Photoacoustic characterisation of vascular tissue at NIR wavelengths.

Thomas J. Allen and Paul C. Beard

Department of Medical Physics and Bioengineering, University College London, Gower Street, London, WC1E 6BT, UK

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ABSTRACT

Photoacoustic spectroscopy has been shown to be able to discriminate between normal and atheromatous areas of arterial tissue in the visible range (410nm-680nm). However, at these wavelengths haemoglobin absorption is also very high. This makes it challenging to apply photoacoustic techniques using an intravascular probe, as a significant amount of the excitation light will be absorbed by the blood present in the artery. In this study we investigate the use of a wider range of excitation wavelengths (740-1800nm) for discriminating between normal arterial tissue and lipid rich plaques and minimise the effect of blood absorption. Special attention will be given to the near infra-red (NIR) wavelength range (900-1300nm) as in this region blood absorption is relatively weak and there are expected to be significant differences in the absorption spectrum of each tissue type. To investigate this, tissue samples were obtained and imaged at a range of wavelengths, the samples were illuminated first through water, then blood. This study demonstrated that the photoacoustic technique can discriminate between normal arterial tissue and lipid rich plaques, even when blood is present.

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

1. INTRODUCTION

Atherosclerosis is a disease in which plaque builds up on the inner lining of arterial walls, resulting in the thickening of the wall. This thickening of the wall will progressively result in the narrowing of the lumen (stenosis), which will have the consequence of reducing the blood supply to different organs. In some cases the plaque can rupture leading to an infarction. The plaques, which are likely to rupture, are known as vulnerable plaques and are generally composed of a thin fibrous cap and a large and soft lipid pool underlying the cap.[1,2] It is generally not possible to identify these vulnerable plaques by only looking at their structure, as stenosis is not always present; information concerning their composition is also required. Several imaging modalities have been investigated for intravascular imaging of vulnerable plaques, such as intravascular ultrasound (IVUS), optical coherence tomography (OCT) and NIR spectroscopy. IVUS[1] provides structural information, but limited information regarding the composition of the plaque. NIR spectroscopy[1,3–5] provides information concerning the composition of the plaque, but low spatial resolution. OCT[1,6,7] provides information concerning the composition of the plaque with high spatial resolution, but has lower penetration depth. Penetrations depths of up to 1mm can be obtained using OCT, but requires the blood present in the artery to be removed from the imaging field of view. This adds to the complexity and invasiveness of the imaging process.

Spectroscopic photoacoustic imaging, which relies on the generation of a broadband ultrasonic wave by the absorption of short pulses of laser light in tissue,[8] has the potential to provide information concerning both, the composition and the structure of the plaque. The detected photoacoustic signal provides a measure of the spatial distribution of the absorbed optical energy which dependent on the optical properties of the tissue. By varying the wavelength of the laser source, spatially resolved spectroscopic information can be obtained from the amplitude of the signals. Early studies showed, that by exploiting the absorption properties of carotenoid in the infrared spectral range of 420-530nm,[9,10] photoacoustic spectroscopy could discriminate between normal and

