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Abstract. Hybrid optical modality imaging is a special type of multimodality imaging significantly used in the recent past in order to harness the strengths of different imaging methods as well as to furnish complementary information beyond that provided by any individual method. We present a hybrid-modality imaging system based on a commercial clinical ultrasound imaging (USI) system using a linear array ultrasound transducer (UST) and a tunable nanosecond pulsed laser as the source. The integrated system uses photoacoustic imaging (PAI) and USI for ocular imaging to provide the complementary absorption and structural information of the eye. In this system, B-mode images from PAI and USI are acquired at 10 Hz and about 40 Hz, respectively. A linear array UST makes the system much faster compared to other ocular imaging systems using a single-element UST to form B-mode images. The results show that the proposed instrumentation is able to incorporate PAI and USI in a single setup. The feasibility and efficiency of this developed probe system was illustrated by using enucleated pig eyes as test samples. It was demonstrated that PAI could successfully capture photoacoustic signals from the iris, anterior lens surface, and posterior pole, while USI could accomplish the mapping of the eye to reveal the structures like the cornea, anterior chamber, lens, iris, and posterior pole. This system and the proposed methodology are expected to enable ocular disease diagnostic applications and can be used as a preclinical imaging system. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: [10.1117/1.JMI.2.3.036003](https://doi.org/10.1117/1.JMI.2.3.036003)]

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1 Introduction

Modern medical imaging modalities are efficient enough to provide the comprehensive structural, functional, and molecular information that will enable highly accurate disease diagnosis. However, the use of each imaging modality in a specific configuration is only suitable for certain diagnostic applications. Multimodality imaging is the use of more than one imaging modalities integrated in a single setting to acquire more information. The modalities chosen for integration should provide complementary and useful information for diagnostic applications. Using this approach, the benefits of each modality can be used to overcome the limitations of the other and to provide more information than could have been provided by only one imaging modality.¹ In addition, multimodality imaging also helps to reduce the patient's level of discomfort when different imaging modalities have to be used. Instead of going through several screenings for different imaging modalities, a multimodality imaging system which has been integrated into a single setting will help to reduce patients' stress. Both the clinician and patient would benefit from the reduced screening duration.

Hybrid-modality imaging refers to a subset of multimodality imaging systems, which employs the use of imaging modalities that have different operation principles. As such, a multimodality imaging system employing reflectance and fluorescence imaging will not be considered as a hybrid as they are both types of optical imaging.

Photoacoustic imaging (PAI) has recently been developing rapidly as it can be used to provide a good spatial resolution beyond the penetration depth limit of optical imaging of about 1 mm in tissue.² PAI is a combination of the optical excitation and the detection of ultrasonic waves. The ultrasonic scattering in PAI is a few orders of magnitude lower compared to optical scattering in biological tissues.³ Depending on the configuration, PAI can provide a finer resolution at a deeper penetration depth up to a few centimeters,^{3–5} which is much more than that of optical imaging.

The initial pressure rise caused by the photoacoustic (PA) effect P_0 is

$$P_0 = \Gamma \mu_a F, \quad (1)$$

where P_0 is directly proportional to the unitless Grüneisen parameter Γ , the optical absorption coefficient μ_a , and the local optical fluence F .^{2,6,7} In PAI, the pulsed optical excitation is irradiated onto the tissue surface, and part of the optical energy is absorbed by the tissue and converted into heat. The proportion of the energy absorbed is directly proportional to μ_a , which is a function of the optical illumination wavelength. The energy absorbed causes a transient temperature rise resulting in thermoelastic expansion, and this relationship is quantified by Γ . This produces an initial pressure rise and broadband acoustic waves, also known as PA waves.^{6,8,9} The PA waves are detected using an ultrasound transducer (UST).

