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Hybrid-modality high-resolution imaging: for diagnostic biomedical imaging and sensing for disease diagnosis

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ABSTRACT

Medical diagnostics in the recent past has seen the challenging trend to come up with dual and multi-modality imaging for implementing better diagnostic procedures. The changes in tissues in the early disease stages are often subtle and can occur beneath the tissue surface. In most of these cases, conventional types of medical imaging using optics may not be able to detect these changes easily due to its penetration depth of the orders of 1 mm. Each imaging modality has its own advantages and limitations, and the use of a single modality is not suitable for every diagnostic applications. Therefore the need for multi or hybrid-modality imaging arises. Combining more than one imaging modalities overcomes the limitation of individual imaging method and integrates the respective advantages into a single setting. In this context, this paper will be focusing on the research and development of two multi-modality imaging platforms. The first platform combines ultrasound and photoacoustic imaging for diagnostic applications in the eye. The second platform consists of optical hyperspectral and photoacoustic imaging for diagnostic applications in the colon. Photoacoustic imaging is used as one of the modalities in both platforms as it can offer deeper penetration depth compared to optical imaging. The optical engineering and research challenges in developing the dual/multi-modality platforms will be discussed, followed by initial results validating the proposed scheme. The proposed schemes offer high spatial and spectral resolution imaging and sensing, and is expected to offer potential biomedical imaging solutions in the near future.

Keywords: Multi-modality imaging, hybrid-modality imaging, photoacoustics, hyperspectral imaging, ocular, colon

1. INTRODUCTION

Medical imaging refers to the concepts and methodologies used to image the body or parts of it for medical diagnostics purposes. It plays a crucial role in the field of medicine because it can highlight functional and structural changes in the body, which lead to eventual diseases. Many types of imaging methods such as optical imaging, ultrasound imaging (USI), magnetic resonance imaging (MRI), X-ray imaging, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are available for varying applications in the biomedical field^{1, 2}. Optical imaging can offer one of the best lateral resolution in tissue (~ 0.1 -100 μm), compared to USI (50-500 μm), MRI (10-100 μm), X-ray imaging (50-200 μm), PET (1-2 mm) and SPECT (1-2 mm)².

Optical imaging has been commonly used in biomedical applications^{3, 4} as it can give very high spatial resolution, one major drawback of optical imaging is its limited penetration depth. The transfer of light in biological tissue is dominated by scattering events as it travels from the ballistic regime and into the diffusive regime. This transition takes place around one transport mean free path, which is of the order of 1 mm⁵. Beyond this depth, optical imaging is unable to offer high-resolution imaging. The maximum penetration depth of about 1 mm in biological tissues represents the main limitation in optical imaging. This constraint therefore restricts the use of optical imaging in diagnostic biomedical imaging only on the surface and sub-surface of tissues, and not suitable for deep tissue layer imaging.

By combining optical imaging with spectroscopic methods, hyperspectral imaging can give rich spectral information of an image. It allows the intensity of narrow and adjacent spectral bands over a large spectral range to be recorded, giving rich spectral information for each point in the image. The information can be used for classification and quantification, and thus useful for many applications such as quality assessment of agro-food products^{6, 7} and for disease diagnosis in biomedical applications^{8, 9}. A hyperspectral imaging system generally can have three main types of configurations, namely the spatial-scanning, spectral-scanning and snapshot imager. Each has its own advantages and limitations and should be chosen based on the respective objective or applications. They are used to achieve the same type of data set,

known as data-cube. A data-cube is a collection of intensity-related values stored in three dimensions (spatial-spatial-spectral). Each voxel in the data-cube (similar to pixel in a two dimensional dataset) contains a value which indicates the intensity of a wavelength band belonging to a particular spatial point in a two dimensional image. Compared to optical imaging, HSI gives more information in the spectral domain as it can detect tens to hundreds of wavelength bands^{10, 11}. Detailed analysis of the information can be performed using a variety of algorithms such as linear discriminant analysis¹² and principle component analysis¹³. However, like other optical imaging techniques, HSI also suffers from a limited penetration depth of approximately 1 mm in biological tissue⁵.

USI is based on the principle of pulse-echo imaging. The ultrasound (US) pulse in USI is produced by the transducer using piezoelectric materials. As the US pulse travels in the biological samples, the density difference in the tissues, fluids and bones provide a mismatch in acoustic impedance which reflects US¹⁴. The echo (reflected US waves) travels back towards the transducer and is subsequently detected. The strength of the reflected US waves is a measure of the mismatch between the different layers. Therefore, USI is often used to provide structural information of the imaged sample.

