Published in final edited form as:

J Ultrasound Med. 2017 October ; 36(10): 2047–2059. doi:10.1002/jum.14259.

Frequency Domain Analysis of Multiwavelength Photoacoustic Signal for differentiating among malignant, benign and normal thyroid in an Ex-vivo study with human thyroid

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Abstract

Objective—This study investigated the capability of spectral parameters, extracted using frequency domain analysis of photoacoustic (PA) signals, to differentiate between malignant, benign and normal thyroid tissue.

Methods—We acquired multiwavelength PA images of the freshly excised thyroid specimens, collected from 50 patients who underwent thyroidectomy after being diagnosed with suspected thyroid lesion. A thyroid cytopathologist marked histology slides of each tissue specimen. These marked histology slides were used as ground truth to identify the region of interests (ROI) corresponding to malignant, benign and normal thyroid tissue. Three spectral parameters, namely slope, midband fit and intercept were extracted from PA signals corresponding to different ROIs.

Results—Spectral parameters were extracted from a total of total of 65 ROIs. According to the ground truth, 12 out of 65 ROIs belonged to malignant thyroid, 28 out of 65 ROIs belonged to benign thyroid and 25 out of 65 ROIs belonged to normal thyroid. Besides slope, the other two spectral parameters and grayscale PA image pixel values were found to be significantly different ($p < 0.05$) between malignant and normal thyroid. Between benign and normal thyroid, all three spectral parameters and PA pixel values were significantly different ($p < 0.05$).

Conclusion—Preliminary results of our ex vivo human thyroid study show that the spectral parameters extracted from radio frequency PA signals as well as the pixel value of 2D PA images can be used for differentiating between malignant, benign and normal thyroid tissue.

Keywords

Photoacoustic imaging; Thyroid cancer; Frequency domain analysis; Tissue characterization

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INTRODUCTION

Over the last few decades, thyroid cancer incidence has been rising significantly in the USA. While thyroid cancer incidence was 3.6 per 100,00 in 1973, it was increased to 8.7 per 100,000 in 2002¹. Among men, the annual percentage change (APC) of incidence for thyroid cancer increased at a rate of 5.3 % for the period of 1996 – 2008, while for women APC for thyroid cancer increased at the rate of 6.5 %². While some of the research groups attribute this strong increase in thyroid cancer incidence to widespread use of ultrasound (US) imaging and US guided fine needle aspiration biopsy(FNAB)^{2,3}, many research groups think that other factors may be responsible for the rise and careful investigation should be undertaken to further explore the factors^{4,5}. According to the estimation of American Cancer Society, about 62450 new cases of thyroid cancer will be reported and about 1980 deaths will be resulted because of thyroid cancer in 2016 in USA⁶.

US imaging is the most popular and widely used diagnostic technique for evaluating malignancy in thyroid gland⁷. Although presence of sonographic features like microcalcification, hyperechogenicity, absence of halo, irregular margin of a nodule increase the risk of thyroid malignancy⁷⁻⁹, there are certain limitations of US imaging for detecting thyroid cancer^{10, 11}. The US features mentioned before are not often reproducible and different experts may evaluate these features differently^{9, 12}. While some researchers believe that size of thyroid nodule may be an important indicator for thyroid cancer⁹, most of the researchers think that it is not helpful for predicting or excluding thyroid malignancy¹¹. Researchers have proposed several sets of guidelines^{7, 13, 14}, which use different combination of the sonographic features, to recommend suspected nodules for FNAB. FNAB is the gold standard for thyroid cancer detection.. There are debates about which set of guidelines is the most the efficient in terms of high sensitivity and low number of FNAB procedures^{14, 15}. A new imaging modality with better tissue characterization ability than US imaging is required for simplifying the diagnosis protocol for thyroid cancer. Photoacoustic (PA) imaging, which is capable of providing functional information of tissue, can be used for efficient diagnosis of thyroid malignancy. Angiogenesis i.e. production of new blood vessels is a primary hallmark for detection of malignant lesions¹⁶. This primary hallmark can be detected by probing thyroid gland with optical radiation at near infra red [NIR] wavelengths. But, pure optical imaging suffers from degrading spatial resolution with increasing depth in soft tissue, whereas PA imaging is capable of maintaining good spatial resolution inside the tissue¹⁶. Using multiwavelength PA imaging, functional information like concentration of different chromophores, and blood oxygen saturation can be found out.

