High-Resolution Ultrasonic Imaging of Blood Flow in the Anterior Segment of the Eye

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PURPOSE. To develop a noninvasive technique to visualize and measure blood flow in the iris and ciliary body.

METHODS. Echo data from 50-MHz ultrasound scans of the iris and ciliary body of rabbits were digitized using a new “swept scan” modality. The method makes use of spatial oversampling to identify regions with scatterers whose range changes with time. The data allowed construction of high-resolution B-mode images with embedded flow information. Pulsatility over the cardiac cycle was evaluated by sending a series of pulses along a single line of sight containing a vessel of interest. Local blood flow and changes over the cardiac cycle before and after application of atropine were quantified.

RESULTS. Flow was identified in the radial vessels and major arterial circle of the iris. Vessels with lumens as small as 40 μm in diameter and flow velocities as low as 0.6 mm/sec were measured. Change in blood velocity over the cardiac cycle was determined to be approximately 27%. Peak systolic velocity after administration of topical atropine increased by 72%.

CONCLUSIONS. This technique allowed visualization of flow using the same type of very-high-frequency transducer now widely used for imaging the anterior segment. The technique can also be used at lower frequencies for more posterior tissues with similar improvement of resolution over Doppler. The ability to examine flow in the anterior segment of the eye offers a new tool for study of glaucoma, hypotony, tumors, and other disorders. (Invest Ophthalmol Vis Sci. 1999;40:1373-1381)

The measurement of blood flow in optically opaque tissues can be accomplished noninvasively using Doppler ultrasonography. The Doppler effect,1 the change in pitch observed when a sound source moves toward or away from a receiver, has been understood since the 19th century. Its first clinical application, ultrasonic measurement of motion in the major vessels of the heart, was described in 1957.2 Doppler ultrasound evaluation of the vessels of the eye and orbit was first reported in 19893 and has subsequently been used to evaluate circulation in the normal eye,4 vascular abnormalities,5,6 glaucoma,7,8 diabetic retinopathy,9 and tumors.10,11

Color-flow Doppler systems provide a high-resolution gray-scale B-mode image on which lower resolution color-coded flow information is superimposed, with red and blue usually signifying flow toward and away from the transducer, respectively. For typical color-flow Doppler instruments operating at 5 MHz to 10 MHz, the minimum diameter vessel that can be resolved is on the order of hundreds of micrometers, with a minimum detectable flow velocity of approximately 1 cm/sec. Thus, conventional instrumentation does not permit visualization or measurement of flow in the small arterioles and venules of the iris and ciliary body.

Spatial resolution in ultrasound systems is directly determined by the frequency (or wavelength) of the system. The central challenge for the detection of low-velocity flow (<1 cm/sec) is the discrimination of flowing blood from tissue motion: The latter may well be on the order of 1 cm/sec. Because tissue motion is uniform over a spatial region that is large in relation to the resolution of the system, this artificial motion can be removed, allowing estimation of low-velocity blood flow in small vessels. Higher frequencies improve resolution and produce a greater (and more easily detected) Doppler frequency shift for a given flow rate.12 Also, at higher frequencies, blood cells are more effective scatterers, improving the signal-to-noise ratio for small vessels. Because of the rapid attenuation of higher frequencies, however, they are necessarily limited to examination of peripheral tissues, such as the skin and anterior segment of the eye.

Very-high-frequency (VHF) ultrasound systems using 50-MHz transducers for B-mode imaging of the anterior segment of the eye were developed in the early 1990s independently by Coleman13-19 and Pavlin20-25 and their respective coworkers. An initial description of methods for high-frequency flow estimation was provided by Ferrara et al.26 Recently, Christopher et al.12,27 described the use of Doppler techniques in this frequency range. However, the application of VHF Doppler...
Acquisition System