Photoacoustic imaging (PAI) has recently been developing rapidly as it can be used to provide good spatial resolution beyond the penetration depth limit of optical imaging of about 1 mm in tissue⁵. PAI is combination of optical excitation and acoustic detection of US waves. In PAI, the detection has an ultrasonic scattering which is a few orders of magnitude lower compared to optical scattering in biological tissues¹⁵. Depending on the configuration, PAI can give finer resolution at deeper penetration depth, up to a few centimeters^{2, 15, 16}, much more than that of optical imaging.

When pulsed optical excitation is irradiated onto the tissue surface, part of the optical energy is absorbed by the tissue and converted into heat. The proportion of the energy absorbed is directly proportional to the local fluence and optical absorption coefficient, which is a function of the optical illumination wavelength. The energy absorbed causes a transient temperature rise resulting in thermoelastic expansion. This produces an initial pressure rise and broadband acoustic waves, also known as photoacoustic (PA) waves¹⁷⁻¹⁹.

The contrast factor in PAI is a function of 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 events²⁰. Blood is also commonly imaged using PAI for vasculature mapping²¹, determining total haemoglobin concentration and blood oxygen saturation^{18, 22}. The rich structural and functional information from blood can be a useful indicator of angiogenesis and hypoxia, both of which are hallmarks of cancer^{23, 24} and can be used for detection of tumors. There has been a renewed interest in the recent past in combining different optical and non-optical modalities of imaging such as USI, PAI and or fluorescence for various potential applications.

This paper in this context focuses on dual-optical and hybrid-optical modality high-resolution imaging for diagnostic biomedical imaging and sensing for disease diagnosis. The research and development of two multi-modality imaging platforms with the respective optical engineering challenges will be detailed. A combined imaging platform integrating USI and PAI for ocular imaging, and an initial study of optical HSI and PAI for diagnostic imaging of the colon using colon tissue phantoms will be discussed in detail.

2. MULTI-MODALITY IMAGING

It is important for modern medical imaging modalities to provide comprehensive structural, functional and molecular information so that a more accurate disease diagnosis can be made. However, the use of each imaging modality in a specific configuration is only suitable for certain diagnostic applications. One approach that can be used to acquire the comprehensive information is multi-modality imaging, which refers to the use of more than one imaging modalities to integrate the benefits of each modality. The modalities chosen for integration should provide complementary and useful information for tissue diagnostic applications. Using this approach, the benefits of each modality can be used to overcome the limitations of the other, and give more information that could have been provided by only one imaging modality²⁵.

Multi-modality imaging can also help to reduce the patient's level of discomfort when different imaging modalities have to be used. Instead of going through multiple tests each using one imaging modality, a multi-modality imaging system or platform will help to reduce patients' stress and discomfort. Both the clinician and patient will also be benefited by spending less time in diagnostic evaluation undergoing such tests.

Multi-modality system can be broadly classified as multi-optical modality^{3, 26, 27} (Figure 1²⁶) or hybrid-modality²⁸ imaging systems. There have been different optical configurations reported in the recent past in this challenging research area. The author's group had proposed specialty fiber optics based dual-modality probes employing image fiber based dual and multi-optical modality and photonic crystal based dual-modality probe systems for early disease diagnosis^{3,4}.

Figure 1: Multi-optical modality imaging system²⁶ (FC: 1x2 Fiber coupler, OF: Optical fiber, M: Mirror, BS: Beamsplitter, FS: Finger splice, CL: Collection lens, Em: Emission, Ex: Excitation).

Here, hybrid-modality imaging refers to multi-modality imaging systems which employ the use of imaging modalities that are different in principle and method. Using this definition, a multi-modality imaging system employing different variants of optical imaging to capture reflectance and absorbance information will not be considered as a hybrid as it employs only optical imaging. However, a system using optical imaging for reflectance and PAI for absorbance can be treated as a hybrid as the former is optical while the latter is acousto-optical in principle.

3. HYBRID-MODALITY IMAGING SYSTEM

3.1 Early diagnosis of diseases such as cancer

Colon cancer accounts for more than half of the total estimated new cases and deaths in the United States, and there is an increasing trend of cancer incidence²⁹. Colon cancer can be divided into five stages and the general trend is in the increasing spread of the cancerous cells. A complete hybrid-modality imaging system for diagnosis of colon cancer should be able to identify colon cancer both in the early and advanced stages without performing tissue biopsy. This enables clinicians to deliver diagnostic results to the patients promptly and implement appropriate medical treatment. The patients can also benefit by avoiding invasive tissue biopsy.

A) In the first study, the use of HSI in reflection mode and PAI are investigated separately for colon cancer diagnostic applications from the tissue surface. The sample used is a tissue phantom (Simulab Corporation) with a piece of black tape pasted on it to represent an abnormality (Figure 2). PAI has been reported to be used for biomedical applications in the gastrointestinal tract to provide absorption and depth information of the sample, with a penetration depth of more than 1 mm^{21,30}. However, this study will only look into the use of PAI for surface imaging though PAI can be configured to give a deep penetration depth.