Send correspondence to T. J. Allen E-mail: tjallen@medphys.ucl.ac.uk,

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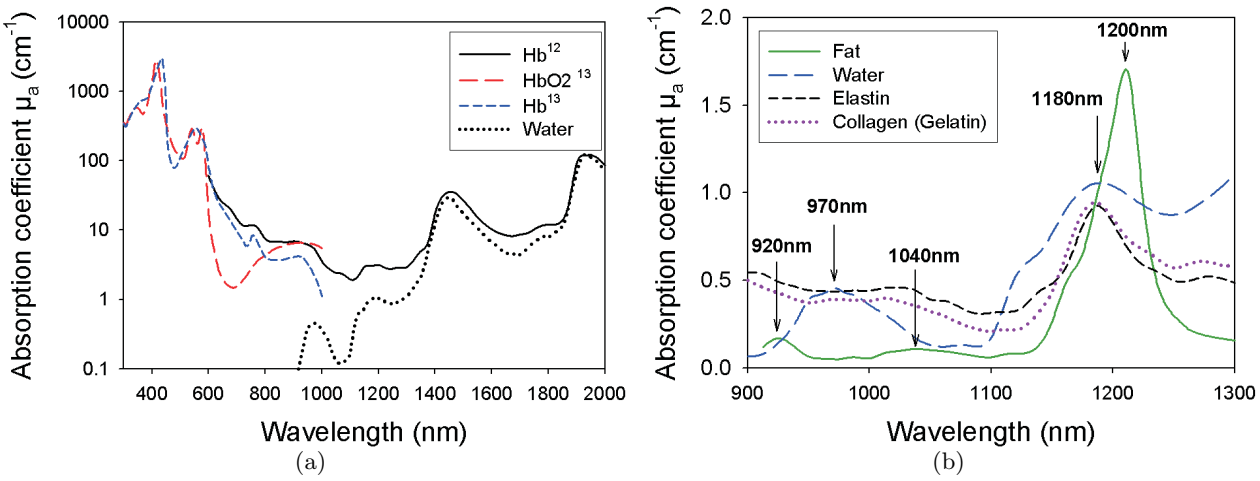

Figure 1. Absorption coefficient spectra of (a) oxyhaemoglobin (HbO2) and deoxyhaemoglobin (Hb)[13,14] (concentration of 150g/liter) and water[15] (b) fat, water, elastin and collagen.[12]

atheromatous tissue. However, the high absorption of blood at these wavelengths (see figure 1 (a)) makes the intravascular implementation challenging, as the excitation light would be strongly absorbed by the surrounding blood present in the artery. One approach to overcome this would be to use a saline flush. However, this increases the complexity and invasiveness of the imaging process. An alternative is to select the wavelength of the excitation source in the NIR part of the optical spectrum. At these wavelengths, the absorption coefficient of blood is several orders of magnitude lower than that in the visible wavelength range (see figure 1 (a)). Also at these wavelengths different tissue components should provide significant distinctive spectral features to discriminate between one another. For example, recently a study reported the possibility of using the wavelength range 680-900nm to discriminate between fibrous and lipid components of atherosclerotic plaques,[11] when using photoacoustic spectroscopy. NIR spectroscopy studies,[3,5,12] have also reported that more distinctive spectral features are present in the 900-1800nm wavelength range. Figure 1 (b) shows the absorption spectra of lipids and the major constituents of arterial tissue, which are collagen, elastin and water, for the wavelength range 900-1300nm. It can be seen that the absorption spectrum of lipids contains distinct absorption peaks around 920nm, 1040nm and 1200nm, whereas the absorption spectrum of normal arterial tissue is expected to have only two broad absorption peaks at 970nm and 1180nm. Therefore, the relatively low absorption coefficient of blood and the distinctive spectral features of lipid rich plaques and normal arterial tissue in the NIR wavelength range (740 -1800nm), suggest that photoacoustic spectroscopy should be able to discriminate between tissue types when implemented *in vivo*.

This study will, in the first instance, demonstrate the possibility of photoacoustic spectroscopy to discriminate between normal and artheromatous areas of arterial tissue in the NIR wavelength range. For this an experimental study was undertaken in which spatially resolved photoacoustic spectra of both tissue types were obtained *ex vivo*. The experimental setup is described in section 2 and the results are described in section 3. In a second instance, this study will look to determine whether this could be implemented if the laser excitation pulses are transmitted through blood, as this would be the case if implemented *in vivo*. This is the focus of section 4.

