Photoacoustic imaging of lipid rich plaques in human aorta.

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ABSTRACT

Recently it has been shown that multiwavelength photoacoustic imaging has the potential to discriminate between normal and atheromatous areas of arterial tissue when operating in the 740-1300nm wavelength range. At this wavelength range the absorption spectrum of lipids and normal arterial tissue are significantly different allowing discrimination between one another. Also, this wavelength range has the advantage of being relatively weakly absorbed by blood. This obviates the need for a saline flush if implemented using an intravascular imaging probe. In this study we investigate the possibility of identifying regions of high lipid concentration from 2D multiwavelength photoacoustic images of vascular tissue by exploiting the unique spectral features of lipids. Recognising regions of high lipid concentration would be useful to identify plaques which are likely to rupture (vulnerable plaques). To investigate this, samples of post mortem human aortas were imaged at a range of near-infrared (NIR) wavelengths and compared to histology. Photoacoustic images were also obtained when illuminating the sample through blood. This study demonstrated that lipid rich atheromatous plaques can clearly be identified using multiwavelength photoacoustic imaging.

Keywords: Photoacoustic spectroscopy, atherosclerosis, vulnerable plaque, vascular tissue

1. INTRODUCTION

Atherosclerosis is a disease which is characterised by the buildup of plaques in the inner lining of arterial walls. The buildup of plaque can cause the arterial wall to thicken, progressively narrowing the lumen (stenosis) and reducing blood supply. In some cases the plaque can rupture, leading to a stroke or heart attack. Plaques, which are likely to rupture, are known as vulnerable plaques and are generally composed of a large lipid pool with a thin fibrous cap.[1, 2] To identify vulnerable plaques it is necessary to obtain information about both their composition and structure. Structural information alone is not sufficient as stenosis is not always present. Several methods have been investigated in order to image vulnerable plaques. These include, intravascular ultrasound (IVUS),[1] NIR spectroscopy[1, 3–5] and optical coherence tomography (OCT).[1, 6, 7] IVUS provides structural information but limited information concerning the composition of the plaque. NIR spectroscopy provides information concerning the composition but limited structural information. OCT can provide information about both the composition and the structure of the plaque. However, the penetration depth is generally limited to 1mm and requires the blood present in the artery to be removed using a saline flush.

Photoacoustic imaging has the potential to provide information concerning both the structure and the composition of plaque. The photoacoustic technique relies on the generation of a broadband acoustic wave by the absorption of a nanosecond pulse of NIR or visible light in tissue.[8] The amplitude of the photoacoustic signal is directly proportional to the local absorbed energy density, which is dependent on the optical properties of the tissue. Spatial information is obtained by measuring the time delay between the generation and detection of the photoacoustic signal. Information concerning the composition of the tissue is obtained by varying the wavelength of the excitation source and analysing the wavelength-dependent photoacoustic response. Previous

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studies have demonstrated the possibility of using the photoacoustic technique to discriminate between normal arterial tissue and artheromatous plaque when operating in the 400-740nm wavelength range.[9–11] However, the strong absorption of blood in this wavelength range (see figure 1 (a)) inhibits the intravascular implementation of the technique, as a significant proportion of the excitation light would be absorbed by the blood present in the artery. A saline flush could be used to overcome this but adds to the complexity of the imaging process. An alternative approach, which has recently been reported,[12] is to use excitation wavelengths in the NIR range (740-1300nm). In this wavelength range the absorption coefficient of blood is several orders of magnitude lower than in the visible wavelength range (see figure 1 (a)). The spectral signature of lipids is also significantly different from the components of normal arterial tissue. This is illustrated in figure 1 (b) which shows the spectral signature of lipids and that of water, collagen and elastin, which are the three main constituents of arterial tissue. It can be seen that the absorption spectrum of lipids is composed of three absorption peaks at 920nm, 1040nm and 1200nm, whereas the absorption spectrum of the arterial tissue is expected to only have two broader absorption peaks at 970nm and 1180nm.

The work presented in this paper investigates the possibility of using photoacoustic imaging to identify lipid rich plaques, by exploiting the strong preferential absorption of lipids in the NIR wavelength range. This work continues from that described in ref. 12 and shows improved photoacoustic images of human aortas and compares them to histology. The experimental setup is described in section 2 and the results are described in section 3. 2D photoacoustic images of arterial tissue were also obtained when illuminating the tissue sample through blood (section 4).