PA imaging, which employs PA effect to acquire information, is an emerging non invasive soft tissue imaging modality^{16, 17}. In PA effect, the object of interest [soft tissue] generates high frequency US waves after being irradiated by short duration [nanosecond] pulsed laser light. The pulsed laser light is absorbed by the light absorbing tissue elements to get heated up. This localized heating produces small increase in temperature which leads to pressure increase. This pressure relaxes in the form of Radio frequency (RF) US waves, which are also known as PA waves¹⁸. Provided uniform illumination is maintained on the surface of the complete ROI, amplitude of the PA waves depend on local optical absorption coefficient. Thus a 2D PA image, formed using the amplitude values of the PA waves, provides a map of

spatially varying optical absorption coefficient of the tissue specimen¹⁶. As mentioned, PA imaging is a hybrid imaging modality between optical and US imaging. Like pure optical imaging, it provides optical absorption property based contrast which can produce better soft tissue characterization than echogenicity based contrast provided by US imaging^{16, 17}. On the other hand, similar to pure US imaging it is capable of providing good spatial resolution at significant depths inside the soft tissue unlike pure optical imaging^{16, 18}.

Spectroscopic application of PA imaging can utilize different absorption properties of major light absorbing tissue constituents or chromophores like deoxy and oxyhemoglobin, water, lipid etc. to provide functional information about a soft tissue specimen by acquiring multiple PA images of the same specimen at the different wavelengths^{16, 19}. The different wavelengths generally coincide with the absorption spectra peaks in NIR region of major chromophores present in the tissue. Multiple images of the same tissue specimen provide different contrast features corresponding to spatially varying concentration of different chromophores. In an ex vivo study, Allen et al.²⁰ performed spectroscopic PA imaging to detect the presence of lipid in human arterial tissue. Zhang et al.²¹ differentiated between arteries and veins on the basis of blood oxygen saturation data, measured using PA spectroscopy on Sprague Dawley rats.

Due to several factors like optical scattering, frequency dependent acoustic attenuation inside soft tissue etc., the relation between PA amplitude values and optical absorption property is nonlinear and complicated. This makes quantitative recovery of spatially varying optical property or chromophore concentration inside the tissue from images formed using PA amplitude values difficult. In addition to analyzing the time domain PA amplitude values, frequency spectra of PA signals can also be analyzed for tissue characterization as the frequency content of RF PA data vary with size and shape of PA absorbers²². Utilization of parameters, extracted from linear models fitted to power spectrum of 1D time dependent signals, is a popular method used for tissue characterization in US imaging^{23, 24}.

Researchers have established relationships among spectral parameters extracted from US signals with tissue parameters like US scatterer size, scatterer concentration and the fractional impedance difference between US scatterers and surroundings, both theoretically and with experimental studies^{23–26}. Recently, researchers have started to use this method for PA imaging also. Patterson et al.²⁷ performed frequency domain analysis of RF PA data and used the results in combination with PA signal amplitudes to differentiate between neoplastic prostate and normal prostate in an in vivo transgenic murine model. Xu et al.²⁸ investigated the correlation between parameters extracted from the frequency domain analysis of PA signals and the Gleason scores for prostate cancer in an ex vivo study with human prostate tissue. Wang et al.²⁹ theoretically determined correlation between spectral slope, extracted from power spectrum of PA signal, and different factors of PA imaging system like laser pulse envelope, US transducer directivity, signal bandwidth etc. In an ex vivo study, Li et al.³⁰ used PA spectral parameters along with features extracted from PA images for ovarian cancer detection. Using simulation and experiments involving osteoporotic rat femoral heads, Feng et al.³¹ explored the capability of PA spectral parameters to perform bone microstructure characterization. Sinha et al. differentiated between malignant, benign and normal prostate tissue using PA spectral parameters in an ex vivo multiwavelength PA study with actual human patients³². In an ex vivo study, Xu et al.³³

utilized frequency domain analysis of PA signal for fatty liver diagnosis in mouse model. Using simulation, Xu et al.³⁴ established a relationship between PA spectral parameters and size as well as concentration of spherical PA absorbers and supported their findings with a phantom study. In an in vivo study with prostate cancer murine model, Kuman et al.³⁵ utilized PA spectral parameters to differentiate between malignant and normal prostate. This study explores the capability of spectral parameters extracted from power spectrum of RF PA signals to differentiate between malignant and nonmalignant thyroid tissue.