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The contrast in PAI is the optical absorption coefficient, and there are many endogenous contrast agents in the tissues that can be imaged directly without administering foreign materials. Lipid has been imaged using PAI for the study of acute coronary events.¹⁰ Blood is also commonly imaged using PAI for vasculature mapping,¹¹ determining the total hemoglobin concentration and the blood oxygen saturation.^{9,12} The rich structural and functional information from blood can be a useful indicator of angiogenesis and hypoxia, both of which are hallmarks of cancer^{13,14} and can be used for the detection of tumors.

Ultrasound imaging (USI) is based on the principle of pulse-echo imaging. The ultrasound (US) pulse in USI is produced by the UST using piezoelectric materials. As the US pulse travels in the biological samples, the density difference in the tissues, fluids, and bones provides a mismatch in the acoustic impedance, which reflects US¹⁵ waves. The echo travels back toward the UST and is subsequently detected. The strength of the reflected US is a measure of the mismatch between the different layers. Therefore, USI is often used to provide the structural information of an imaged sample.

PAI is commonly integrated with USI because both the imaging modalities are detecting acoustic waves using an UST. USI has already been widely used and accepted in many clinical applications. By combining these two imaging modalities, it also makes it easier for clinicians to accept PAI as a new imaging modality.¹³ It has been reported in literature that systems use single-element USTs, which require mechanical scanning to form a B-mode image.^{16,17} Such scanning makes the overall speed of the system slow and more susceptible to motion artifacts, thus reducing the image quality. From these perspectives, this paper details a novel integrated hybrid-modality imaging platform, where a fast clinical USI system is easily integrated with a tunable nanosecond pulsed laser. The developed system uses a linear array UST, which facilitates the data acquired in each scan to form a B-mode image devoid of mechanical scanning. In this integrated hybrid-modality imaging system, the optical absorption-based information is available through PAI and structural information through USI. The system's ability to derive such complementary information is demonstrated by using enucleated pig eye sample as a test sample. The system can find clinical applications in the diagnosis of uveal melanoma, a type of ocular cancer, which can arise in the iris leading to blindness or death.¹⁸ PAI can be used to differentiate between the healthy iris and the tumor, and to determine the tumor size, spread, and type. By combining this information with that obtained from USI, the location of the tumor with respect to other ocular structures is revealed.

Fig. 1 Experimental setup.

2 Methods

2.1 Experimental Setup

The experimental setup (Fig. 1) consists of a commercial clinical USI scanner (UltraVision 64B Research Platform, Winprobe) and a laptop with dedicated software (UltraVision Control Panel) to process the data acquired from the UST and the display of PA and US images. The UltraVision software comes with several functions that are commonly seen in many clinical USI systems, such as the selection of image depth and focal depth, time-gain compensation, and spatial compounding. PA and US images were acquired using a 128-element linear array UST set within a clinical-style imaging probe (L15, Winprobe). The UST has a center frequency of 15 MHz and a bandwidth of >60%. The elements are placed on a 0.1-mm pitch, thus the probe has an azimuthal length (width of view at the surface) of 12.8 mm. A tunable nanosecond pulsed laser (Vibrant 355II, Opotek Inc), utilizing optical parametric oscillator technology for wavelength generation, was used as the excitation source of the PA images. It operates at 10 Hz, and the wavelength selection and the output intensity of the pulsed laser can be controlled using the laptop. Whenever the laser fires a pulse, the laser Q-switch synchronization sends a trigger to the scanner.

US excitations (US pulses) are delivered by the multielement UST and the detected echoes form US images. The scanner calculates the number of US images that it can produce between the PA triggers, as affected by the user-defined parameters, such as the imaging depth and settings for spatial compounding. It creates those US images and waits for the next PA trigger. Once the scanner receives the PA trigger from the laser, US excitation from the UST stops and the UST will only receive signals for a short time (~ 50 to $200 \mu\text{s}$). The time duration is dependent on the imaging depth and during this time the signals acquired are used to form a PA image. USI resumes after the PA image is formed. In this study, PAI and USI run at 10 Hz and ~ 40 Hz, respectively. An exposure time of 8 s is sufficient to acquire the data for each set of measurements.