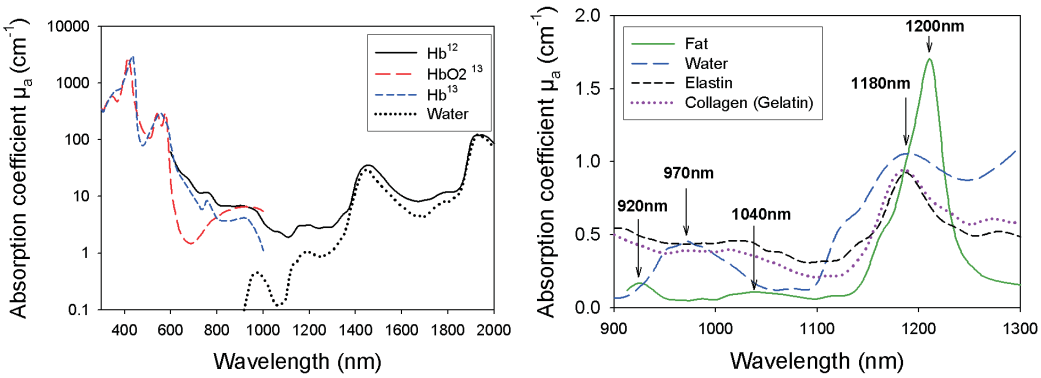

Figure 1. Absorption coefficient spectra of (a) oxyhaemoglobin (HbO2) and deoxyhaemoglobin (Hb)[13,14] (concentration of 150g/L) and water[15] (b) fat, water, elastin and collagen.[16]

2. EXPERIMENTAL METHOD

Figure 2 shows a schematic of the experimental setup used to generate and detected photoacoustic signals in arterial tissue. The excitation source was a fibre coupled tuneable optical parametric oscillator (OPO) based laser system, which provides ns pulses of tens of mJ in the 740 to 1400nm wavelength range. The sample was immersed in a saline bath and illuminated on one side via a glass window. The beam diameter at the tissue surface was in the range of 4 to 6mm. A spherically focused PVDF (25MHz) ultrasound detector was placed on the opposite side of the sample to detect the generated photoacoustic signals. The focal spot diameter of the detector was approximately $240\mu m$ and the focal length was approximately $24mm$. In order to obtain 2D photoacoustic images, the sample was mechanically scanned along a line in the x direction in steps of $500\mu m$. For each step a photoacoustic signal was detected, amplified (40dB) and signal averaged 20 times, before being downloaded

to a personal computer. These signals were then mapped to distance using the speed of sound ($1480m/s$) and integrated to obtain a depth profile of the absorbed optical density. These depth profiles or A-lines were also corrected for the exponential decay of the light distribution through tissue. A 2D image was then formed from the A-lines by converting the amplitude of the signals to a grey scale. The tissue samples were all human aortas fixed in formalin and suspended in ethanol and were obtained from the UK Human Tissue Bank (UKHTB). The start and finish point of the scan were marked on the tissue samples so that they could be registered with the histological section obtained after the experiment.

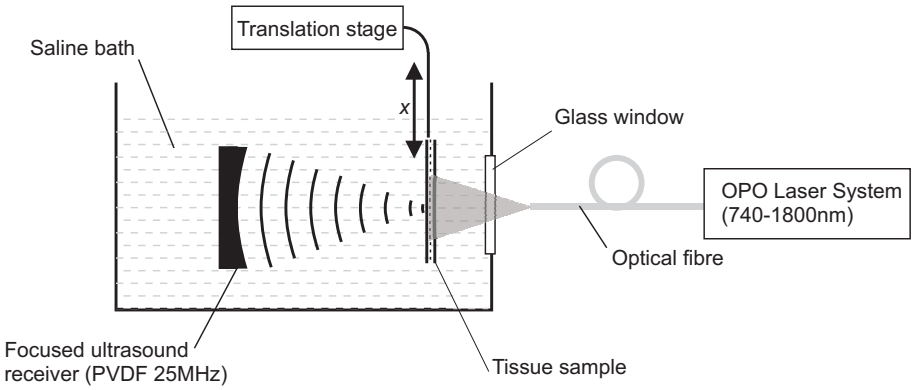

Figure 2. Experimental setup used to generate and detect photoacoustic signals in human aorta, for a range of excitation wavelengths (740-1400nm).