2. EXPERIMENTAL METHOD

The experimental setup used to generate and acquire photoacoustic signals in arterial tissue is shown in figure 2. The tissue sample was immersed in a saline bath and illuminated through a glass window by nanosecond

pulses of NIR light. The excitation source consisted of a fibre coupled tunable optical parametric oscillator (OPO) based laser system. This excitation system allowed photoacoustic signals to be generated and acquired at wavelengths ranging from 740nm to 1800nm, in increments of 20nm. The beam diameter at the tissue surface was in the range of 4-6mm and the pulse energy was kept bellow 20mJ. On the opposite side of the sample a custom built 25MHz PVDF spherical focused detector was used to acquire the photoacoustic signals. The sample was placed at the focus of the ultrasound detector (24mm) and the focal spot diameter was $240\mu m$. In order to acquire photoacoustic signals at different spatial points, the tissue sample was mechanically scanned in the x direction using a translation stage. Tissue samples used in this study were all human aortas fixed in formalin and suspended in ethanol and were obtained from the UK Human Tissue Bank (UKHTB).

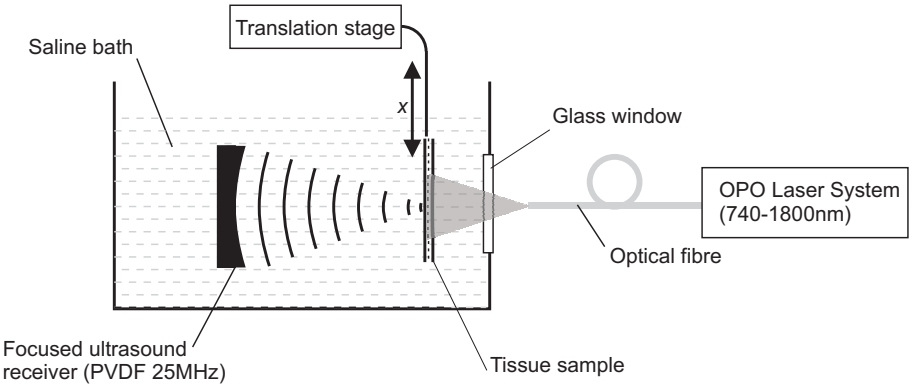

Figure 2. Experimental setup used to generate and detected photoacoustic signals in human aorta, for a range of excitation wavelengths (740-1800nm).

3. RESULTS

3.1. Normal tissue

A tissue sample identified as being normal was selected. The sample was opened out flat and scanned along the x axis over a distance of 10mm in steps of $400\mu m$, using the setup shown in figure 2. For each step a photoacoustic signal was acquired and an image was formed by converting the amplitudes of the signals to a colour map and mapping the time domain signals to distance using the speed of sound (1480m/s). The image was acquired at an excitation wavelength of 1200nm and a depth dependent scaling factor was used to compensate for optical attenuation. The obtained image shown in figure 3 (a), shows the structure of the vessel wall indicating that the thickness of the wall is approximately 2mm. The excitation source was incident on the lower side of the sample. Photoacoustic spectra (740-1800nm) were then obtained at 8 different spatial locations as indicated on the image. The spectra were normalised for the wavelength dependence of the laser source energy. Figure 3 (b) and (c) shows the photoacoustic spectra for spatial points obtained along the lower (position 2, 4 and 6) and upper (position 1, 3 and 5) surface of the arterial wall, respectively. Figure 3 (d) show the spectrum obtained at the centre of the tissue sample (position 7 and 8). In all cases, the spectra are broadly similar. It can be seen that for the low wavelength range (780-1300nm), the absorption spectrum of arterial tissue resembles that of water, with absorption peaks at 970nm and 1180nm. This is illustrated in figure 3(b), which shows the absorption spectrum of normal arterial tissue superimposed with that of water. Water constitutes a significantly greater fractional part of arterial tissue (70%[16]) than elastin and collagen ($< 25\%$) and therefore the absorption spectrum of normal arterial tissue is dominated by the spectral features of water. For the higher wavelength range (1300-1800nm) the measured spectra of arterial tissue does not correlate with that of water. For example, the water absorption peak which should occur at 1400nm is not observed in the spectrum of normal arterial tissue. This is due to the presence of water between the glass window and the tissue sample (see figure 2), which strongly attenuates the excitation light. In the wavelength range 1600-1800nm, where the absorption coefficient

of water is relatively weak (see figure 1 (a)), the excitation light is less attenuated resulting in a peak occurring in the measured spectrum at around 1700nm (see figure 3 (b) and (d)). This peak is however not apparent in the spectra shown in figure 3 (c), as the excitation light has been strongly attenuated, when propagating through the full thickness of the tissue.

