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2019

Sivasubramanian, K., Das, D., & Pramanik, M. (2019). High framerate photoacoustic imaging of blood clots. Proceedings of SPIE - Photons Plus Ultrasound: Imaging and Sensing 2019.

https://hdl.handle.net/10356/102638

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ABSTRACT

Deep vein thrombosis (DVT) is a disorder that occurs when a blood clot (thrombus) forms in one or more of the deep veins in your body, usually in your legs. Deep vein thrombosis can cause leg pain or swelling, but also can occur with no symptoms. If the clot moves to the vital organs like lungs, heart, brain etc., it can be very fatal and can cause death to the individual. Diagnosing it at early stages is very crucial to decide the treatment strategy. The most commonly used techniques that are used for the diagnosis includes ultrasound, x-ray, CT, etc. For definitive diagnosis contrast agents are required for better visualization of the blood clots and harmful radiations are used. For label free imaging of the blood clots, photoacoustic imaging can be used. To perform in-vivo photoacoustic imaging, high framerate imaging is needed as the velocity of the blood in the veins is between 3 cm/s to 14 cm/s. In this work, we have shown high framerate photoacoustic imaging at different framerates of 5, 10, 50, 100, 500, 1000, 2000 and 3000 fps using a pulsed laser diode of 7000 Hz frequency. We have demonstrated label free imaging of blood clots at 803 nm. Blood clot has at least 1.5 times higher SNR compared to blood and can be clearly visualized against blood as background. High framerate photoacoustic imaging can be used for label free diagnosis of deep vein thrombosis.

Key words: Photoacoustic imaging, clinical imaging system, blood clot imaging, handheld imaging.

1. INTRODUCTION

Photoacoustic imaging[1, 2] is a rapidly growing imaging modality and constantly gaining more importance in the field of biomedical imaging. It is a non-invasive imaging modality which combines the optical (high contrast) and ultrasound imaging (high resolution) into a single imaging system. Sample (e.g., biological tissues) are irradiated using non-ionising laser pulses,[3] the absorption of light energy by the sample leads to increase the local temperature (in the order of a few milli kelvin) which leads to thermoelastic expansion. This further leads to the release of pressure waves known as photoacoustic (PA) waves which can be acquired using ultrasound detectors (single or multiple or array detectors) outside the sample. Photoacoustic imaging[3] has several advantages in comparison to other optical imaging techniques like deeper penetration depth, good spatial resolution, and high soft tissue contrast. As the scattering from ultrasound waves is two to three orders of magnitude less than the optical scattering in biological tissues,[4] it helps photoacoustic imaging in overcoming the fundamental depth limitations pure optical imaging. It has a range of potential clinical applications, including breast cancer imaging, brain imaging, sentinel lymph node imaging,[5-7] blood vessel imaging,[8] tumor monitoring,[9] temperature monitoring,[10, 11] and many others.[12-15] PA imaging requires contrast agents for strong light absorption. These contrast agents can be intrinsic (blood, melanin etc.),[16] or extrinsic contrast agents (methylene blue, gold nanoparticles, nanospheres, carbon nanotubes etc.).[17-21] Typically, expensive, bulky, heavy lasers are used for PA imaging which often needs to be mounted on an optical table. Low cost, light weight, portable lasers[22-24] are needed for clinical translation of PA imaging. We have previously demonstrated high frame rate photoacoustic imaging of up to 7000 frames per second using a pulsed laser diode.[25] Pulsed laser diodes[22, 26] are small, compact, portable which emits light in the near-infrared region with very high pulse repetition frequency.