3. RESULTS

3.1. Normal arterial tissue

A sample of human aorta identified by visual inspection as normal (see figure 3 (a)) was opened out flat and scanned over a distance of 10mm in steps of $500\mu m$ (The scan line is illustrated in figure 3 (a) by a dotted line). The histological section of the sample, which was obtained after the experiment, is shown in figure 3 (b). This shows that the imaged cross section is relatively uniform with no evidence of atheromatous plaque. Figures 3 (c) and (d) shows 2D photoacoustic images obtained at excitation wavelengths of 970nm and 1200nm respectively. The excitation source was incident on the upper side of the sample. Both photoacoustic images show a similar structure of the vessel wall; a wall thickness of approximately 2mm and a uniform contrast without any apparent lesions, which correlates well with the histological section.

Photoacoustic spectra (740-1400nm) were obtained at 3 different spatial points as indicated on figure 3 (d). The spectra are shown in figure 3 (e). For all three points the spectra are broadly similar, with two main absorption peaks evident at 970nm and 1180nm. These peaks correspondent to the absorption peaks of water, which is the dominant NIR chromophore in arterial tissue (Arterial tissue is composed of 70 % of water and less than 25 % of elastin and collagen[17]).

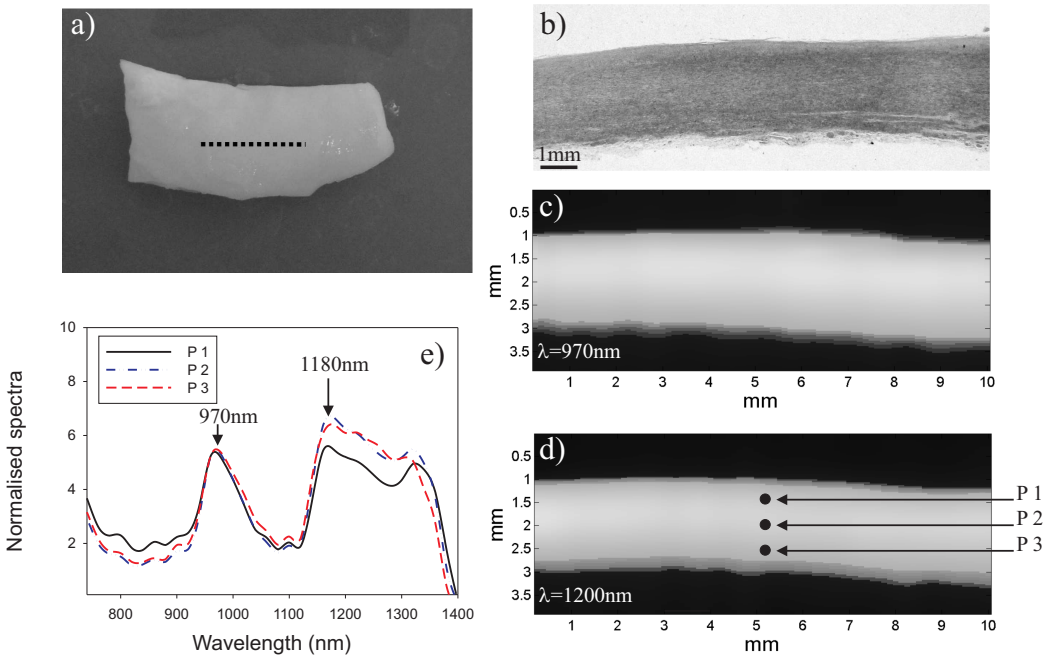

Figure 3. (a) Photograph of the sample of human aorta (The dotted line represents the scan line). (b) Histological section (c) photoacoustic image obtained at 970nm (d) photoacoustic image obtained at 1200nm (e) photoacoustic spectra obtained at points 1, 2 and 3.