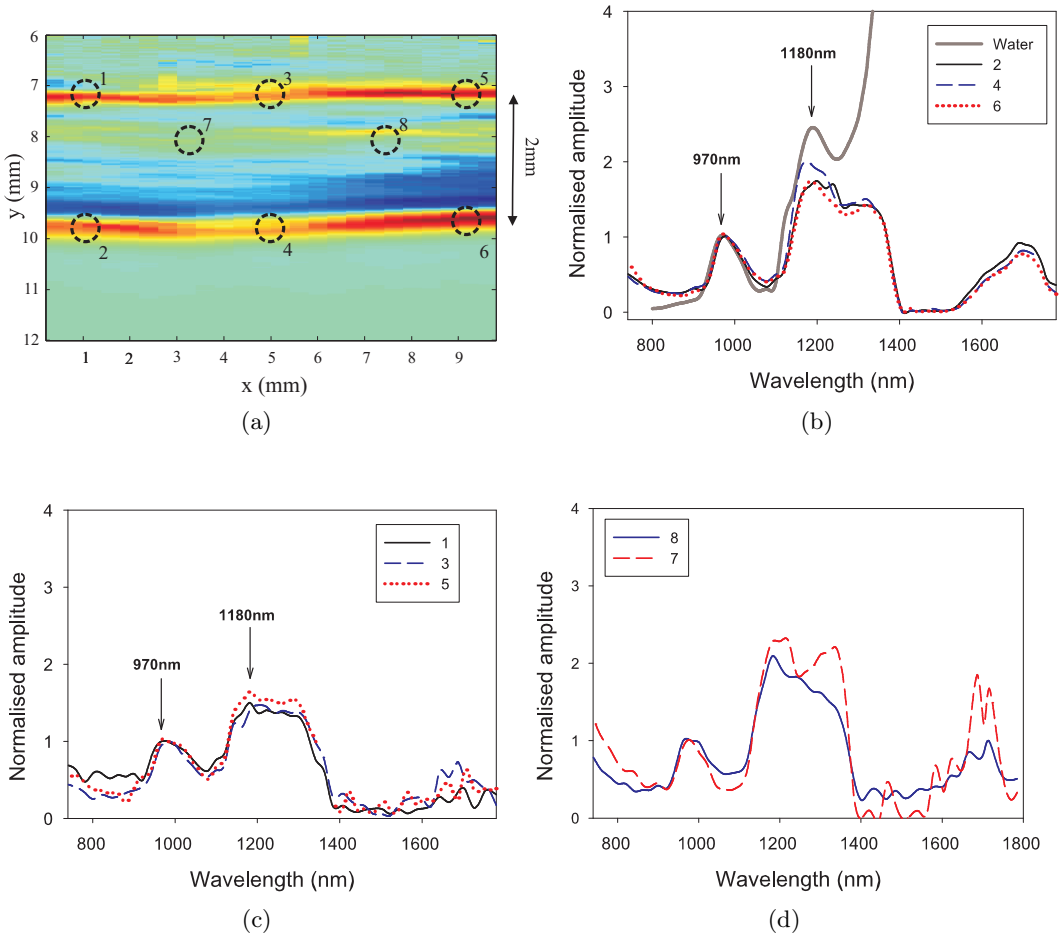

Figure 3. Photoacoustic measurements obtained in a sample of normal human aorta. (a) Line scan of the sample obtained for an excitation wavelength of 1200nm -the excitation laser pulse was incident on the lower side of the sample (b) photoacoustic spectra obtained from the lower surface of the sample (The grey curve is the absorption spectrum of water.) (c) photoacoustic spectra obtained from the upper surface of the sample (d) photoacoustic spectra obtained from the centre of the sample.

