 0.01$. Therefore we choose $thr = 0.01$ as the percentage of activation for a low-resolution cell. The area of the plaque affects only the absolute value of MIFR (Eq. 1) and not the distribution of MIFR over different values of thr . Apparently, for $thr = 0$ the MIFR is infinite since all cells will be activated. In order to have a threshold of larger precision (>0.01) a larger spatial resolution (more than $10\times$) should be applied, which however increases computational time. Finally, the error trend as depicted in Figure E.1 is linear and therefore is not expected to increase significantly for $thr < 0.01$.

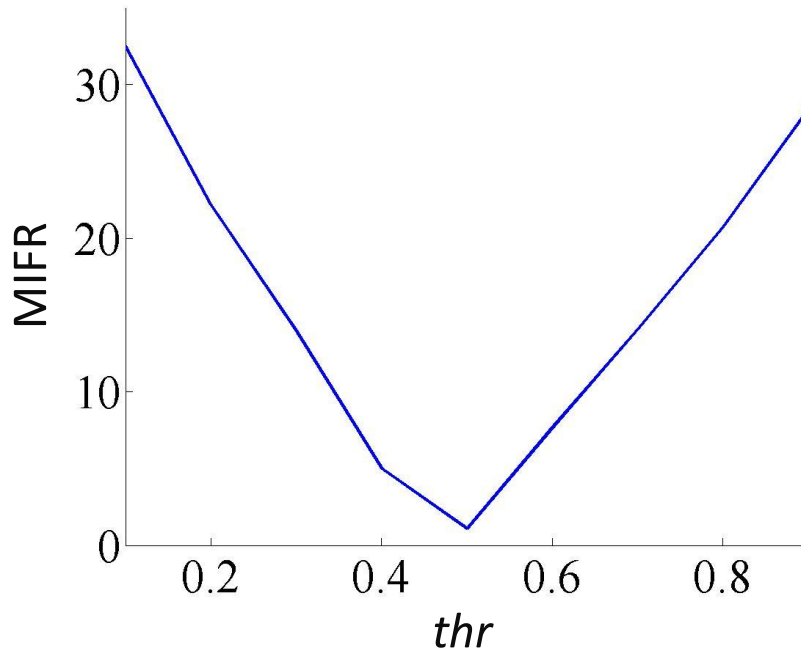


Figure E.1: MIFR calculation using different threshold values thr (range 0.1-0.9).

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Short CV

Lambros S. Athanasiou was born in Ioannina, Greece, in 1985. He received the diploma degree in the Department of Information and Communication Systems Engineering from the University of Aegean, Greece, in 2008. He received his Ph.D. degree in the Unit of Medical Technology and Intelligent Information System at the Dept. of Materials Science and Engineering at the University of Ioannina. His research interests include medical image processing, biomedical engineering, decision support and medical expert systems.