For demonstration purposes, the eye sample is held in place by a holder and orientated such that the anterior segment is facing upward. The lens of the eye is placed about 2 cm away from the UST and the focusing depth of the US mode is also set at 2 cm. The line illumination is performed across the diameter of the eye and on its anterior segment. The UST is placed above this line and is just in contact with the water surface. The fluence of the line illumination on the eye is not uniform. On a flat surface, the fluence of the line illumination is higher near the center of the line. When applied to the eye, the fluence is further distorted due to the shape of the eye.

2.2 Preparation of Pig Eye Sample

Due to the similarities in morphology between pig and human eyes,¹⁹ the pig eye sample is chosen as an *ex vivo* animal model in this study. Pig eye samples have been used in vision sciences' research involving corneal transplant and glaucoma.^{19,20} The main differences between the pig and human eyes includes the absence of the Bowman's layer in the pig eye and that the cornea thickness of the pig eye is twice that of humans.²¹

Six randomly selected eyes from four pigs (*Sus scrofa domestica*) were enucleated from the local abattoir. Extraocular tissues, such as the conjunctiva and lacrimal gland, were

removed from the samples. The *ex vivo* samples were placed and transported on ice until the experiments began to maintain the “freshness.” Each sample was inspected visually and found to be free of signs of deterioration before testing. The eye samples were used within 6 h of death. A total of eight pig eye samples have been used in this study, all of which were conducted according to NTU’s regulations on biosafety and the regulations of Agri-Food & Veterinary Authority of Singapore. As the experimental results from the pig eye samples are reproducible (refer to [Appendix](#)), only the results from one eye sample are presented in the main text of this paper.

3 Quantification of System Resolution

A human hair measured to have a diameter of about $105\ \mu\text{m}$ is used as a test target to evaluate the system’s axial and lateral resolution in both the US and PA modes. The target was held horizontally and placed perpendicularly to the UST. Similar to the imaging setup for the *ex vivo* pig eye sample, the hair is kept at a distance of about 2 cm away, with the focusing depth of the US mode set at 2 cm. The US and PA images (Fig. 2) acquired from the human hair are acquired and analyzed.

The maximum amplitudes of the signals in both images are located, and Gaussian fit is applied to the amplitude profiles in both the vertical and horizontal directions. The full widths at half maximum of the Gaussian fittings were determined to quantify the system’s axial and lateral resolutions (Fig. 3). The US mode was found to have axial and lateral resolutions of about

Fig. 2 (a) Ultrasound (US) image and (b) photoacoustic (PA) image to determine axial and lateral resolutions.

Fig. 3 Normalized Gaussian fittings of axial and lateral profiles of (a) US image and (b) PA image.

0.42 and 0.97 mm, respectively, whereas the PA mode was found to be 0.25 and 1.35 mm, respectively. The axial resolutions in both modes are better than the lateral resolution, which can be seen in Fig. 2, where the signals appear to spread more in the horizontal directions. The resolutions in the two modes are different and one reason may be because the US mode has a function to determine the focusing depth, but the PA mode does not.

4 *Ex vivo* Pig Eye Sample Imaging

4.1 Experimental Results

Using the UltraVision software, the system is able to capture and display PA and US images side by side on the laptop. The settings can also be configured such that a pseudocolored PA image is overlaid on the US image. In order to improve the contrast of PA images, they are processed in MATLAB after the experiment before being presented in the following sections. However, no change is made on the US images.

Figure 4(a) shows the schematic of the eye and Fig. 4(b) shows the US image of the enucleated pig eye sample. The US image is able to show the ocular structures, including the cornea, anterior chamber, lens, iris, and posterior pole.

Fig. 4 (a) Schematic of the eye and (b) US image (C: cornea, AC: anterior chamber, L: lens, I: iris, PP: posterior pole).

Fig. 5 (a) PA image and (b) combined US/PA image of enucleated pig eye sample.