MATERIALS & METHODS

For this study, compliance with the Health Insurance Portability and Accountability Act was maintained and approval from Institutional Review Board (IRB) was obtained. This study involved a total of 50 patients, out of which 41 were female and the rest were male. Written informed consent was obtained from each patient involved in this study. The patients underwent thyroidectomy after being diagnosed with suspected thyroid lesion. Three dimensional (3D) PA signals generated by the freshly excised thyroid specimens, were acquired by an ex vivo PA imaging system at multiple wavelengths. Although, a detail description of experimental protocol and PA image acquisition system are available in some of our earlier reports^{32, 36–38}, a concise description of those aspects are provided here for continuity.

We developed an ex vivo PA imaging system with acoustic lens based focusing in our laboratory. Figure 1 shows the schematic of the actual ex vivo PA imaging system developed in our laboratory. The system was based on transillumination geometry. The tissue specimen to be imaged, was held in a sample holder placed in between the light source and the US detection system. A fiber optic cable, attached to the pulse laser, was used to deliver laser light to the tissue specimen from below the sample holder. A tunable laser (wavelength: 700 – 1000 nm, pulse repetition frequency: 10 Hz, pulse duration: 5ns, EKSPLA, Vilnius, Lithuania) was used to generate pulse laser light at multiple wavelengths. The cylindrical container, suspending on top of the tissue specimen, as shown in Figure 1, was the US detection system. The US detection system contained an acoustic lens followed by a linear transducer array. For maintaining unit magnification between the object plane and the image plane, the tissue specimen as well as the acoustic lens (focal length: 39.8 mm, diameter 32 mm) and the linear transducer array (center frequency: 5 MHz, bandwidth: 60%, pitch: 0.7 mm, elevation: 1 mm, OLYMPUS NDT, State College, PA, USA) were arranged in 4f setup where f is the focal length of the acoustic lens. In this setup, both object distance [distance between the tissue specimen and the acoustic lens] and image distance [distance between the acoustic lens and the transducer array] were equal to 2f. The fiber optic cable was attached with the US detection system via the adjustable laser arm and together they were referred as PA camera. The US detection system was water sealed.

Before the start of PA imaging using this system, the excised tissue specimen was held in the sample holder and the sample holder was filled with saline water. The pulse laser was switched on at a particular wavelength and the tissue specimen emitted PA waves after absorbing the pulsed laser light. The emitted PA wave was captured by the US detection system. The US detection system was synchronized with the laser. For each pulse generated

by the laser, a trigger signal was sent to the US detection system to receive the PA wave generated by the laser pulse. Inside the US detection system, the acoustic lens focused the PA wave on the linear transducer array. For acquiring 3D PA signal, generated by an excised thyroid specimen, the tissue specimen was held stationary in the sample holder and the PA camera raster scanned the tissue specimen in the cross sectional plane. In each step during raster scanning, one set of 1D RF PA signals along the depth of the tissue specimen i.e. one PA A line signal was acquired. A custom made data acquisition system (APCON, Rochester, NY, USA) was connected to the transducer array. It sampled the RF PA data at a sampling frequency of 30 MHz, digitized and stored the samples in the computer. Using a Matlab (v2012a, Mathworks Inc., Natick, MA, USA) based software developed by us, different C-scan and B-scan 2D grayscale PA images were generated from the stored 3D PA signal. To ensure that the laser intensity was well below the safe human exposure limit according to American National Standards Institute guidelines (ANSI 2007)³⁹, 5 mJ (milli Joules)/cm² laser intensity on the excised thyroid specimens was maintained during all experiments.

Immediately after surgery, the excised thyroid specimen was sent to surgical pathology laboratory from the operating room. A thyroid cytopathologist, inked and divided the thyroid specimen into 2 – 3 mm thick multiple specimens in the surgical pathology laboratory. From those multiple specimens, two specimens, one with completely normal thyroid tissue and another with at least one grossly visible thyroid nodule were selected and sent for performing multiwavelength PA imaging. The average dimensions of the thyroid specimens were 2 cm × 2 cm × 2 mm. After PA images was completed, the specimens were returned to the surgical pathology laboratory for further processing. For preventing dryness of the excised thyroid specimens, they were kept in normal saline. To ensure that PA signal acquisition procedure would not affect the histopathological evaluation of the thyroid specimens, the complete thyroid specimen handling protocol was planned by the thyroid cytopathologist. The complete process of PA image acquisition and returning the specimens to the surgical pathology laboratory for histopathology was completed within 1 hour of surgery.