3.2. Lipid rich plaque

A tissue sample identified as being a lipid rich plaque was then obtained and scanned over 18mm, as described in section 3.1. The image is shown in figure 4 (a) and shows the structure of the vessel wall. It appears from the image that the thickness of the vessel wall is approximately 2.5mm, the lesion is approximately 10mm wide and extends to a depth of approximately 1.5mm into the vessel wall. In order to obtain information concerning the composition of the plaque, photoacoustic spectra for 5 different spatial locations were then acquired as indicated on the image. Position 1, 2 and 3 in figure 4 (b) correspond to regions identified as normal. The photoacoustic spectra here resemble those shown in figure 3, with the same water absorption peaks occurring at 970nm and

1180nm. The spectrum obtain at position 5 at the centre of the lesion, is shown in figure 4 (c)(dotted curve) and is characterised by several distinct spectral features; which are not present in the spectrum of the normal tissue (see figure 4 (b)). These include two small peaks at 920nm and 1040nm and two larger peaks at 1200nm and 1720nm. There are also absorption peaks around 1200nm and 1720nm exhibited by the normal tissue but these peaks are much broader. It should also be noted that the absorption peak present at around 1350nm in normal arterial tissue (see figure 4 (b)) is clearly not visible in the absorption spectrum obtained at position 5. These features suggest that the spectrum for position 5 is fat. To verify this, a photoacoustic spectrum was obtained from a lump of fat using the setup shown in figure 2 and superimposed onto the spectrum obtained at position 5 (see figure 4 (c)). It can be seen that both spectra correlate well, demonstrating that photoacoustic spectroscopy could identify lipid rich plaques. Figure 4 (c) also shows the spectra obtained at position 4 and 6, which can be considered as areas of normal arterial tissue.