The images in Fig. 5 are acquired from the same eye sample and at the same position as in Fig. 4. Figure 5(a) shows the PA image using a 500-nm pulsed laser illumination. It can be observed that the PA signals are produced from certain specific regions, as shown in the image. Without a clear understanding of the structures of the eye, it can be difficult to determine the exact location from which the PA signals are produced. In Fig. 5(b), the PA image is overlaid onto the US image to form a combined image. Both the ocular structural features as seen from USI and the absorption-based information from PAI appear in one image. With the combined image, it is now evident that strong PA signals are produced from the pigmented iris, and weaker PA signals from the anterior lens surface (ALS) and the posterior pole. As the fluence of the line illumination is not uniform, it is not suitable to compare the properties of the iris, ALS, and posterior pole. Therefore, another set of experiments is conducted so that the lens and iris can be illuminated separately under the same fluence.

The line illumination is reduced to a shorter length of about 3 mm by blocking the path of the illumination from the two ends. Only the center region of the line illumination is allowed to pass. The UST is positioned such that its center axis is in line with this illumination. First, the eye sample is moved into position such that the illumination is now on the center of the lens. The short illumination is much smaller than the lens, thus only the lens is illuminated and not the iris. The results are shown in Figs. 6(a) and 6(b). Next, the eye sample is repositioned such that the iris appears in the middle of the US image. Now only the iris region is illuminated, and the results are shown in Figs. 6(c) and 6(d).

The results in Fig. 6, where the lens and iris were illuminated separately, were acquired under the same fluence. Therefore, Eq. (1) is reduced to

$$P_0 = k\Gamma\mu_a, \quad (2)$$

where k is a constant.

From Figs. 6(a) and 6(b), it is observed that the top PA signals originated from the ALS, and the bottom PA signal is from the posterior pole. From Figs. 6(c) and 6(d), a PA signal is acquired from the pigmented iris. By comparing the PA amplitude and using Eq. (2), where fluence is the same, it can be concluded that $(\Gamma\mu_a)_{\text{Iris}}$ is much higher compared to $(\Gamma\mu_a)_{\text{ALS}}$. This is attributed to the pigmented iris containing melanin, which is highly absorbing compared to the optically clear lens ($\mu_{a,\text{Iris}} \gg \mu_{a,\text{ALS}}$).

As the short illumination travels across the lens and further into the eye toward the posterior pole, the illumination path cannot be reliably estimated. Therefore, the fluence on the posterior pole is not known. Though the ALS and posterior pole produced PA signals of comparable amplitude, no additional information can be drawn from the obtained results. It is known that the posterior pole contains blood vessels¹⁶ (highly absorbing), which could be producing PA waves.

PA signals from the pigmented iris containing melanin and from the posterior pole of the eye which contains blood vessels were expected, as both melanin and blood are highly absorbing. On the other hand, if only the absorption property of the lens is considered, then it is unexpected for the ALS to be producing

Fig. 6 (a) PA image and (b) combined image with lens illumination, and (c) PA image and (d) combined image with iris illumination.

Table 1 Parameters.^a

λ (nm)	PRF (Hz)	t_{pulse} (ns)	T_{max} (s)	C_A	Area (m ²)
500	10	5	8	1.0 for 400 nm $\leq \lambda < 700$ nm	9.62113E- 06 ^b

^a λ , excitation wavelength; PRF, pulse repetition frequency; t_{pulse} , pulse duration; T_{max} , exposure duration; C_A , spectral correction factor related to melanin absorption.

^bArea of 3.5-mm diameter limiting aperture.

PA signals, as it has an apparently low optical absorption. The result of the ALS producing PA waves is similar to those already reported.¹⁷ Although this phenomenon is still not clarified, possible explanations include the postmortem changes and an unidentified chromophore,¹⁷ all of which has a relation only to μ_a . However, by taking a closer look at Eqs. (1) and (2), the amplitude of the PA wave is not only directly proportional to μ_a , but also to Γ . Therefore, any investigation into how the ALS is generating PA waves can be done through two approaches, μ_a and Γ .