The imaging system comprises the data acquisition and display systems, plus the scan assembly, consisting of the transducer and motion systems. The polyvinylidene fluoride transducer has a nominal center frequency of 50 MHz, a 6-mm aperture, and a 12-mm focal length. The observed output spectrum peaks at 58 MHz, with a $-15$ dB bandwidth extending from 10 MHz to 60 MHz. The scan assembly produces motion of the transducer so that its focal point describes an arc with a radius that can be adjusted from 8 mm and 13 mm, which are optimal for scanning the anterior of the eye. A second axis of motion permits acquisition of sequential scans that are offset in a meridional fashion by fixed angular increments. Motion is actuated by DC servomotors, with an encoder that provides a resolution of approximately $0.003^\circ$ of arc. The acquisition system consists of a conventional computer with a 233-MHz Pentium II processor (Intel, Mountain View, CA) and 64 megabytes of random access memory. A broadband pulser/receiver with a remote pulser provides excitation pulses of 1 $\mu$s to the transducer. A digitizer allows acquisition sampling rates of up to 1000 MHz, with 8 bits of resolution. The acquisition program allows control of digitizer parameters (sample rate, delay, samples per vector, and vectors per plane), pulser/receiver parameters (gain and attenuation), and motion parameters (arc angle and angular increment between planes). During scanning, data are presented as images on the computer’s display.

In acquisition of conventional B-mode images, pulses are emitted, and echo data are digitized at equal angular increments so that adjacent vectors do not exceed independence—that is, they are offset from one another by no more than the lateral resolution of the transducer in its focal plane. Placing vectors closer may improve the signal-to-noise ratio but provides no improvement in lateral resolution. A B-mode image can thus be considered to be a plot of echo amplitude as a function of range (on the $y$-axis) versus lateral position (on the $x$-axis). In an M-mode image, a sequence of vectors is acquired at a fixed pulse-repetition frequency along a single line of sight. An M-mode image can therefore be considered to be a plot of echo amplitude as a function of range (on the $y$-axis) versus time (on the $x$-axis). M-mode images of flow in detected vessels are particularly useful for evaluation of pulsatility over the cardiac cycle.

The swept-scan method involves a simple modification of the standard B-mode acquisition process that provides a conventional B-scan and flow data, simultaneously. This requires acquisition of scan vectors at spatial intervals much smaller than the lateral resolution of the transducer at a constant pulse-repetition frequency (Fig. 1). If a “resolution cell” is defined to be any group of successive vectors that are within a lateral beam-width of each other, then all vectors within such a cell are highly correlated and may be considered to be interrogating the “same” spatial position. The resultant image represents echo amplitude as a function of range (on the $y$-axis) versus lateral position (on the $x$-axis), exactly as in a conventional B-mode image. However, because groups of adjacent vectors within the lateral beam width of the transducer are obtained from an overlapping region in space, the local $x$-axis can be considered to represent time instead of space (as in an M-scan). Should blood flow exist along a particular line of sight, it appears as an oblique echo trace (range changing with time) within the corresponding resolution cell, whereas stationary structures produce a horizontal trace (range is constant). The axial speed of the flow can easily be determined from the slope of the oblique line (because the local $x$- and $y$-axes represent time and axial position, respectively). Data acquired using this technique yield a conventional B-scan with embedded time-domain information. Flow rate estimates can be refined by tracing the spatial orientation of a blood vessel in a series of spatially independent resolution cells. By doing so, we can determine the angular orientation of the vessel presented to the transducer and correct for it by using a cosine term.