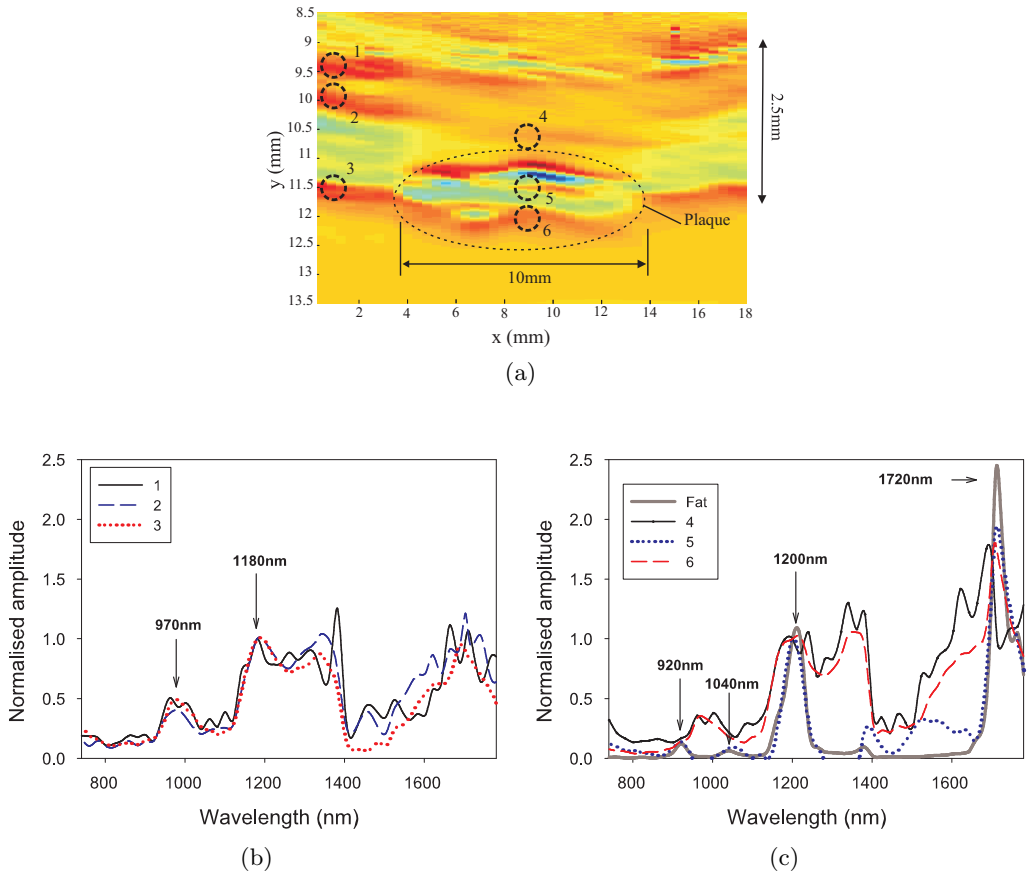

Figure 4. Photoacoustic measurements obtained in a sample of lipid rich plaque. (a) Line scan of the sample obtained for an excitation wavelength of 1200nm -the excitation laser pulse was incident on the lower side of the sample (b) photoacoustic spectra corresponding to different depth at x =1mm where the tissue was identified as being normal and (c) photoacoustic spectra corresponding to different depths at x =9mm at the centre of the region of plaque. The photoacoustic spectrum obtained in pure fat is represented by a grey curve.

A second lipid rich plaque was also imaged and showed in figure 5 (a). It appears from the image that the thickness of the vessel wall was approximately 2.8mm and the lesion was approximately 7mm wide. Photoacoustic spectra were obtained for 5 different spatial locations which are shown in figure 5 (b) and (c). Once again the spectrum at the centre of the lipid rich plaque (position 2) showed the absorption peaks found in fat, whereas the spectra obtained from the area designated as atheromatous free (position 4 and 5) manifested

similar absorption peaks to normal arterial tissue, as shown in figures 3 (b-d) and 4 (b).