Figure 2: a) Sample of black tape on tissue phantom placed on glass slide, b) Image area 4 x 4 mm².

The setup of the point-scanning PAI system featuring an angled transducer probe is shown in Figure 3. A tunable pulsed laser provides the excitation source of the PA images using a wavelength of 500 nm. It is then split into two beams using a beam sampler, directed towards a photodiode and the objective lens. A focused single element transducer detects PA signal. The photodiode and the transducer are both attached to a digitizer inside a PC. A x-y-z motorized stage is used to position the sample.

Figure 3: Setup of conventional PAI system.

A customized LabView software, which is in-house developed, is used for proper instrument control and data acquisition. First, the pulsed laser output (500 nm) is directed to both the photodiode and sample. The PA waves produced by the sample will be detected by the transducer and amplified by the pre-amplifier. The photodiode detects the incoming pulse and it serves as a trigger for the digitizer to capture the signal from both the photodiode and pre-amplifier. Here, 40 measurements were taken from each position and the averaged data is subsequently saved. Then the stage moved to the next position and this process is repeated until scanning is completed. In this study, the stage was controlled to move 32 incremental steps of 125 μm , in both X- and Y-direction, covering an area of $4 \times 4 \text{ mm}^2$. The data acquired is subsequently processed and displayed in Matlab.

Figure 4: a) Imaged area $4 \times 4 \text{ mm}^2$, b) Point-scanning PAI results with 33×33 data points.

Figure 4 shows the result from the point-scanning PAI system. The black tape which represents abnormality can be easily imaged using PAI as it has a much higher absorption at 500 nm compared to the surrounding tissue phantom.

The same sample and region is also imaged using a push-broom HSI system which is in-house developed (hardware and software). This system incorporates a video camera (VC) and the software allows user to choose a region of interest (ROI) from the full view of the VC. Thereafter, scanning is done over the ROI only, and data which is saved comes from only within the ROI. This enables correct measurement with minimized data acquisition time and data storage requirement. The system is able to capture 756 spectral bands from a spectral range of 400 – 1000 nm, covering the visible light to near-infrared. A broadband white light source and an infrared lamp were used for wide-field illumination. The ROI selected and the corresponding cut data-cube are shown in Figure 5a and 5b respectively. Figure 6 shows the intensity mapping of three selected wavelength band (600, 700 and 850 nm out of 756 available spectral bands).

Figure 5: a) Imaged area $4 \times 4 \text{ mm}^2$, b) HSI cut data-cube.

Figure 6: Intensity mapping of a) 600 nm, b) 700 nm and c) 850 nm using HSI.

Figure 7: Spectra of tissue and black tape measured using HSI.

The tape and tissue phantom spectra are calculated from the selected regions in the ROI, indicated by the red and yellow boxes in Figure 5a respectively. Each box contains 20 x 20 spatial points from the data-cube. Each spectrum presented in Figure 7 is an average of 400 spectra. By plotting the spectrum of the black tape and the tissue phantom, the two can be clearly distinguished from each other.

B) In the second study, the use of HSI for fluorescence imaging and PAI are investigated separately for colon cancer diagnostic applications from the tissue surface. Instead of the black tape, a Rhodamine 6G fluorescent film is placed on the tissue phantom and the imaged area is 3 x 4 mm² (Figure 8). The illumination wavelength of 500 nm was used during fluorescence HSI with a supercontinuum source and an acousto-optic tunable filter. All other imaging parameters and systems used is the same as in Section 3.1A.

The results from PAI can be seen in Figure 9 where the fluorescent film shows a higher PA signal being detected using a pulsed excitation at 500 nm. Figure 10 shows the intensity mapping of 548, 563 and 578 nm using HSI. They are selected as they are close to the measured emission peak of 563 nm in this study. The emission spectrum is shown in Figure 11 and is calculated from the area indicated by the red box in Figure 10. The excitation spectrum which is measured separately is also shown.

Figure 8: a) Sample of fluorescent film on tissue phantom placed on glass slide, b) Image area 3 x 4 mm²

Figure 9: a) Imaged area 3 x 4 mm², b) Point-scanning PAI results using 500 nm with 25 x 33 data points.

Figure 10: Intensity mapping of a) 548 nm, b) 563 nm (emission peak) and c) 578 nm using HSI (images same color scale).