Signal Processing

In human subjects, small saccadic eye movements during scanning are inevitable. The axial component of such movements causes a shift in range of all structures along the given line of
FIGURE 1. Top: schematic representations of scan modes that can provide flow information. In M-mode, many vectors are acquired along a single line of sight. In the discrete scan mode, many vectors are acquired at each of a series of independent positions. In swept-scan mode, the transducer is moved continuously while closely spaced overlapping vectors are acquired. Bottom left: schematic representation of moving blood cell in vessel. In the swept scan, the transducer is moved at a constant speed and ultrasound vectors (vertical lines) are acquired at constant time intervals. In this case, all measurements are within a lateral beam width (dashed lines) of one another. Dashed lines represent the beam profile when centered around the central vector (bold line) in the schematic. Bottom right: because all vectors within a beam width can be treated as interrogating the same position, spatial content can be disregarded and the data plotted as an M-scan. In the M-scan, stationary structures (e.g., blood vessel walls) appear as horizontal lines because changes in range within one beam width are not discernible. However, motion occurring over time, as is generated by the moving blood cell, would be detectable, producing a diagonal trace on the M-scan. The slope of this diagonal defines the axial flow rate.

sight. To limit the effect of such motion, the new strategies detailed in this article allow acquisition of a frame of data in 1 to 2 seconds. To improve vascular maps further, flow-induced motion is separated from physiological motion by realigning received signals between adjacent vectors before the estimation of blood velocity. Two correlation-based alignment strategies have been evaluated and their performance described. In this report we showed how such strategies can compensate for tissue motion in scans of human subjects. Once vectors are aligned using the strong and spatially uniform tissue echoes, these are removed to allow detection of blood flow. A high-pass (wall) filter is used, removing slowly varying tissue echoes without removing the higher frequency blood echoes. An especially useful aspect of this technique relates to the continuously overlapping resolution cells. This facilitates the use of a wall filter to remove clutter (stationary structures) because the filter can be applied in a similarly continuous manner. Because of our overlapping resolution cells, the wall filter continuously processes hundreds, rather than tens, of pulses. The result is that tissue echoes are removed far more effectively than in a conventional Doppler system. This novel result is of particular importance for the detection of very low flow velocities.

The velocity of blood flow can then be determined with the traditional estimation technique known as the autocorrelator, first reported by Kasai et al. With this technique, velocity resolution is limited, because velocity is estimated based on the change in phase of successive echoes. With the combination of alignment algorithms, wall filter, and autocorrelator, axial ve-
FIGURE 2. Top: B-mode image of angle in rabbit eye derived from 1024 consecutive vectors spaced 2.2 μm apart at a rate of 500/sec. In this case, only one of eight vectors was used to construct the image. This is similar to conventional high-frequency image reconstruction. The iris is seen in cross section from the top to the bottom of the image, with the angle near the top of the image. Corneal echoes are not evident (because of oblique angle to ultrasound beam). Bottom: same image as shown above reconstructed from all 1024 vectors. In this case, flow in the major arterial circle (large arrow), not seen in the conventional image, is evident because of the improvement in temporal resolution. Possible radial vessels (small arrows) are also seen. Inset: magnified representation of major arterial circle in which positive and negative phase are indicated. The slope of the phase shift, indicated by the arrow, shows a maximum uncorrected flow rate of 3.8 mm/sec.

Examination of Rabbit Anterior Segment

Animal experimentation adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

We tested the technique described earlier by scanning the anterior segment of Dutchbelt rabbit eyes. Rabbits received general anesthesia (35 mg/kg ketamine HCl plus 5 mg/kg xylazine) and the proptosed eye was coupled to the transducer with a sterile normal saline water bath. The transducer was placed to position the area of interest in its focal zone. Scans consisting of 1024 vectors were acquired at a pulse-repetition frequency of 500 Hz over an arc of 12° with a radius of curvature of 11 mm. A sample rate of 250 MHz was used, and 512 samples were acquired for each vector. This arrangement produced vectors 2.2 μm apart, and groups of approximately 30 vectors in each (overlapping) lateral resolution cell. These parameters produced scan planes 2.25 mm wide by 1.5 mm deep in 2 seconds. Consecutive planes were acquired at 0.5° intervals, with the center of each plane displaced 20° from the apex. Adjacent radially offset scan planes had a minimum separation of 35 μm and a maximum separation of 50 μm.