In our laboratory, PA imaging of the excised thyroid specimens was performed at five different wavelengths. In this report results obtained after analyzing PA data acquired at three different wavelengths are shown. The three wavelengths are 760 nm, 800 nm and 850 nm. In the NIR region, absorption peak of deoxyhemoglobin occurs at 760 nm while 850 nm is the corresponding wavelength for the absorption peak of oxyhemoglobin^{40, 41}. At 800 nm, absorption coefficient of both oxyhemoglobin and deoxyhemoglobin are equal^{40, 41}. Deoxyhemoglobin and oxyhemoglobin are two significant chromophores that can be used for tissue characterization, using in vivo AP imaging. In case of ex vivo imaging, the major contributing chromophores may be different than those for in vivo imaging. We adhered to the standard practice of in vivo imaging as far as wavelength selection was concerned and in this ex vivo study, analyzed PA images acquired at the three wavelengths mentioned before.. Once the PA imaging was completed, each tissue specimen was returned to the surgical pathology laboratory where histopathology was performed. Using digital images of the marked histology slides as ground truth, the region of interest (ROI) in the PA data corresponding to normal, benign and malignant thyroid tissue were selected.

Figure 2A and 2B show a freshly excised thyroid specimen and its histology slide. Figure 2C shows the digital images of the histology slide overlaid on the image of the thyroid specimen. Figure 2D, 2E and 2F are the C scan PA images formed using the PA data generated by the thyroid specimen in Figure 2A at 760 nm, 800 nm and 850 nm.

After the ROI selection was completed, the RF PA A line signals corresponding to particular ROIs were processed for further analysis. . Before PA imaging of a tissue specimen, laser intensity at different wavelengths was measured. Using these data, each PA A line signal was corrected for the input laser intensity variation at different wavelengths. Each PA A line signal was time gated into small sections using a set of sliding Hamming windows of 1 μ s length. Fourier transform (FT) of each windowed PA Aline signal was computed using fast Fourier transform (FFT) algorithm. Power spectrum was obtained by squaring the amplitudes of the PA signal FT. Windowing was performed to reduce the truncation artifacts in fast Fourier transform (FFT) computation. Successive Hamming windows were overlapped by 30% as a compromise between the truncation artifact and scale factors of the signal amplitudes. Power spectrum of each windowed PA A line signal was divided by the one way power spectrum of the transducer array in the usable frequency range [2.4 MHz – 7.4 MHz]. The usable frequency range was determined by selecting the 10 dB fall of points on the both sides of the peak of the one way power spectrum of transducer. The one way power spectrum of the transducer array was computed from the two way amplitude spectrum of the transducer array provided by the manufacturer. Calibration of each power spectrum using one way transducer power spectrum was performed to reduce the artifacts introduced due to the limited bandwidth of the transducer. After calibration, the power spectrum was converted to dB scale and then linear regression was performed to find out the best fit straight line to the calibrated power spectrum. Three spectral parameters, namely slope [dB/MHz], midband fit [dB] and intercept [dB] were extracted from the best fit straight line. Figure 3 shows the example of power spectrum analysis process performed on a single A line signal. Figure 3B shows the PA A line signals corresponding to the blue colored pixel in the C scan PA image shown in Figure 3A. The windowed version of the signal, obtained by applying a Hamming window of the same length as that of the signal, is shown in Figure 3C. Figure 3D shows the normalized amplitude spectrum of the windowed PA signal along with the normalized one way amplitude spectrum of the transducer array in the usable frequency range. The calibrated power spectrum of the A line signal in the usable bandwidth is shown in Figure 3E. The red line in Figure 3E shows the straight line best fit to the calibrated power spectrum. Spectral parameters, slope and intercept are the slope and intercept of the straight line while the midband fit is the height of the straight line at the middle frequency [5 MHz] of the usable bandwidth.