Figure 11: Normalized excitation and emission spectra

The results from PAI and HSI demonstrate that different information from the same sample can be acquired. By combining the results from these two types of imaging modalities, both the reflection and absorption-based characteristic of the different components in the sample can be determined. Coupled with appropriate algorithm capable to perform classification and quantification, the information can be used for diagnostic applications.

3.2 Ocular imaging using PAI and HSI

Vision trouble is defined as having difficulty in seeing, even with the aid of glasses and contact lenses. Age has been identified as being positively associated with vision trouble³¹, thus vision trouble can be a significant problem in many aging societies such as Singapore. Vision trouble can be caused by a variety of ocular diseases such as close-angle glaucoma (closed anterior chamber angle)³² and uveal melanoma (intraocular cancer)³³. Ocular imaging can be performed to identify causes of vision trouble and disease progression.

USI is commonly integrated with PAI because both the imaging modalities are detecting acoustic waves using an US transducer. USI has already been widely used and accepted in many clinical applications and by combining these two imaging modalities, it also makes it easier for physician to accept PAI as a new imaging modality²³.

The use of USI and PAI for ocular imaging is not new. It has been reported in literature where systems use single element transducers, which require mechanical scanning to form a B-mode image³⁴. In this study, an USI system was integrated with a tunable nanosecond pulsed laser to form a hybrid-modality imaging system comprising of both USI and PAI (Figure 12). Using linear array transducers, the data acquired in each scan can form a B-mode image without any mechanical scanning.

Figure 12: Setup of hybrid-modality imaging using PAI and USI.

A dedicated scanner (UltraVision 64B Research Platform, Winprobe) and a laptop are used to process the data acquired from the transducer and display of US and PA images. US and PA images were acquired using a linear array transducer (L25, Winprobe) with 128 elements in a clinical-style probe. During USI, the signals from all 128 elements were processed. However, when performing PAI only the signals from the center 64 elements were used. The transducer has a center frequency of 18 MHz, bandwidth of more than 70%, elements placed on a 0.1 mm pitch and an azimuth length (width of view at the surface) of 12.8 mm.

A tunable pulsed laser is used as the excitation source of the PA images. Wavelength selection and output intensity of the pulsed laser can be controlled via the same laptop. The pulsed laser illuminates the anterior segment of the eye sample. One PA image is captured each time the scanner is triggered by the laser Q-switch synchronization, running at 10 Hz. Between each trigger, more than one US image can be captured. In this study, the combined frame rate from both USI and PAI falls between 30 – 40 Hz, depending on the setting such as imaging depth.

Figure 13: a) US image, b) PA image without illumination, c) PA image using 500 nm wavelength (C: Cornea, AC: Anterior chamber, L: Lens, I: Iris).

Figure 13 shows the different images acquired from an enucleated pig's eye sample. Figure 13a shows the US image of the eye. Figure 13b shows the PA image but with the path of the pulsed laser blocked. There is no illumination of the eye, and therefore no PA wave is produced but still there is a weak signal in the center of the image. This image shows the background noise of the PA channel. Figure 13c shows the PA image using a 500 nm pulsed laser. By comparing Figure 13b and 13c, the actual PA signals can be seen.

Looking at the US image in Figure 13a, the structural features of the eye can be seen. From Figure 13c, the regions of the eye which has a higher absorption at 500 nm can be observed using the PA image. However, Figure 13c does not clearly show from which region in the eye are the PA signals produced. By looking at both Figure 13a and 13c, the top PA signal is observed to originate from the lens and the pigmented iris, and the bottom PA signal from the posterior pole of the eye. PA signals from the pigmented iris containing melanin and from the posterior pole of the eye which contains blood vessels are expected, as both melanin and blood exhibit high optical absorption. On the other hand, it is unexpected for the lens to be producing PA waves due to its apparent low optical absorption. However, this result is similar to those already reported and the explanation to this phenomenon is still not yet determined³⁴.

4. CONCLUSION AND FUTURE WORK DIRECTION

The designed and developed multi or hybrid-modality imaging system is able to provide comprehensive information in a single setting. This allows a more reliable disease diagnosis which can be completed within a shorter period of time. The hybrid-modality imaging systems presented in this paper have shown the potential of using hybrid-modality imaging systems to provide complementary information through different approaches. With prior measurement calibration, the results obtained from HSI or PAI can be coupled with appropriate algorithms to perform classification and quantification. When used with appropriate algorithmic analysis, the information gathered can be processed to give diagnostic results, helping clinicians make better medical decisions and the benefits will be transferred to the patients.

Future work will be directed towards developing a hybrid-modality imaging system which integrates both PAI and HSI into a single setting. In this work, there is also a need to reconfigure the PAI system to achieve coaxial optical and

acoustic beams which is much more suitable for deep tissue layer imaging, compared to the current angled probe configuration³⁵ presented in Section 3.1.

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