Scans were performed as described immediately before topical administration of 2 drops of 1% atropine sulfate. After vessels were identified, M-mode acquisition (pulse-repetition frequency, 500 Hz) was used to evaluate cardiac cycle pulsatility.
FIGURE 3. Color-flow image showing a major arterial circle near iris root and smaller vessels in the iris. Ciliary processes are seen posterior to iris plane. The angle-corrected flow reaches a maximum of 1 cm/sec.

RESULTS
Figure 2 is a B-mode representation of a single-scan plane of the angle. In this image, we have determined the signal envelope by taking the absolute value of the echo data and averaging. The top image is reconstructed from every eighth vector—that is, with vectors spaced approximately 18 \( \mu \)m apart, or approximately four per lateral resolution cell. Immediately below is an image reconstructed from all 1024 vectors spaced 2.2 \( \mu \)m apart, or approximately 30 vectors per resolution cell. Comparison shows no improvement in spatial resolution by oversampling, as would be expected. However, in the lower highly oversampled image, a circular region measuring approximately 200 \( \mu \)m diameter near

FIGURE 4. Color-flow image showing radial iris vessel. Intermittent appearance of flow may be because of the vessel moving in and out of scan plane and/or pulsatile flow during the 2-second acquisition time. Prominent ciliary processes are seen posterior to the iris.
the iris root shows a speckle pattern, the slope of which is steep relative to its surround. This is consistent with blood flow. A magnified version of this region presenting positive- and negative-phase information is also provided at the bottom of the figure. This allows us to track more readily the slope of the flow-induced phase deviation relative to stationary structures. In this case, an angle uncorrected flow velocity of approximately 3.8 mm/sec was determined.

Figures 3 and 4 are examples of color-flow images derived from signal processing of echo data. In Figure 3, we observe color-coded flow in the major arterial circle. In Figure 4, color flow is evident in a radial vessel in the iris stroma that apparently weaves in and out of the scan plane. Note, however, that the intermittent appearance of the vessel could have been caused by changes in flow rate and echogenicity associated with the cardiac cycle, because the 2-second acquisition time encompassed several cycles. Both these factors affect vessel continuity in ultrasound flow images.

To evaluate the feasibility of this technique and to provide initial estimates of reliability and sensitivity, sequential sets of flow maps were acquired from a small series of rabbits before and after the application of topical atropine. A substantial
variation in flow velocity was recorded between individual rabbits within the series, although repeated evaluation of the same rabbit produced nearly identical results. Thus, we report velocities recorded from individual rabbits.

Evaluating the major arterial circle, we calculated the maximum flow velocity and volume flow within the vessel. An evaluation of the changes in velocity and flow rate was conducted in M-mode and is reported in this section. From a 2-D image (such as in Fig. 3), a single line of sight within the major arterial circle was interrogated, producing the M-mode image shown in Figure 5 (left). The signal-processing techniques described in the previous section were then applied to estimate blood velocity within this region. Resultant maps of blood velocity before and after the application of atropine are shown in Figure 5 (center and right). Note that the effect of cardiac pulsatility is evident in this figure because of the periodic variation in velocity magnitude shown in the displayed color image.

We conducted these preliminary experiments to evaluate the ability of the technique to monitor changes in blood velocity over the cardiac cycle and evaluate the effects of vasoactive drugs. It is important to quantify changes occurring over the cardiac cycle to assess the sources of variance in 2-D and 3-D maps of blood flow. From data collected over a sequence of seven cardiac cycles, the angle-corrected spatial and temporal peak velocities in the major arterial circle were found to be $4.57 \pm 0.54$ mm/sec (SD) and $3.32 \pm 0.15$ mm/sec during systole and diastole, respectively. Thus, the variation in peak velocity estimated over the cardiac cycle was 27% of peak systolic velocity.

