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Research paper

Dark-field imaging in coronary atherosclerosis

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ABSTRACT

Objectives: Dark-field imaging based on small angle X-ray scattering has been shown to be highly sensitive for microcalcifications, e.g. in breast tissue. We hypothesized (i) that high signal areas in dark-field imaging of atherosclerotic plaque are associated with microcalcifications and (ii) that dark-field imaging is more sensitive for microcalcifications than attenuation-based imaging.

Methods: Fifteen coronary artery specimens were examined at an experimental set-up consisting of X-ray tube (40 kV), grating-interferometer and detector. Tomographic dark-field-, attenuation-, and phase-contrast data were simultaneously acquired. Histopathology served as standard of reference. To explore the potential of dark field imaging in a full-body CT system, simulations were carried out with spherical calcifications of different sizes to simulate small and intermediate microcalcifications.

Results: Microcalcifications were present in 10/10 (100%) cross-sections with high dark-field signal and without evidence of calcifications in attenuation- or phase contrast. In positive controls with high signal areas in all three modalities, 10/10 (100%) cross-sections showed macrocalcifications. In negative controls without high signal areas, no calcifications were detected. Simulations showed that the microcalcifications generate substantially higher dark-field than attenuation signal.

Conclusions: Dark-field imaging is highly sensitive for microcalcifications in coronary atherosclerotic plaque and might provide complementary information in the assessment of plaque instability.

1. Introduction

X-rays, as well as other forms of electromagnetic radiation, are subject to several physical effects including attenuation, refraction and scattering. So far, attenuation is the only source of contrast in medical X-ray imaging including plain radiography, fluoroscopy or computed tomography (CT).

Imaging techniques, which are capable of exploiting other physical phenomena, are subject to intensive research efforts, since they might provide additional and potentially complementary information to attenuation contrast imaging [1]. Grating-interferometry allows the simultaneous acquisition of data on X-ray attenuation, phase-shift and small-angle scattering [2]. It has been shown that phase-contrast imaging yields a substantially improved contrast in low-absorbing materials like biologic soft tissue [3–6]. Small-angle scattering occurs predominantly in areas with multiple density fluctuations [7,8]. This has been referred to as dark-field signal [9]. It has been shown that the dark-field signal is highly sensitive for calcifications and especially microcalcifications, e.g. in breast imaging [10,11].

Calcification is an important phenomenon in the development of atherosclerotic plaque. Macrocalcifications, which are easily detected in X-ray attenuation imaging, are the result of long standing, subsiding vessel wall inflammation and are often detected in late, generally stable plaque stages. Microcalcifications, which are too small to be visualized by clinical scanners, are more often found in areas of active inflammation, such as lipid-rich necrotic core as well as the fibrous cap

Abbreviations: ATS, attenuation signal; CT, computed tomography; DFS, dark-field signal; HE, Hematoxylin-Eosin; PET, positron emission tomography

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and thus might be an interesting target in the assessment of plaque vulnerability [12]. For example, ex vivo studies have shown that microcalcifications increase local tissue stress and cause fibrous cap rupture [13].

The purpose of this study was to assess the potential of grating-based dark-field imaging for the detection of macro- and microcalcifications in human coronary atherosclerotic plaque. We hypothesized that dark-field imaging is more sensitive for calcifications than both phase contrast and attenuation contrast with histopathology serving as the standard of reference. Since our experiments were carried out using an ex vivo experimental set-up, we simulated attenuation and dark-field signal for calcifications in a clinically more realistic setting using bigger pixel sizes and measurement parameters that are comparable to clinical computed tomography.

2. Materials and methods

2.1. Ethics statement

The project was approved by the local ethics committee and complies with the Declaration of Helsinki of 1975, as revised in 2008. All heart specimens were provided by the International Institute for Advancement in Medicine (IIAM, Edison, New Jersey, USA). Written informed consent to provide tissue for research purposes had been obtained by the donor or the donor's relatives.