Deep vein thrombosis (DVT)[27, 28] is a disease that occurs when a blood clot (thrombus) forms in one or more of the deep veins in the body, more commonly in the legs. Deep vein thrombosis may cause pain in the leg or can lead to swelling, but it can also occur without any symptoms. It can occur if there are some medical conditions that can lead to blood clot formation. It can occur after surgery or an accident. Deep vein thrombosis can prove to be very dangerous if the blood

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clots break loose and reaches any vital organs like the brain, lungs, etc.[29] It can block the blood flow causing embolism, which can be very fatal. Therefore, it is essential to diagnose and monitor the blood clots.[29, 30] Currently, they are imaged using ultrasound imaging with external tracers. But it still requires the injection of external tracers in the body and does not provide the best quality images. Therefore, an alternative imaging modality is required for imaging fast moving blood clots. The blood clots can travel in the blood vessel at varying velocities, depending on the type of blood vessel. Usually, the velocity of blood in the veins range from 5 to 15 cm/s. Using a low framerate photoacoustic imaging system, the blood clots can be easily missed as they are not suitable for imaging fast moving objects. Therefore, high framerate PA imaging system is essential for imaging blood clots in the blood vessels. Till now, imaging frame rate of up to 200 frames per second have been shown.

In this work, we report a high framerate photoacoustic imaging system for blood clot imaging up to 3500 frames per second using a low cost, light weight pulsed laser diode (PLD). The maximum achievable pulse repetition frequency using this system is 7000 Hz. The PLD is combined with a with clinical ultrasound imaging system which can pave way for clinical translation. Phantom experiments were conducted with samples of blood, clot in saline and clot in blood at a flow rate of 5 cm/s. Imaging was done at different framerates of 5, 10, 50, 100, 500, 1000, 2000, and 3000 fps. Imaging of the blood clots in blood vessel can be useful for diagnosis and monitoring of diseases like deep vein thrombosis.

2. METHODS

For photoacoustic imaging, clinical ultrasound system (ECUBE 12R, Alpinion, South Korea) which can simultaneously perform photoacoustic imaging along with ultrasound imaging was used. Laser excitation is essential to initiate the acquisition of the photoacoustic imaging and operating in that mode. The laser unit is synchronized with the ultrasound system using a laser controlling unit which provides the trigger for the ultrasound system for image acquisition. Excitation can be provided by different types of lasers. Here, a pulsed laser diode (Quantel DQ-Q1910 SA-TEC) is used for performing high frame rate photoacoustic imaging. It produces light pulses in the near-infrared wavelength of 803 nm. Each laser pulse width is approximately 136 ns with a maximum pulse energy of ~1.4 mJ. The maximum pulse repetition frequency of the laser is 7000 Hz. The pulsed laser diode produces a diverging laser beam rectangular in shape. The PLD is controlled by a laser driver unit (LDU) which consists of a temperature controller (LaridTech, MTTC1410), a 12 V power supply (Voltcraft, PPS-11810), a variable power supply (to vary the laser output power), and a function generator (to control the laser repetition rate). The pulse repetition rate and pulse energy can be controlled individually with variable power supply (BASETech, BT-153), and function generator (FG250D, Function Generator), respectively. The function generator provides a TTL (Transistor-Transistor Logic) signal which synchronizes the laser excitation and the ultrasound data acquisition by the clinical ultrasound system. A cylindrical lens was placed in front of the laser to make the light focused and the laser beam narrower. After passing through the cylindrical lens, the laser spot size was 16 mm X 6 mm.

The photoacoustic signals generated from the sample were acquired using a linear array transducer (L3-12 transducer, compatible with the ultrasound system) consisting of 128 array elements which has an active area of 3.85 cm X 1 cm. This setup is call as PLD-PAT system. For photoacoustic imaging the ultrasound system was operated in research mode. When the system is being operated in this mode, a python script is used to assign and manage various parameters. Fig. 1 shows the schematic representation of the PLD-PAT imaging system. Data acquisition is done by 64 channels parallel data acquisition card. Therefore, to acquire data from all the 128 channels, two light pulses are needed. The system can be operated in two different modes. In the first one, data from all the 128 channels are taken and therefore, it requires 2 light pulses as trigger to form a single PA image. Therefore, the effective imaging frame rate is half the laser repetition rate (3500 frames per second). In the second mode, data from only 64 elements are used to generate an image and hence requires only one laser pulse trigger to obtain photoacoustic image. Therefore, the frame rate of the PA images is same as the laser pulse repetition rate giving us a framerate of 7000 frames per second. For all the experiments here, the system was operated in the first mode with a maximum framerate of 3500 frames per second. For image processing, the acquired PA signals pass through a series of inbuilt filters in the imaging system and the final processed PA image is seen on the monitor. Apart from the final image seen on the monitor, other data types can be obtained from the system and saved separately like the beam-formed or IQ demodulated data or scan converted data. The saved frames were later processed in MATLAB for measurement of signal to noise ratio, etc.