3.2. Lipid rich plaques

A sample of human aorta identified by visual inspection as containing a raised lipid rich plaque was obtained. Figure 4 (a) shows a photograph of the tissue sample with the plaque identified by a dotted box. The sample was scanned along a distance of 25mm in steps of $500\mu m$. A histological section of this tissue sample was obtained after the experiment and is shown in figure 4 (b). The histological section shows a small artheromatous plaque about 5mm in length and extending to a depth of 1mm. Figures 4 (c) and (d) shows 2D photoacoustic images obtained at excitation wavelengths of 970nm and 1200nm respectively. The contrast in the photoacoustic image obtained at 970nm is relatively uniform. At this wavelength the contrast between areas of high lipid content and normal arterial tissue is small as the absorption coefficient of lipids is relatively weak (see figure 1(b)). The only indication of the presence of plaque is the thickening of the arterial wall. The arterial wall was measured to be approximately 2mm in the areas surrounding the plaque and about 3mm in the area of the plaque itself. These dimensions seem to match with those obtained from the histological section. The plaque is however visible in the photoacoustic image obtained at 1200nm. This is due to the high absorption coefficient of lipids at this wavelength (see figure 1 (b)). The location, geometry and size of the plaque shown in the photoacoustic image are in broad agreement with those obtained from the histological section.

Photoacoustic spectra (740-1400nm) were also obtained for several different spatial points as indicated on figure 4 (d). The spectra are shown in figure 4 (e). Point 1 corresponds to a point above the plaque boundary, whereas point 2 corresponds to a region within the plaque. The spectra obtained at both of these points have similar trends, however differences can be seen in the wavelength regions 920nm, 1040nm and 1200nm. This is more apparent in figure 5 which shows the difference between the spectra obtained at P1 and P2. The resulting spectrum has three absorption peaks occurring around 920nm, 1040nm and 1200nm. From visual inspection this spectrum appears to match that of lipids, shown in figure 1 (b), suggesting that the plaque is lipid rich.

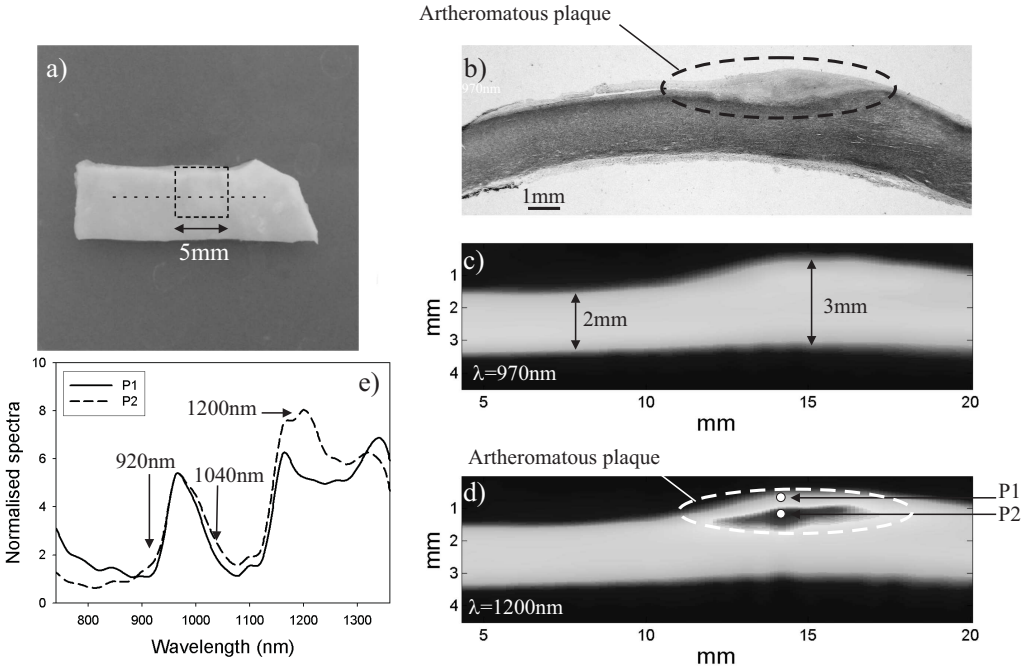

Figure 4. (a) Photograph of an aorta sample with a raised lipid rich plaque. (The horizontal dotted line represents the scan line). (b) Histological section (c) photoacoustic image obtained at 970nm (d) photoacoustic image obtained at 1200nm (e) photoacoustic spectra obtained at points 1 and 2.