2.2. Study design and experimental overview

The study was designed as a prospective, post-mortem, ex vivo experimental study. Human heart specimens were gathered within 24 h of the patient's death. Coronary arteries including the right, the left main, the left anterior descending and left circumflex coronary artery were excised including parts of the myocardium and epicardial fat. The samples were fixed in 10% buffered formalin and kept in phosphate buffered normal saline. Experiments were carried out at a laboratory-based imaging set-up consisting of an X-ray tube, a grating-interferometer and a detector. Cross-sections with high signal areas in dark-field contrast but no indication of potential calcification in phase and attenuation contrast were identified. An equal number of cross-sections serving as positive controls and negative controls were selected (Table 1). Afterwards, samples underwent histological work-up and were matched with the corresponding dark-field, attenuation and phase contrast data. Histopathological images were analyzed for macro- and microcalcifications by an expert pathologist blinded to all imaging data.

2.3. Principles of dark-field imaging

The principles of a grating-based interferometry have been explained in detail elsewhere [2,9,14]. Briefly, X-ray phase information is obtained using a conventional x-ray source, a grating interferometer, the sample and a detector. The first grating, the so-called source grating, is placed in front of the x-ray source and increases the ability of the beam to interfere. The phase grating is placed at a certain distance downstream of the source grating and creates an interference pattern. The analyzer grating is required since the interference pattern usually is too small to be detected by a normal X-ray detector. The grating is moved in a number of steps perpendicular to the beam and several images are acquired. In this so-called phase-stepping approach intensity variations in a pixel can be measured as a function of the analyzer grating position.

Three image contrasts that arise from different physical processes can be obtained simultaneously by using the same set of images: the attenuation image is calculated based on X-ray attenuation caused by the sample. The phase image originates due to the shift of the stepping curve, acquired with the sample, compared to the reference curve. The dark-field image is formed by small angle scattering. Especially on boundaries between materials with big differences in density x-rays are scattered creating a pattern as shown in Fig. 1. Dark-field contrast is calculated by dividing the amplitude of the stepping curve with the sample by the amplitude without the sample.

Homogeneous specimens present a neglectable small-angle X-ray scattering and therefore show no significant dark-field signal. However, specimens with high density fluctuations provide a strong small-angle X-ray scattering and therefore a strong dark-field signal. The small-angle scattering signal measured with this technique is particularly sensitive to density variations in a sample on the length scale of a few micrometers.

Table 1
Definition of study samples, negative and positive controls according to dark-field, attenuation, and phase-contrast.

	Dark-field	Attenuation	Phase Contrast
Study Sample	High signal	No high signal	No high signal
Negative Control	No high signal	No high signal	No high signal
Positive Control	High signal	High signal	High signal

2.4. Experimental imaging set-up

The X-ray source was a rotating molybdenum anode X-ray tube operated at a tube voltage of 40 kV and a tube current of 70 mA. The grating interferometer consisted of a golden source grating, a nickel phase grating and a golden analyzer grating that were placed at a distance of 80 cm from each other. All gratings were fabricated at the Karlsruhe Institute of Technology (KIT) and Microworks GmbH (Karlsruhe, Germany) with periods of 5.4 μm. The height of both gold gratings was about 50 μm, the height of the nickel grating was either 8.5 or 9.5 μm. The specimens were put into cylindrical plastic tubes filled with phosphate buffered saline and were submerged in a water bath in front of the phase grating. The detector (single photon-counting detector Pilatus II, Dectris, Baden, Switzerland) was positioned 4 cm behind the analyzer grating. This set-up configuration resulted in a sample magnification of 1.72 and an effective pixel size of $100 \times 100\ \mu m^2$. For a full tomographic scan 1200 projections were recorded with 5 reference projections without the specimen every 20 projections. A stepping approach was used to determine the phase shift and the source grating was stepped over one period for each projection and reference projection while 11 images were acquired. Exposure time was 5 s per image. The attenuation data was reconstructed using a standard filtered back projection with a Ram-Lak filter and the phase-contrast data was reconstructed using an imaginary Hilbert filter. The images with a slice thickness of 100 μm were digitally stored in DICOM format and analyzed using open source software OsiriX 4.0 (32 bit).

2.5. Radiologic data analysis

An expert radiologist analyzed attenuation, dark-field and phase contrast images separately. Images with high signal intensity in dark-field and no high signal in attenuation and phase-contrast were identified. An equal number of positive controls with high signal intensity in all three contrast modalities and negative controls with no areas of high signal intensity in all three modalities were selected.

2.6. Histology workup

All samples were dehydrated and fixed en bloc in paraffin. Subsequently, specimens were cut with a slice thickness of 10 μm every 2 mm. Hematoxylin-Eosin staining (HE) and Movat's pentachrom staining were performed.