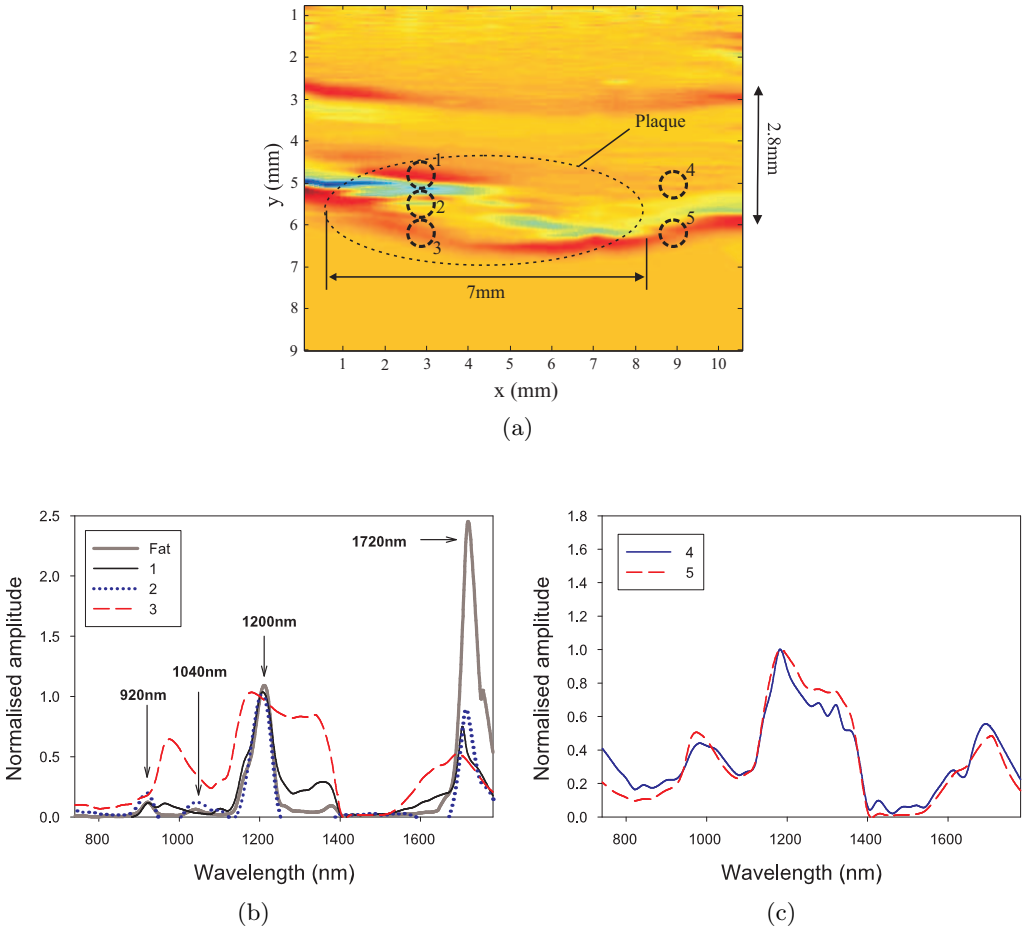

Figure 5. Photoacoustic measurements obtained in a sample of lipid rich plaque. (a) Line scan of the sample obtained for an excitation wavelength of 1200nm -the excitation laser pulse was incident on the lower side of the sample (b) photoacoustic spectra corresponding to different depth at x =3mm at the centre of region of plaque (The photoacoustic spectrum obtained in a pure bit of fat is represented by a grey curve.) and (c) photoacoustic spectra corresponding to different depth at x =9mm where the tissue was identified as being normal.

Although the measurements have been made on a limited number of samples it appears that photoacoustic spectroscopy is able to distinguish between normal and lipid rich plaques in the NIR wavelength range, by simply inspecting the tissue spectral signature. It is thought that a more sophisticated spectroscopy inversion approach such as that described in Ref 18 could provide a more sensitive and specific identification. This approach could also potentially allow for the discrimination of a wider range of tissue types (fibrous and calcification), for which the difference in their spectral signatures are more subtle. Ultimately, such approach could provide a quantitative indication of the content of lipids and other plaque constituents.

4. MEASUREMENTS THROUGH BLOOD

In order to use photoacoustics for intravascular imaging of vulnerable plaques, it is necessary to consider the effect of the blood, present in the artery, on the measured spectrum. Although the absorption coefficient of blood is not significantly higher than that of water at these wavelengths (740-1800nm), the scattering property

of blood will strongly attenuate the excitation light. It is therefore necessary to investigate, if photoacoustic signals can be generated and detected with sufficient SNR to discriminate between tissue types, when the excitation pulse is transmitted through several mm of blood. For this, photoacoustic signals were generated in normal and atheromatous areas of arterial tissue for a range of wavelengths (740nm to 1800nm) using the experimental setup shown in figure 6. This setup was identical to that previously shown in figure 2 except that a 4mm thick cuvette filled with human blood was inserted between the output of the optical fibre and the tissue sample. For these measurements a 15MHz focussed PZT detector, with a focal length of 20mm and a spot size of 150μm, was used.

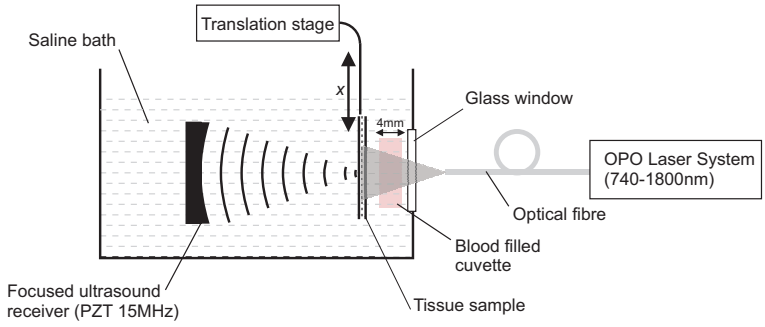

Figure 6. Experimental setup for simulating the effects of intravascular blood.