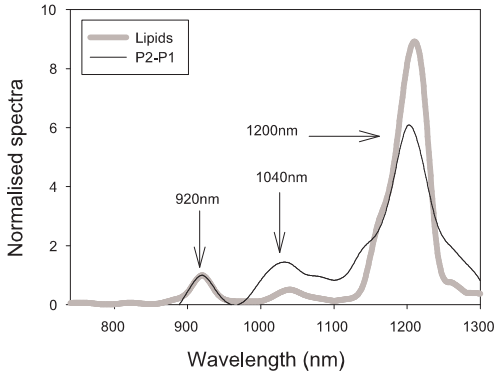

Figure 5. Photoacoustic spectrum obtained by subtracting the spectrum obtained at point 2 with that obtained at point 1. The photoacoustic spectrum of lipids is also shown.

Although these measurements have been made on a limited number of tissue samples, it appears that multiwavelength photoacoustic imaging should be able to identify vulnerable plaques, as it can provide adequate structural information and most importantly identify regions of high lipid concentration. It is also thought that using a more sophisticated spectroscopic inversion method such as that described in references 18 and 19 could enable the identification of a wider range of tissue types such as calcified and fibrous plaques.

4. IMAGING LIPID RICH PLAQUES THROUGH BLOOD

The long-term aim is to image the arterial wall using an intravascular photoacoustic probe. This would require transmitting the excitation laser beam through several mm of blood. To investigate the feasibility of this, the previous experimental setup was modified. The saline bath was partitioned in two sections (see figure 6), one filled with saline and the other with blood. The partition consisted of an acoustically transparent wall made from a piece of $5\mu m$ thick PVC film. The blood was human obtained from the blood bank. In order to avoid the blood cells settling at the bottom of the bath, a pump was used to keep the blood constantly circulating (not illustrated in figure 6).

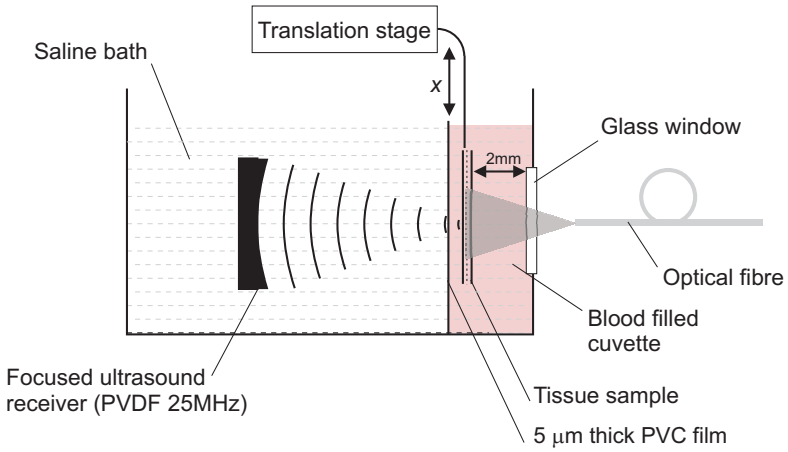

Figure 6. Experimental setup used to generate and detect photoacoustic signals in human aorta when illuminating through blood, for a range of excitation wavelengths (740-1400nm).

A tissue sample containing a raised lipid rich plaque was obtained and scanned over a distance of 25mm in steps of 500 μm. The sample is shown in figure 7 (a) and the line scan is shown by the horizontal dotted line. Initially the tissue sample was imaged when immersed in saline. Figures 7 (b) and (c) shows the images obtained for excitation wavelengths of 970nm and 1200nm respectively. The photoacoustic image contrast at 970nm is relatively uniform, as lipids are weakly absorbent at this wavelength. Once again the only indication of plaque is the thickening of the arterial wall. The arterial wall was measured to be approximately 2mm in the areas surrounding the plaque and about 3mm in the area of the plaque itself. The plaque becomes apparent in the photoacoustic image obtained at 1200nm, as lipids strongly absorb at this wavelength. The width of the plaque obtained from the photoacoustic image correlate with the actual width of the lesion ($\sim 10mm$). The tissue sample was then imaged when immersed in blood. The distance between the tissue sample and the glass window was approximately 2mm. Figure 7 (d) shows the photoacoustic image obtained when illuminating at a wavelength of 1200nm. It should be noted that the quality of the image is degraded by the presence of the strong background photoacoustic signal generated in the blood surrounding the tissue sample. However, the plaque is still visible and the size, shape and location of the plaque correlates well with the image obtained in water (figure 7 (c)). A photoacoustic spectrum was also obtained in the area of the lipid rich plaque (P1) and is shown in figure 7 (e). The spectral features of lipids, which consist of three absorption peaks at 920nm, 1040nm and 1200nm are still visible, demonstrating that the presence of blood does not corrupt the spectral signature of lipids.

