# Development of a fast-scanning combined ultrasound-photoacoustic biomicroscope

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## ABSTRACT

Recently a realtime photoacoustic microscopy system has been demonstrated. Unfortunately, however, displayed B-scan images were sometimes difficult to interpret as there was little structural context. In this work, we provide structural context for photoacoustic microscopy images by adding ultrasound biomicroscopy as a complementary and synergistic modality. Our system uses a voice-coil translation stage capable of 1" lateral translation, and can operate in excess of 15 Hz for 1-cm translations, providing up to 30 ultrasound frames per second. The frame-rate of the photoacoustic acquisitions is limited by the 20-Hz pulse-repetition rate of the laser, but can be increased with a faster-repetition-rate laser. Data from the system is streamed in real time from a 2GS/s PCI data acquisition card to the host PC at rates as high as 200 MB/s. The system should prove useful for various in vivo studies, including combined ultrasound Doppler and photoacoustic imaging.

**Keywords:** photoacoustic imaging, high-frequency ultrasound, Q-switched lasers, biophotonics, cardiovascular research

## 1. INTRODUCTION

Recently photoacoustic microscopy has emerged as a promising technology for visualizing optically-absorbing structures in vivo with ultrasonic spatial resolution. Short laser pulses are fired into tissue producing rapid but minimal heating in optically-absorbing pigments, and causing temperature-induced expansion. This transient mechanical perturbation serves as an internal source of ultrasonic waves which are detected by an ultrasound transducer in the imaging system. The received photoacoustic signals are subsequently reconstructed to form images of subcutaneous optical absorption. Spatial resolution in this case is determined primarily by ultrasonic focusing and receiver frequency response, and multiply-scattered light is well-tolerated, enabling optical-contrast imaging with high-resolution at new depths. Maslov et al [1] and Zhang et al [2] have demonstrated images of subcutaneous microvasculature using dark-field confocal photoacoustic microscopy (PAM), a raster-scanning-based reflection geometry imaging system. Leveraging known oxy-hemoglobin and deoxy-hemoglobin absorption spectra, photoacoustic methods possess the ability to image blood oxygen saturation as well as relative concentration of total hemoglobin [2-4]. Emerging photoacoustic methods are enabling molecular imaging in small animals, including imaging of gene expression [5] and distribution of cell receptors [6].

C-scan and accompanying 3-dimensional imaging methods currently require fairly long scan times. Recently, a 50-frame-per-second B-scan system has been reported using a high-frequency array transducer, a high-repetition-rate laser, and a multicore computer for realtime beamforming and display [7-9]. This system has been used to image the beating hearts of small animals in realtime. Unfortunately, these images were sometimes difficult to interpret, and were without structural context.

While optical imaging technology, and in particular, photoacoustic methods, have made significant inroads for imaging small animals, high-frequency ultrasound [10, 11] has seen an explosion of growth in the pre-clinical imaging sector, partially due to recent availability of commercial pre-clinical high-frequency ultrasound systems. This adoption of high-frequency ultrasound by the biomedical research community is timely considering the vast array of animal models used in preclinical research.

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We would like to piggyback photoacoustic imaging on a high-frequency ultrasound imaging system to allow for combined ultrasonic and photoacoustic information in a single system. To pursue this goal, a cost-benefit analysis is valuable. High-frequency arrays [12] are very expensive as are their parallel-receive electronics. Fast-scanning systems using a single element transducer are possible, however frame-rate will be limited by laser pulse-repetition rate. Presently, however, there are very few options for tunable high-repetition-rate (>KHz) Q-switched lasers, and they are very expensive.

We would like to develop a photoacoustic imaging system that is easy to use for biologists, and that could be accessible in a price-regime accessible to an average mouse biology lab. Our approach to this is to develop a fast-scanning high-frequency ultrasound imaging system with a mode for photoacoustic imaging at an albeit slower frame rate as limited by the pulse-repetition rate of the laser (~1s for a 120-Hz laser). Presently we use a 10/20-Hz laser.

A number of synergistic techniques could be applied using such a system. Interlaced photoacoustic and ultrasonic pulse sequences could pave the road for slow-scan photoacoustic imaging motion compensation. Furthermore, color or power Doppler [13] could be used to visualize blood flow in concert with photoacoustic visualization of the microvasculature. Synergistic Doppler-photoacoustic signal processing methods could offer interesting possibilities. Additionally, molecular imaging via photoacoustic could be visualized with structural context.