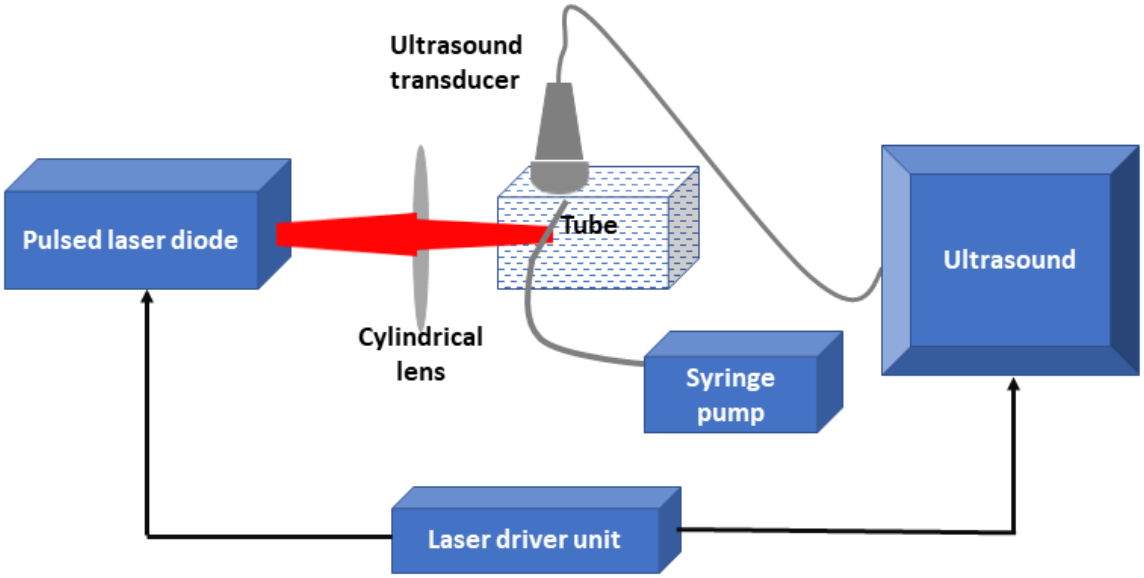

Figure 1: Schematic representation of the high framerate PLD-PAT imaging system

Experiments were performed with blood and clot at different framerates. Blood was obtained from the rats. A small amount of the blood obtained was kept separately in the open for 10 to 15 minutes without the presence of any anticoagulants to allow the formation of the blood clots. These clots were taken for imaging.

Imaging was done using three different samples namely blood, clot in saline and clot in blood. All these samples are inserted into a low density polyethyelene (LDPE) tubes of diameter 1.63 mm. the LDPE tubes were immersed in water and placed in a water tank. The tubes with samples were placed at the focus of the laser light and the movement of the liquids in the tube was controlled with a syringe pump. The linear array ultrasound transducer was placed parallel to the LDPE tube and placed at a distance of 1 cm from the tube. The transducer covered the entire area of laser light illumination. Total imaging distance was set as 2 cm. Imaging of the three samples were done at 5, 10, 50, 100, 500, 1000, 2000, 3000 fps respectively. Imaging was done when the sample was stationary and when the sample was moving at a velocity of 3 cm/s.