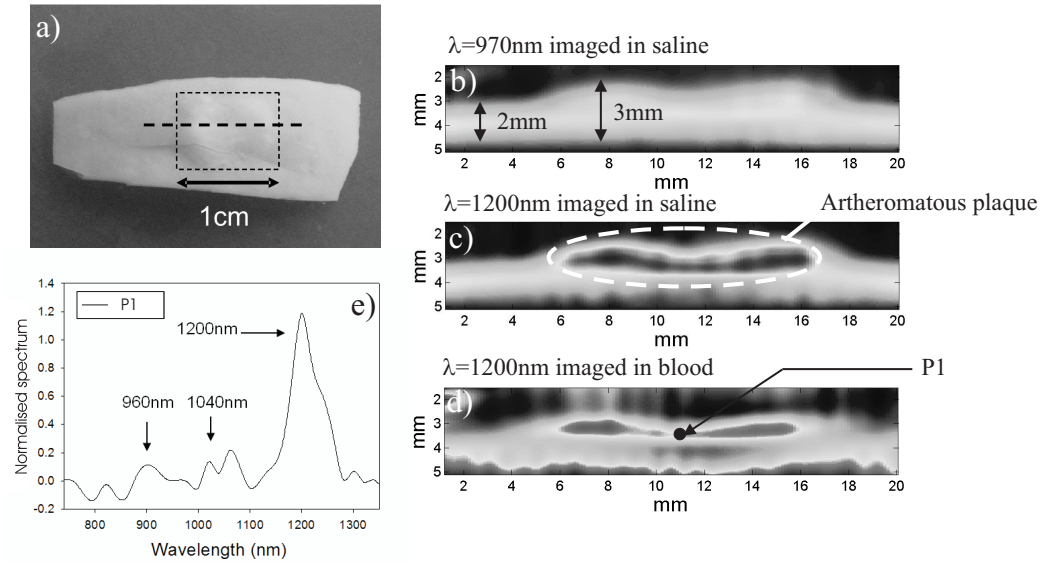

Figure 7. (a) Photograph of an aorta sample with a raised lipid rich plaque (The horizontal dotted line represents the scan line). (b) 2D photoacoustic image obtained at 970nm when illuminating through saline (c) photoacoustic image obtained at 1200nm when illuminating through saline (c) photoacoustic image obtained at 1200nm when illuminating through blood (e) photoacoustic spectrum obtained at point 1.

5. CONCLUSION

This study has shown that multiwavelength photoacoustic imaging can identify regions of high lipid concentration by exploiting the strong preferential absorption of lipids in the NIR wavelength. It was also shown that the structure of the arterial wall and plaque boundaries could be identified. This suggests that the photoacoustic technique could potentially be a powerful imaging tool used to identify vulnerable plaques. It is also thought that other tissue types, such as calcified and fibrous plaques could be identified using more complex spectroscopic inversion methods.[18, 19] This study also demonstrated the possibility of imaging plaque when illuminating the sample through blood. This suggests that the photoacoustic technique could be implemented using an intravascular probe without the need for a saline flush. Future work will focus on obtaining measurements from a wider range of tissue samples and developing an intravascular probe.

ACKNOWLEDGMENTS

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REFERENCES

1. Z. A. Fayad and V. Fuster, "Clinical imaging of the high-risk or vulnerable atherosclerotic plaque," *Circulation Research* **89**(4), pp. 305–316, 2001.
2. F. D. Kolodgie, A. P. Burke, A. Farb, H. K. Gold, J. Y. Yuan, J. Narula, A. V. Finn, and R. Virmani, "The thin-cap fibroatheroma: a type of vulnerable plaque - the major precursor lesion to acute coronary syndromes," *Current Opinion in Cardiology* **16**(5), pp. 285–292, 2001.
3. J. D. Caplan, S. Waxman, R. W. Nesto, and J. E. Muller, "Near-infrared spectroscopy for the detection of vulnerable coronary artery plaques," *Journal of the American College of Cardiology* **47**(8), pp. C92–C96, 2006.
4. L. A. Cassis and R. A. Lodder, "Near-IR imaging of atheromas in living arterial tissue," *Analytical Chemistry* **65**(9), pp. 1247–1256, 1993.