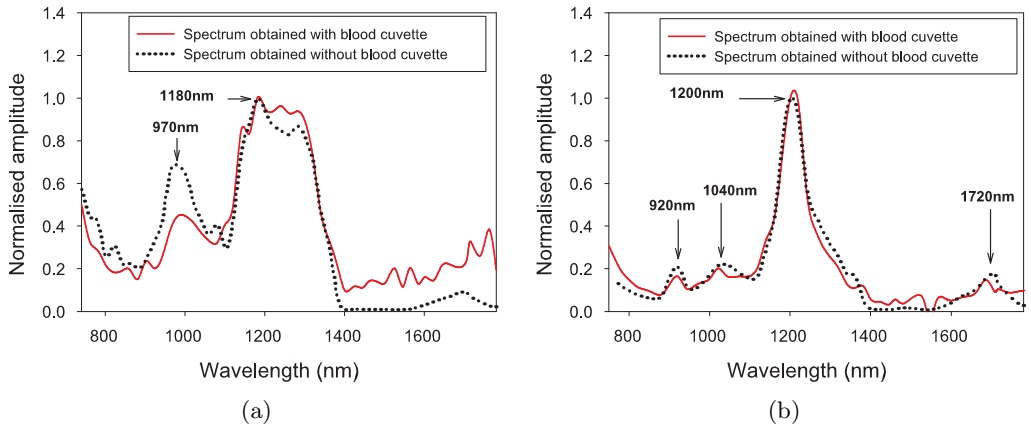

Figure 7. Measurements obtained using the setup shown in figure 6 with and without a 4mm blood filled cuvette. Photoacoustic spectra of (a) normal tissue and (b) fatty tissue.

Figure 7 (a) shows the spectra obtained from the normal tissue, with and without the cuvette in place. It can be seen that the absorption peaks at 970nm and 1180nm which are characteristic of normal arterial tissue are present for both cases. Figure 7 (b) shows the spectra obtained from a lipid rich plaque, with and without the cuvette in place. It can be seen that the absorption peaks at 920nm, 1040nm, 1200nm and 1720nm which are characteristic of lipid rich plaques are also present for both cases. This suggests, that the effects of blood on the ability to discriminate between tissue types, based on their spectral characteristics are unlikely to be significant. Although, not visible on these graphs, the SNR of the generated photoacoustic signals decreased by a factor of 20 when the blood filled cuvette was present. This decrease in SNR is due to optical scattering by blood, which strongly attenuated the excitation light. However, despite this, adequate SNR (∼20dB) was still

obtained, suggesting that it may be possible to detect photoacoustic signals through as much as 10mm of blood.

5. CONCLUSION

This preliminary study has demonstrated that spectroscopic photoacoustic imaging, could be used *in vivo* to discriminate between normal arterial tissue and lipid rich plaques, when operating in the wavelength range 740-1800nm. However, in order to implement this, an intravascular photoacoustic probe will have to be developed. Such a probe could use current technologies developed for IVUS imaging,[10,11] which would allow for both modalities which are ultrasound and photoacoustic imaging to be combined. Fibre optic ultrasound detectors[17] could also be used. It is also thought that, if more sophisticated spectroscopic inversion techniques were used,[18] it may be possible to discriminate between a wider range of tissue types such as fibrous and calcified tissues, as well as quantify the abundance of specific plaque constituents. This suggests that photoacoustic spectroscopy could potentially become a powerful investigation tool to identify vulnerable plaques.

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