The first and second authors performed the PA signal analysis and PA image acquisition. Together, these two authors have 12 years of experience in the field of PA image acquisition and PA signal processing. The third author is a practicing radiologist who has 30 years of experience in US imaging and 8 years of experience in PA imaging. The fourth author is an imaging scientist who has 25 years of experience in US imaging and 10 years of experience in PA imaging. The third and fourth authors jointly supervised the complete study.

RESULTS

Number of patients involved in this study was 50. 3D PA data from a total of 65 ROIs corresponding to different thyroid tissue pathologies are analyzed in this study. The thyroid cytopathologist diagnosed 12 out of 65 ROIs as malignant, 28 out of 65 ROIs as benign and 25 out of 65 ROIs as normal thyroid tissue. ROIs under benign and the normal tissue categories were clubbed together to form another category known as nonmalignant tissue category.

Four different bar plots showing average values of four parameters [slope, midband fit, intercept & pixel value] computed over the ROIs corresponding to malignant, benign and thyroid tissue categories at three wavelengths are shown in Figure 4. Each bar in the plots represents average value of a particular parameter, computed over all the values of that parameter extracted from A line signals corresponding to all ROIs belonging to a particular tissue category at a particular wavelength. The error bar on each average value represents the standard deviation of that particular parameter over all the ROIs corresponding to a particular tissue category at a particular wavelength. In Figure 5 similar bar plots of average values of four parameters are shown for two different tissue categories; malignant and nonmalignant thyroid tissue at the three wavelengths.

To find out whether statistically the parameters are significantly different between different tissue categories, two tailed two sample t tests were performed. Results of the t tests performed at 5 % significant levels with the parameter values corresponding to four different pairs of tissue categories are shown in four plots in Figure 6. For a particular parameter, p-value below 0.05 indicates the corresponding parameter is significantly different between a given pair of tissue type. According to Figure 6A, besides slope at three wavelengths, all other parameters at all wavelengths are significantly different ($p < 0.05$) between malignant and normal thyroid tissue. Figure 6B shows that, between malignant and benign thyroid tissue, intercept at 760 nm and slope as well as intercept at 800 nm and 850 nm are not significantly different ($p > 0.05$). According to Figure 6C, all four parameters at three wavelengths are significantly different ($p < 0.05$) between benign and normal thyroid tissue whereas Figure 6D shows that intercept at 760 nm and slope at 800 nm and 850 nm are not significantly different ($p > 0.05$) between malignant and nonmalignant thyroid tissue. If a parameter is significantly different between a given tissue pair, then it is likely to be highly efficient in differentiate between the given tissue pair.

DISCUSSIONS

This study was performed to evaluate the potential of frequency domain analysis of 3D PA data for thyroid tissue characterization. Capability of the extracted spectral parameters to differentiate between different thyroid tissue categories was evaluated by applying t tests to extracted parameters corresponding to particular tissue categories. As PA data were acquired at three different wavelengths, for the ease of discussion, we refer to each parameter at each wavelength a separate parameter which implies a total of 12 parameters [each of slope, midband fit, intercept and pixel value at 760 nm, 800 nm and 850 nm] are discussed here. Out of these 12 parameters, 9 are spectral parameters [slope, midband fit & intercept at 3

wavelengths] and 3 are pixel value parameters [pixel value at 3 wavelengths]. Thyroid tissue specimens belonging to three categories, malignant, benign and normal were imaged in this study while during analysis a fourth category, nonmalignant under which all specimens belonging to benign and normal specimens were grouped, was created. Results of this study show that spectral parameters were most efficient in differentiating between benign and normal thyroid [9 out of 9 spectral parameters were significantly different], while they were least efficient in differentiating between malignant and benign thyroid tissue [4 out of 9 spectral parameters were significantly different]. Between malignant and normal thyroid 6 out of 9 spectral parameters were significantly different and same number of spectral parameters were significantly different between malignant and non malignant thyroid. So effectiveness of the spectral parameters in differentiating between different tissue categories were similar for malignant vs normal thyroid and malignant vs non malignant thyroid. The 3 pixel values parameters were significantly different between different tissue categories for all 4 tissue category pairs.