5. J. Wang, Y. J. Geng, B. Guo, T. Klima, B. N. Lal, J. T. Willerson, and W. Casscells, "Near-infrared spectroscopic characterization of human advanced atherosclerotic plaques," *Journal of the American College of Cardiology* **39**(8), p. II, 2002.
6. H. Yabushita, B. E. Bourna, S. L. Houser, T. Aretz, I. K. Jang, K. H. Schlendorf, C. R. Kauffman, M. Shishkov, D. H. Kang, E. F. Halpern, and G. J. Tearney, "Characterization of human atherosclerosis by optical coherence tomography," *Circulation* **106**(13), pp. 1640–1645, 2002.
7. M. Zimarino, F. Prati, E. Stabile, J. Pizzicannella, T. Fouad, A. Filippini, R. Rabozzi, O. Trubiani, G. Pizzicannella, and R. D. Caterina, "Optical coherence tomography accurately identifies intermediate atherosclerotic lesions - an in vivo evaluation in the rabbit carotid artery," *Atherosclerosis* **193**(1), pp. 94–101, 2007.
8. M. H. Xu and L. H. V. Wang, "Photoacoustic imaging in biomedicine," *Review of Scientific Instruments* **77**(4), 2006.
9. P. C. Beard and T. N. Mills, "Characterization of post mortem arterial tissue using time-resolved photoacoustic spectroscopy at 436, 461 and 532 nm," *Physics in Medicine and Biology* **42**(1), pp. 177–198, 1997.
10. S. Sethuraman, S. R. Aglyamov, J. H. Amirian, R. W. Smalling, and S. Y. Emelianov, "Intravascular photoacoustic imaging using an ivus imaging catheter," *Ieee Transactions on Ultrasonics Ferroelectrics and Frequency Control* **54**(5), pp. 978–986, 2007.
11. S. Sethuraman, J. H. Amirian, S. H. Litovsky, R. W. Smalling, and S. Y. Emelianov, "Spectroscopic intravascular photoacoustic imaging to differentiate atherosclerotic plaques," *Optics Express* **16**(5), pp. 3362–3367, 2008.
12. T. J. Allen and P. C. Beard, "Photoacoustic characterisation of vascular tissue at nir wavelengths," *Proc. of SPIE* **7177**, p. 71770A, 2009.
13. J. T. Kuenstner, K. H. Norris, and W. F. MCCarthy, "Measurement of hemoglobin in unlysed blood by near-infrared spectroscopy," *Applied Spectroscopy* **48**(4), pp. 484–488, 1994.
14. S. Prahl, "Optical absorption of hemoglobin." http://omlc.ogi.edu/spectra/hemoglobin/index.html, 1999.
15. G. M. Hale and M. R. Querry, "Optical-constants of water in 200-nm to 200-mum wavelength region," *Applied Optics* **12**(3), pp. 555–563, 1973.
16. C. L. Tsai, J. C. Chen, and W. J. Wang, "Near-infrared absorption property of biological soft tissue constituents," *Journal of Medical and Biological Engineering* **21**(1), pp. 7–14, 2001.
17. F. Duck, "Physical properties of tissue: A comprehensive reference book," *Academic Press Limited* , 1990.
18. J. Laufer, D. Delpy, C. Elwell, and P. Beard, "Quantitative spatially resolved measurement of tissue chromophore concentrations using photoacoustic spectroscopy: application to the measurement of blood oxygenation and haemoglobin concentration," *Physics in Medicine and Biology* **52**(1), pp. 141–168, 2007.
19. J. Laufer, B. Cox, E. Zhang, and P. Beard, "Quantitative determination of chromophore concentrations from 2d photoacoustic images using a nonlinear model-based inversion scheme," *Applied Optics* **in press**, 2010.