In this paper, we describe the present state of development of our fast-scanning ultrasound-photoacoustic biomicroscope and provide data acquired from it, including fast-scan realtime imaging of a finger, and separate photoacoustic imaging of a phantom.

## 2. METHODS

One key to our targeted high-frame-rate ultrasound system is a fast scanning actuator. An ultrasound transducer or combined light-delivery & ultrasound probe is mounted on such a scanning actuator, and ultrasonic or photoacoustic A-scan lines are acquired at a sequence of defined positions along the scan trajectory. A B-mode image is assembled from the sequence of A-scan lines along a scan direction.

We use a voice coil positioning stage (VCS-10-023-BS-01, 1.0" travel, 2.3lbs continuous force, 6.9 lbs peak force, purchased from H2W technologies, Inc., Valencia, CA) assembled on a linear scanning stage (non-commutated DC Linear Actuator, H2W Model Number NCC10-15-023-1X), with a magnetic hall-effect position sensor, and closed loop controller and driver (Elmo Harmonica HAR 5/60, Elmo Motion Control Inc., Nashua, NH, USA). This unit has 1-inch translation capabilities with micron-scale positioning accuracy and is able to oscillate over 1-cm distances in excess of 15 Hz. Since each scan direction is used to acquire an image, this offers 30 frames per second imaging rates.

Fig. 1. Photograph of our voice-coil stage scanning an ultrasound transducer.

Presently our system provides two separate scanning modes: ultrasound and photoacoustic. We envision that realtime ultrasound scanning could be used to first to visualize a region of interest. Subsequently, the photoacoustic imaging mode could be engaged. In the future, ultrasonic and photoacoustic pulse-sequences could be interlaced for some interesting imaging possibilities.

In the ultrasonic scanning mode, the motion controller drives the voice coil stage to oscillate at a fixed scanning rate. During the scanning, a digital output bit from the controller is set to toggle high for a duration at the end of each scan trajectory. This end-of-scan trigger is used to mark the beginning of each image. This trigger event triggers a sequence of N A-scan line trigger events generated by a digital input-output card which are sent to the ultrasound pulser-reciever. This DIO card (NI PCI-6542, National Instruments Inc.) is a PCI-device, has 32 parallel input-output channels, and a clock-rate of up to 100 MHz. This card is controlled via C/C++ software leveraging a manufacturer-provided software development kit. The card also provides digital control for time-gain compensation (which is presently not used in the current results).

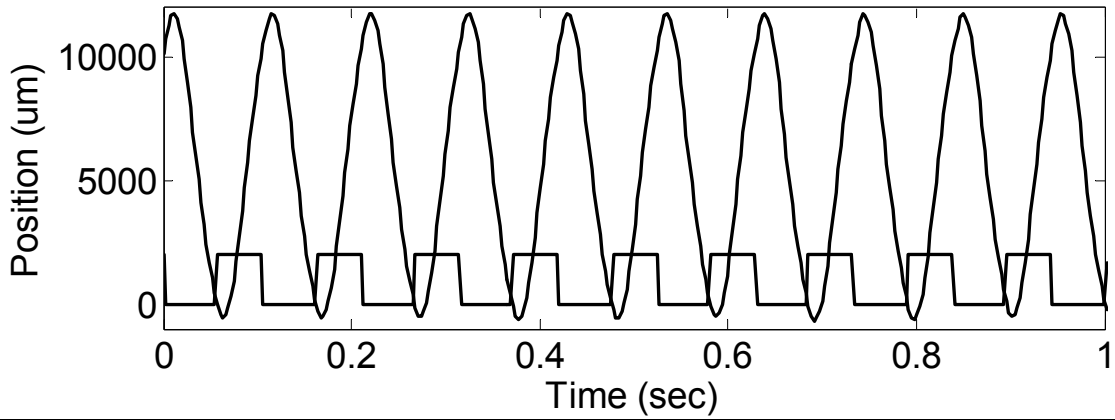


Fig. 2. Motion capture of the fast-scanning stage position as a function of time. A digital output bit is toggled high at the end of each scan trajectory, and this is used to trigger the sequence of A-scan lines for each image via a programmable digital input-output card.