For tissue characterization using PA images, conventionally pixel values of the grayscale PA images corresponding to different tissue types are compared³⁹. In this study, we used spectral parameters extracted from the PA signals for thyroid tissue characterization and compared their performance with the performance of pixel value for tissue characterization. Among the spectral parameters, performance of the midband fit was found to be best for thyroid tissue characterization as it was significantly different between different tissue types for all 4 different tissue category pairs at all of the three wavelengths. Performance of slope was worst as it was significantly different between different tissue types only 5 times out of 12 [for 4 tissue categories at 3 wavelengths]. Intercept performed in between midband fit and slope as it was significantly different 8 times out of 12 between different tissue types. When performance of spectral parameters and pixel value were compared, it was found out that the performance of pixel value was better than that of midband fit [the best performing spectral parameter] as it was significantly different between different tissue types for all 4 tissue categories at 3 wavelengths and the corresponding p values were quite smaller [maximum p value = 4.9×10^{-7} , minimum p value = 6.9×10^{-21}] than the p values corresponding to midband fit [maximum p value = 0.015, minimum p value = 4.2×10^{-7}].

As the PA imaging and analysis were performed at three different wavelengths, performance of the spectral parameters corresponding to different wavelengths were also compared. From Figure 6, it is evident that among the three wavelengths, the spectral parameters at 760 nm performed best. Considering all 4 different thyroid tissue category pairs, spectral parameters were not significantly different between different tissue types only 4 times out of 12 [considering 3 spectral parameters for 4 different tissue pairs] at 760 nm while at both 800 nm and 850 nm, the spectral parameters were not significantly different between different tissue types 5 times out of 12.

Parameter values, averaged over all the ROIs corresponding to different tissue categories are plotted in Figure 4 and Figure 5. From the figures, it is evident that average pixel value corresponding to malignant thyroid was higher than that of benign and normal thyroid. Generally additional network of blood vessels grow in malignant tissue regions^{16, 42}. As the optical absorption coefficient of blood is high at all the three wavelengths used in this study,

the malignant tissue regions were expected to generate PA signals having amplitudes higher than that corresponding to benign or normal tissue regions. The plots also show that average values of midband fit and intercept corresponding to malignant thyroid were also greater than that of benign and normal thyroid. In one of our earlier study³², we have shown that both midband fit and intercept depend on the optical absorption coefficient of tissue in a non linear fashion. As the optical absorption coefficient of malignant tissue is generally expected to be higher for the particular wavelengths used in this study, midband fit and intercept values corresponding to malignant thyroid tissue are also expected to be higher than that corresponding to non malignant thyroid tissue. In that same study, we have also shown that slope is independent of optical absorption coefficient of the tissue. For this study, the average slope value corresponding to benign thyroid was highest while average slope corresponding to malignant thyroid was lowest.

When the freshly excised thyroid tissue specimen was immersed in normal saline water, the blood content of the specimen would be diluted. This dilution would produce decrease in the optical absorption coefficient of the blood content of tissue^{43, 44}. Amount of blood dilution and reduction of absorption coefficient of blood would depend on the time gap between the surgery [when the tissue specimen was removed] and the PA imaging⁴⁴. We maintained a short time gap between the surgical procedure and PA image acquisition [~ 30 minutes]. Because of this short time gap, we think that the blood content of the tissue specimen was not diluted severely and thus optical absorption coefficient of the blood content would not decrease by a large amount.

We believe that although total blood content of the tissue specimen would reduce when immersed in normal saline, there would be some difference between the blood content of the malignant region and that of the nonmalignant region. Due to the difference of blood content mentioned before, PA signals of higher amplitudes were produced by the malignant thyroid tissue. In the NIR region, the absorption coefficient of water and lipid are two orders magnitude smaller than that of blood, specially in the 600 nm to 800 nm range⁴¹. In case of ex vivo PA imaging of tissue specimen immersed in normal saline, the absorption coefficient of blood might decrease due to penetration and dilution. In that case, it is possible that the absorption coefficient values of water and lipid present in the tissue might become comparable to that of the blood, but this was most likely to happen in the 800 nm to 1000 nm range where water and lipid absorption coefficient values begin to increase. This study considered PA signals acquired at 850 nm and below that wavelength. Therefore we believe that major part of the PA signal was generated by blood microstructure. However, for our ex vivo study, since the overall water content of the tissue specimen increased, water and lipid might have significant contribution towards PA signal generation. We believe that in case of ex vivo imaging, the major contributing chromophores may be different than those for in vivo imaging. We adhered to the standard practice of in vivo imaging as far as wavelength selection was concerned and in this ex vivo study, analysed PA images acquired at the three wavelengths corresponding to the absorption peaks of deoxyhemoglobin, oxyhemoglobin and whole blood. But we did not attribute the difference in PA amplitudes corresponding to malignant and nonmalignant thyroid in terms of deoxyhemoglobin and oxyhemoglobin concentration. This study did not attempt to characterize thyroid tissue in terms of different chromophore concentration. In future, we want to perform in vivo PA imaging for thyroid

tissue characterisation and explore the differences in the PA signals obtained in case of in vivo PA image and ex vivo PA image.