In the present study, a linear array UST was used for the detection of the PA and reflected US waves. The vertical distance between the linear array UST and the eye increases when moving away from the center of the eye. For both PAI and USI, this space needs to be filled up by an acoustic coupling medium, such as the ultrasound gel, which is commonly used in clinical environments. If this space is smaller, less of the gel is required and it will become more clinically convenient. This issue can be overcome if a curved array UST is used. An optical fiber can also be used to deliver the illumination for PAI when the curved array UST is placed closer to the eye. Future modifications to the developed system will be along these directions.

4.2 Adherence to Guideline on Exposure Limit to Laser Radiation

For potential diagnostic clinical applications of the iris for detection of uveal melanoma, the exposure limit (EL) of the system is subjected to guidelines defined by International Commission on

Non-Ionizing Radiation Protection for the skin.²² For this purpose, where the illumination is targeted at the iris and not the cornea and retina, the EL for skin is used as the guideline to protect the anterior parts of the eye.²² Table 1 shows the parameters used for the below calculations for repetitive pulse exposures.

Two general rules are applied when using repetition pulsed systems and the EL for skin exposure. Rule 1 states that the exposure from a single pulse should not exceed the EL for one pulse of that pulse duration. In this case, the pulse EL is $EL_{\text{SP}} = 200C_A = 200 \text{ Jm}^{-2}$. Consider a 3.5-mm diameter limiting aperture, the pulse energy EL is $EL_1 = EL_{\text{SP}} \times \text{Area} = 1.92 \text{ mJ}$. Rule 2 states that the exposure from any group of pulses delivered in time T should not exceed the EL for time T . For a T_{max} of 8 s, the EL is $EL_{\text{Rep}} = 11C_A T_{\text{max}}^{0.25} = 18.50 \text{ kJm}^{-2}$. Considering a 3.5-mm diameter limiting aperture and that there are multiple pulses in T_{max} , the pulse energy EL is $EL_2 = EL_{\text{Rep}} \times \text{Area} / (T_{\text{max}} \times \text{PRF}) = 2.22 \text{ mJ}$. Since the pulse energy EL for a single pulse is lower than that of an exposure for the full 8 s, EL_1 will be applied for this study.

Based on the current experimental setting, the measured pulse energy using a power meter (Nova Display and 12A-V1, Ophir) is 0.131 mJ, which is only about 7% of EL_1 . Therefore, the current configuration can potentially be used for diagnostic applications of the iris to detect diseases like uveal melanoma.

5 Conclusion

In this paper, we presented a hybrid-modality imaging system based on a commercial clinical USI platform with a linear array UST set within a clinical-style imaging probe and a tunable nanosecond pulsed laser. The integrated system uses PAI and USI to provide complementary absorption and structural information, respectively. PAI in this system forms a complete B-mode image at 10 Hz, while US B-mode images are acquired at about 40 Hz based on the user-defined parameters used in this study. Using a linear array UST, the system captures B-mode images at a much faster rate compared to other ocular imaging systems using a single element UST. The system and the proposed methodology are validated by using a pig eye as the test sample. The obtained results showed that the proposed instrumentation is able to perform PAI and USI under the same

Fig. 7 (a), (b), (c), and (d) are four sets of combined images from pig eye samples.

setting. PAI could successfully capture PA signals from the iris, ALS, and posterior pole, whereas USI could accomplish the mapping of the eye to reveal structures like the cornea, anterior chamber, lens, iris, and posterior pole. This system and the proposed methodology are expected to be used as a preclinical imaging system in ocular imaging and other relevant diagnostic medical applications. One of the future work directions is to improve the spatial resolution. This can be achieved by using better image processing algorithms or adding a galvanometer to scan the focused beam for PAI.²³

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Appendix: Experimental Data Set

Figure 7 shows the combined US/PA images from four sets of experimental results acquired from different pig eye samples. Figure 7(a) is also Fig. 6(d) that appeared earlier in Sec. 4.1. It can be observed from Fig. 7 that the combined images are similar to each other, therefore, the experimental US and PA results are reproducible.

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