In the photoacoustic mode, the DIO card is used as a clock source to trigger the laser and provide a step command to the motion controller. Thus in photoacoustic mode, the controller operates in externally-triggered stepping mode rather than fast scanning mode as used for ultrasonic imaging. The motion controller is programmed using a microcontroller scripting environment and each motion program is uploaded from a PC to the microcontroller memory via RS-232. There is an unacceptable jitter of multiple nanoseconds between the time the laser flashlamp is triggered and the expected time of the lasing output, so we use the laser's Q-switch trigger out or a fast photodiode to trigger data acquisition initiation at a jitterless time-zero.

One difficulty with the present motion controller is that is produces noise events due to a technique called digital pulse-width modulation. Rather than use a digital to analog current driver, digital pulse-width modulation turns on current pulses for various number of clock cycles to give an effective average current. We found that these noise events were synchronous with the clock rising edge of controller, set at 44kHz. Prior to laser trigger or ultrasound trigger events being routed from the DIO card to the trigger-in ports, the signals are routed through a D-flip-flop such that they are toggled high only at the rising edge of the clock signal. In this way we have 22.7μs to acquire an ultrasound signal before the next noise event, which represents 34 mm of two-way ultrasound propagation or 1.7 cm imaging depth – which is perfect for our application. During ultrasound fast scanning, this technique produces some small ambiguity as to where along a lateral scan each ultrasound pulse will occur. However, for 15-Hz scanning rate over 8-mm scanning distance, we expect a position uncertainty of plus or minus ~10 μm, which is much less than the ~200μm focal width of our 25-MHz ultrasound transducer. This position uncertainty was computed as $(22.7\mu s/2)v_{max}$, where $v_{\max} = 2\pi(15Hz)(0.01m) = 0.94m/s$.

A PCI data acquisition card (CS22G8, Gage Cobra, Gage Applied Systems, Inc.) was used to acquire both the amplified scan data and the trigger- or photodiode signal. Recording the photodiode signal affords the opportunity to normalize the recorded photoacoustic data by the laser pulse energy during post-processing to normalize for laser pulse-to-pulse energy fluctuations. The Gage card provides 8-bit sampling rates as high as 2GSamples/s, and can stream data to the PC RAM at rates exceeding 200 MSamples/s – more than adequate for realtime visualization of the acquired images. To see this,

consider 1024x256 pixel images to be displayed at 30 frames per second. This corresponds to a required transfer rate of 7.9MSamples/second - thus utilizing only 4% of the possible data transfer rate. The unused data transfer bandwidth may prove invaluable for Doppler ultrasound studies where multiple pulses per A-scan line are required. A diagram of our system is shown in Fig. 3.