In this study, we demonstrated the capability of spectral parameters to differentiate among malignant, benign and normal thyroid tissue using PA images of freshly excised thyroid specimen from human patients. The results of this ex vivo study can motivate towards designing an in vivo study for thyroid tissue characterization using spectral and pixel value parameters from 3D PA data. In case of an in vivo study, a detailed 2D or 3D optical model based inversion scheme^{45, 46} has to be incorporated to correct for the excessive optical scattering and absorption in the overlying tissue on the ROI. In addition to that, the PA imaging system will also be changed to a reflection mode imaging system [light source and the US transducer will be on the same side of the region to be imaged] from the transillumination imaging system [light source and US transducer are on the opposite side of the tissue specimen] used in the present study. One weakness of this study is the small number of malignant thyroid tissue specimens incorporated in this study. Only twelve out of sixty five specimens were malignant. As the number of patients with thyroid cancer in the hospital was small, the number of malignant tissue specimens was small. This affected the statistical significance of this study. If the number of malignant samples was more, the statistical analysis results would be better.

In conclusion, it can be said that the preliminary results of this ex vivo study show that the spectral parameters extracted from RF PA signals as well as the pixel value of 2D PA images can be used for differentiating between malignant, benign and normal thyroid tissue.

Acknowledgments

This work was partially supported by a R15 research grant (Grant No: R15EB019726) awarded by National Institute of Health (NIH) and an Instrumentation grant from Lang Memorial Foundation.

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Figure 1.
Ex-vivo PA imaging system

Figure 2.

Ex-vivo C-scan PA images of a freshly excised thyroid tissue specimen acquired at three different wavelengths. (A) Thyroid tissue specimen (B) Histology slide of the thyroid specimen (C) Histology slide overlaid on the thyroid tissue specimen (D) PA image acquired at 760 nm (E) PA image acquired at 800 nm (F) PA image acquired at 850 nm. White circles in A, D, E, F and blue circles in B, C show ROI corresponding to malignant tissue.

Figure 3.

Power spectrum analysis on a single A line PA signal (A) C scan PA image of the thyroid specimen shown in Figure 2A (B) PA A line signal generated by the tissue corresponding to the blue colored pixel (C) PA signal in Figure 3B is windowed [multiplied] by a Hamming window of the same length (D) Normalized amplitude spectrum of PA A-line signal in Figure 3C and one way transducer spectrum in the usable bandwidth region [2.4 MHz – 7.4 MHz] (F) Calibrated power spectrum (black line) along with the best fit straight line (red line) fitted to the calibrated power spectrum.

Figure 4.

Average values of the power spectral parameters namely slope, midband fit, intercept along with pixel values corresponding to malignant, benign and normal thyroid tissue at three different wavelengths (A) Average slope values (B) Average midband fit values (C) Average intercept values and (D) Average PA pixel values. Error bars show the standard deviation of the corresponding parameter over the particular tissue types.

Figure 5.

Average values of the power spectral parameters namely slope, midband fit, intercept along with pixel values corresponding to malignant and nonmalignant thyroid tissue at three different wavelengths (A) Average slope values (B) Average midband fit values (C) Average intercept values and (D) Average PA pixel values. Error bars show the standard deviation of the corresponding parameter over the particular tissue types.

Figure 6.

Comparison of the four parameters using two sample two tailed student t test between pairs of different thyroid tissue types (A) Malignant vs normal (B) Malignant vs benign (C) Benign vs normal (D) malignant vs nonmalignant. The red line in each plot show the level corresponding to the decision criterion ($p = 0.05$). A p-value below 0.05 indicates that the corresponding spectral parameter is likely to be highly efficient in differentiating between a given pair of tissue type. For example in order to differentiate between malignant and normal tissue in Figure 6A, three parameters, midband fit, intercept and pixel value at all three wavelengths are deemed highly efficient but slope is not.