3. RESULTS AND DISCUSSION

PA imaging was done for three different samples, blood, clot in saline and clot in blood when stationary and moving. Imaging was done at different framerates namely 5, 10, 50, 100, 500, 1000, 2000 and 3000 fps respectively. Figure 2 shows the PA B-mode images obtained from the system for 3 different samples when stationary. Figure 2(a) shows the PA image of blood in the LDPE tube, Fig. 2(b) shows the PA image of the clot in saline background and Fig. 2(c) shows the PA image of the clot in blood background. The images shown in Fig. 2 are obtained by averaging 25 image frames and the signal to noise ratio (SNR) was calculated. SNR can be defined as SNR = V/n, where V is the photoacoustic signal amplitude and n is the standard deviation of the background noise. From Fig. 2(a) the SNR of blood was calculated to be 150. From Fig. 2(b) the SNR of the clot in saline was found to be 340. In Fig. 2(c) SNR was calculated from blood and clot separately as 127 and 333 respectively. It can be noted that SNR from clot was two times that of the blood. As the SNR from the clot is at least two times more than that of blood, it can be used for in-vivo imaging of clots in blood vessels.

Figure 3 shows the PA B-scan images obtained from the PLD-PAT system when the blood clot is moving inside the tube. The images shown here are of blood clot with blood in the background. Flowing at a velocity of 5 cm/s. The flow of the clot in the tube at various given time points can be seen in the images. For the purpose of representation only 5 fps and 3000 fps are shown here. In Figs. 3 (a-c), PA images of the blood clot flowing at 5 cm/s is shown at a frame rate of 5 fps.

Figure 3(a) shows the 0th frame where the blood clot cannot be seen, Fig. 3(b) shows the 1st frame and the blood clot can be visualized clearly in this image, Fig. 3(c) shows the 2nd frame and the blood clot can no longer be visualized anymore.

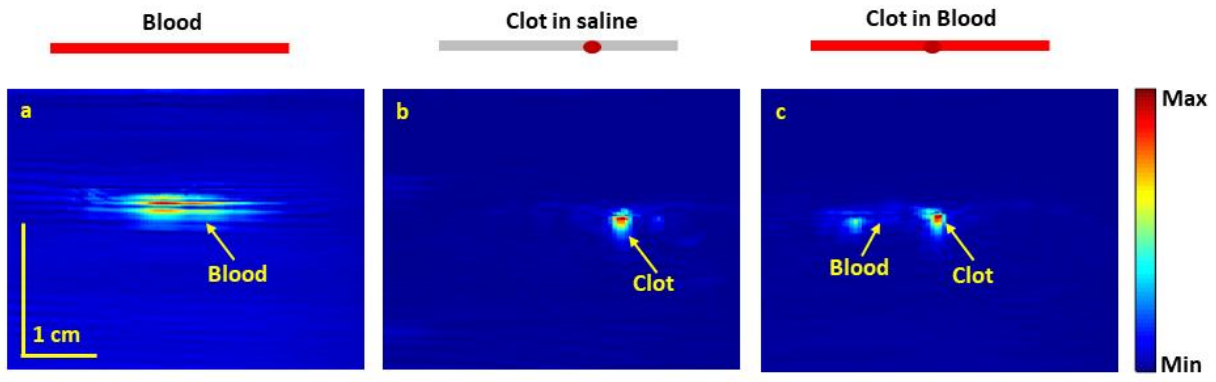

Figure 2: (a) PA image of blood in LDPE tube, (b) PA image of clot in saline, (c) PA image of clot in blood. Scale bar and color bar are mentioned in the image.

Figures 3(d-i) shows the PA images of the blood clot moving at a velocity of 5 cm/s in the LDPE tube at a framerate of 3000 fps. Figure 3(d) shows the 0th frame and the blood clot cannot be seen in this frame. Figure 3(e) shows the PA image of 200th frame and it can be noted that the blood clot becomes visible in the left corner of the tube. Figure 3(f) shows the PA image of the 500th frame and it can be observed that the blood clot is moving along the tube. Figure 3(g) shows the PA image of the 800th frame and it can be seen that the blood clot has moved further along in the tube. Figure 3(h) shows the PA image at the 1000th frame and the clot has moved further along in the LDPE tube. Figure 3(i) shows the PA image at the 1500th frame and the clot can no longer be visualized in the tube.