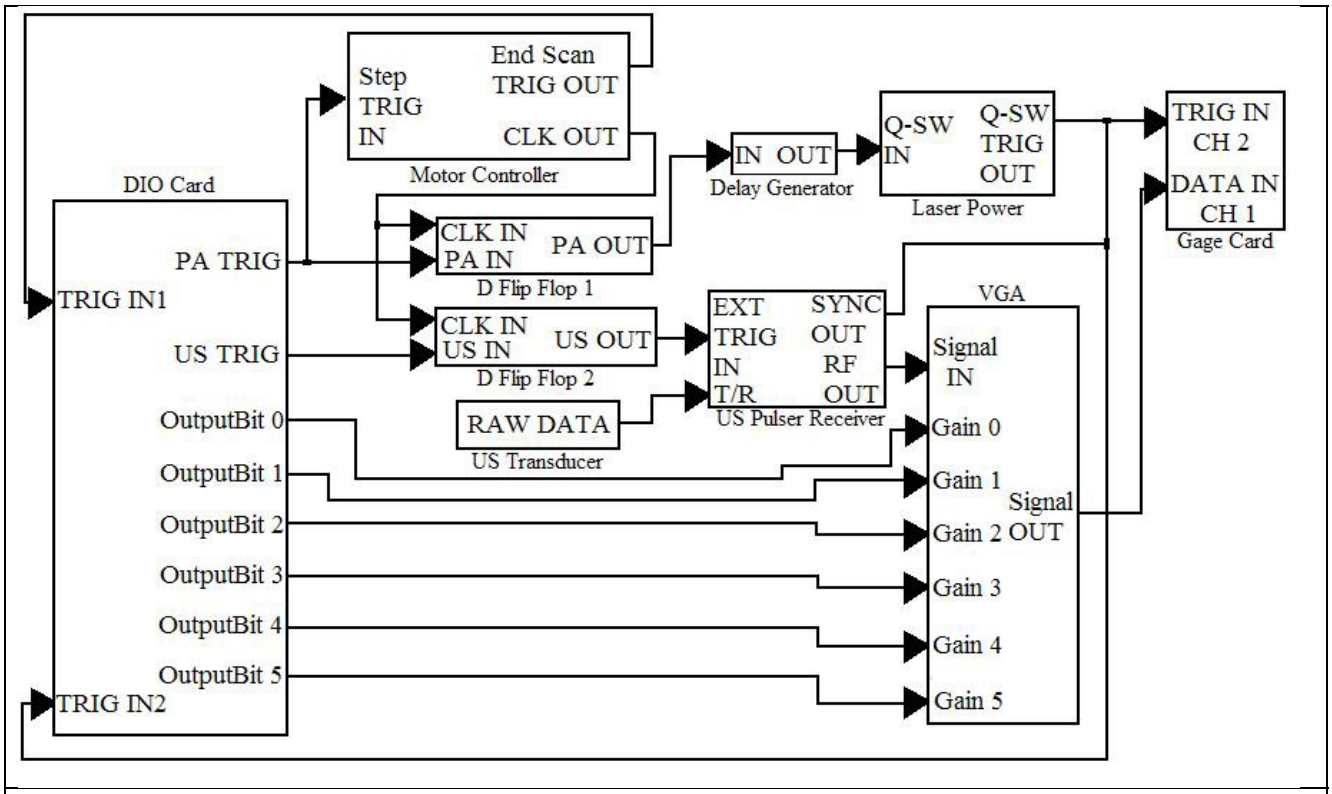


Fig. 3. System block diagram.

B. Probe Design

To deliver light in the photoacoustic mode, we tried two different designs. In the first design, we use a variation of the dark-field illumination approach first described by Maslov et al [1]. Rather than use a conical lens, we use a reflective cone to divert downward-incident light to a 45° polished surface of an acrylic tube holding the ultrasound transducer. Another polished face, machined at an angle to deflect light confocally around the transducer focal axis, was created at the bottom of this tube.

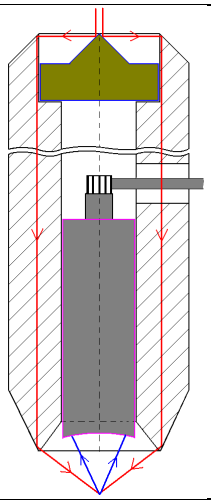

Fig. 4. Diagram illustrating our light-delivery design system based on a reflective cone.

In the second design, somewhat similar to the light-delivery system described by Maslov et al, [14], our ultrasound transducer faces a downfacing optical prism such that photoacoustic signals directed upwards to the prism's diagonal will be deflected to the transducer. Optical index-matching fluid is used as ultrasonic coupling, and allows for top-down laser illumination to be directed to the tissue surface without optical refractive path variation. Further details about this probe is given in another paper presented in this proceedings.

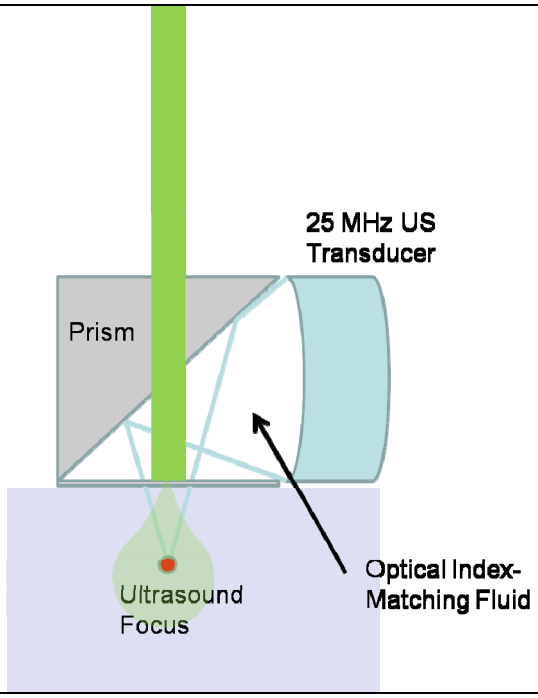


Fig. 5. Light-delivery probe based on an optical prism and optical-index-matching fluid.

### 2.1 Imaging Results

We used our fast scanning system to acquire images of a human finger immersed in a water bath. Movies of the finger were acquired at 20 frames per second and visualized offline for now. A representative image frame is shown in Fig. 6. Images were formed by performing standard envelope detection, followed by median filtering, then intensity-scaling.

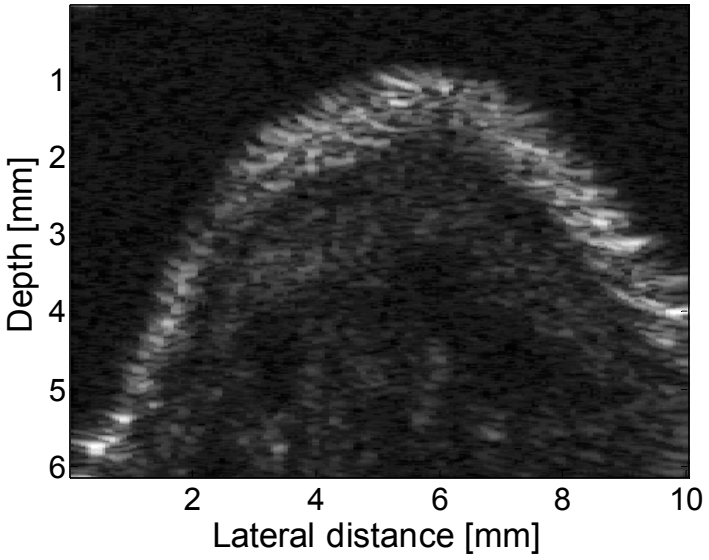


Fig. 6. A high-frequency ultrasound image of a human finger acquired using our system. Penetration to depths greater than 5-mm is demonstrated.

Photoacoustic imaging was performed separately for now on a phantom consisting of a human hair mounted across an acrylic holder. Photoacoustic images of this hair were acquired for multiple hair depths using the light-delivery mechanism shown in Fig. 5. The images of the different hair positions were then combined in a composite image, shown in Fig. 7.

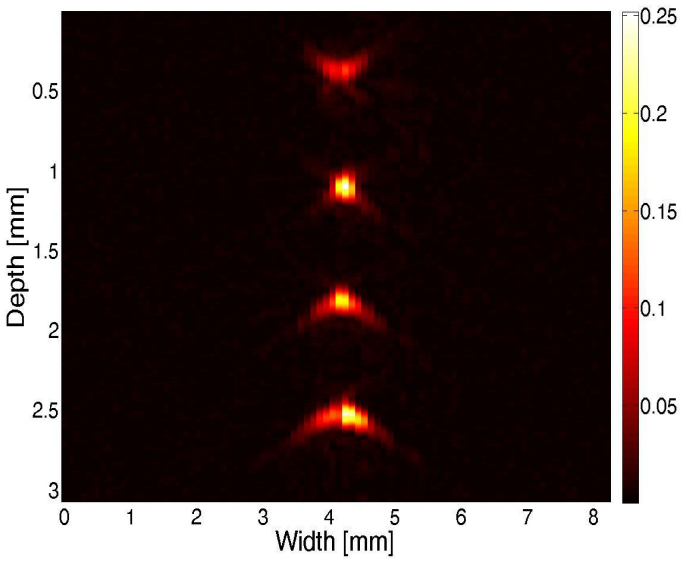


Fig. 7. Photoacoustic point-spread functions acquired using our system by scanning a human hair in water.

## 3. SUMMARY AND CONCLUSIONS

We have developed a combined ultrasound-photoacoustic imaging system which allows for up to 30-frames-per-second ultrasound imaging rates with slower photoacoustic B-scan rates limited by the pulse-repetition rate of the laser. Future work will focus on further combining ultrasonic and photoacoustic modes by interlacing laser and ultrasound pulse sequences, and overlaying the photoacoustic image on the B-mode image. In vivo photoacoustic imaging will be pursued. Additionally, we will work to develop color and power Doppler, and synergistic ultrasound-photoacoustic signal processing strategies. The instrument should be valuable for preclinical imaging of small animals and perhaps clinical imaging applications in depth-limited pathologies. Additionally, the system should afford unprecedented opportunities for molecular and functional imaging in vivo. With the ability to perform both high-frequency ultrasound and photoacoustic microscopy simultaneously with the same imaging instrument, we hope that photoacoustic imaging will become easy to use for biologists and pre-clinical researchers.

## 4. ACKNOWLEDGEMENTS

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