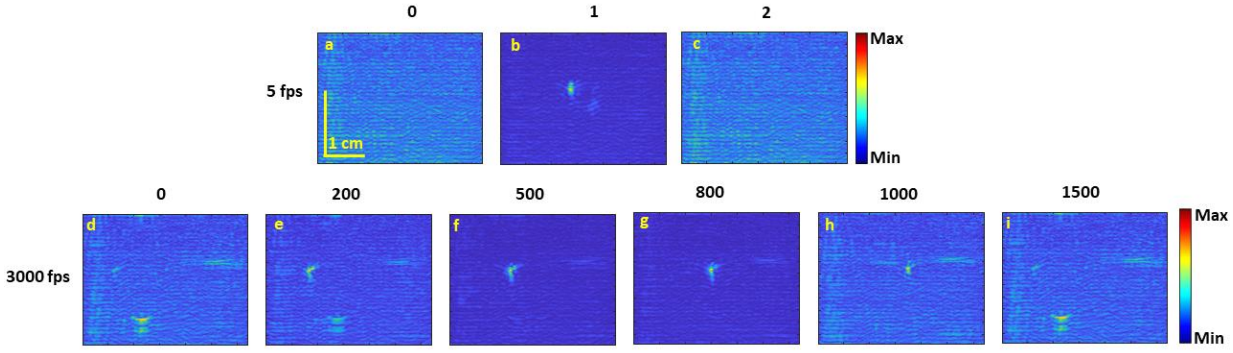

Figure 3: (a-c) PA images of the clot in blood flowing at a velocity of 5 cm/s images at a framerate of 5 fps; (d-i) PA images of the clot in blood flowing at a velocity of 5 cm/s imaged at a framerate of 3000 fps. Scale bar, color bar and frame number are mentioned in the images.

It is evident from the above results that the at low framerate imaging, the movement of the blood clot could not be visualized, and it is easy to miss the blood clot when flowing. Using high framerate PA imaging, visualization of the gradual movement of the blood clot can be done when the movement of the clot is at 5 cm/s. We have previously demonstrated that high framerate imaging can be used visualization of the movement of samples at higher velocity up to 15 cm/s.[25] Theoretically, with the high framerate PLD-PAT imaging system samples moving at a velocity of up to 134 m/s can be monitored.

For deep vein thrombosis and other diseases, visualization of the blood clot moving in the blood vessels can be observed using high framerate photoacoustic imaging. Here, we have shown a proof of concept that fast-moving blood clot can be imaged using high framerate photoacoustic imaging. All experiments are shown here are done on phantoms. In order to show that this imaging system can be translated to the clinics additional studies in small animal models are needed for validation.

4. CONCLUSION

We have shown that high frame rate photoacoustic imaging of blood clots up to 3000 fps is possible using the PLD-PAT clinical ultrasound system. Blood, blood clot in saline and blood clot in blood was imaged at different imaging framerates of 5, 10, 50, 100, 500, 1000, 2000, 3000 fps was shown at a velocity of 5 cm/s. The SNR was calculated for the same and it was noted that the SNR of the blood clot was at least 2 times that of blood, which can prove to be very useful for *in vivo* imaging. It was noted that using high framerate imaging the movement of the clot can be accurately visualized and monitored in diseases like deep vein thrombosis. Further studies will be conducted on small animal disease models to translate this imaging system to the clinics.

5. ACKNOWLEDGEMENT

The research is supported by Tier 1 grant funded by the Ministry of Education in Singapore (RG48/16: M4011617). Authors have no relevant financial interests in the manuscript and no other potential conflicts of interest to disclose.

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