After the application of atropine, the angle-corrected spatial and temporal (systolic) peak velocity in the major arterial circle was $7.88 \pm 0.49$ mm/sec. The spatial peak velocity during diastole was $4.85 \pm 0.44$ mm/sec. Thus, the variation in velocity estimated over the cardiac cycle after the application of atropine was 38% of the peak systolic velocity. The 72% increase in peak systolic velocity and 46% increase in diastolic velocity after atropine administration were clearly detectable with this technique ($P < 0.01$). Thus, single-line-of-sight M-mode evaluation provided a tool to evaluate pulsatile flow within small blood vessels and the effect of vasoactive therapies.

Next, the variation of maximum velocity ($V_{\text{max}}$) and volume flow in a single 2-D frame was estimated. To determine the variance in velocity estimates, 2-D frames from a single spatial region were acquired before and after application of topical atropine. Because each 2-D frame was acquired in approximately two seconds, individual frames contained echoes acquired from cardiac systole and diastole. The resultant variation in peak velocity between successive 2-D frames was 34% of its peak value and therefore was of magnitude similar to the fluctuations over the cardiac cycle recorded in M-mode, as described earlier. The vessel area could also be estimated from the 2-D view to produce a volume flow rate. The estimated volume flow rate based on the average velocity for this vessel was $0.13 \pm 0.04$ μL/sec.

After the application of atropine, the spatial $V_{\text{max}}$ was recorded and averaged over a sequence of six frames. The increase in spatial $V_{\text{max}}$ produced by atropine in the 2-D frames was 54%, between the estimates of 72% for systole and 46% for diastole made in M-mode. This is expected because each 2-D frame contains echoes acquired during systole and diastole.

**DISCUSSION**

This new approach relies on several changes from existing flow-measurement systems and signal processing strategies. The center frequency of the transducer is far higher than conventional systems, providing a significant improvement in spatial resolution. Acquisition of a 2-D or 3-D survey of blood flow is made in a "swept mode" in which the transducer is continually moved across the region of interest. This greatly speeds data acquisition and provides the opportunity to use new signal-processing strategies. These strategies include signal alignment to remove physiological motion and a new form of wall filter that eliminates the echoes from tissue. Velocity estimation can then be based on Doppler estimators or time-domain correlation algorithms. The data are well suited for generation of conventional high resolution B-mode images and for simultaneous detection, quantification, and depiction of flow. Small motions of the eye can be compensated for by correlation and alignment of adjacent vectors. The method can be implemented using either mechanically scanned single-element transducers or linear arrays.

Current Doppler instrumentation, operating at 10 MHz or less, allows visualization of flow primarily in large vessels, such as the central retinal artery. Several techniques have been considered for increasing the sensitivity of Doppler ultrasound systems for detection of slow flow in smaller vessels. One now widely used technique is power, or amplitude, Doppler. This technique increases detection and circumvents the angular dependence of color-flow Doppler, but information related to the direction and velocities of flow are lost. Power Doppler is also sensitive to tissue motion artifacts.

Another approach to increasing sensitivity is the use of ultrasound contrast agents. These enhance the acoustic scattering of blood, improving detection of slow flow. Further enhancement can be obtained using "harmonic" imaging of contrast agents. This takes advantage of the resonance of microbubbles at the second harmonic of the ultrasound frequency to improve signal-to-noise ratios for contrast agents suspended in flowing blood versus stationary structures. Disadvantages of contrast agents include their invasive nature, the

In a separate experiment, we produced a 3-D volume-rendered model from a group of 64 sequential scan planes. In Figure 6 (left), after reducing the opacity of stationary structures, color-coded flow in the major arterial circle is evident, with a peak velocity of 1.6 cm/sec. A small branch from this circle can be visualized entering the ciliary body. Visualization of flow and vessel diameter in the small branches from the major arterial circle is particularly important for the assessment of vasoactive drugs used to treat glaucoma. A 2-D slice from this 3-D volume is then presented in Figure 6 (right) to aid in visualization of the anatomy. These 3-D reconstructions of the vascular architecture provide the opportunity to determine the angle between the ultrasound beam and each vessel, allowing computation of angle-corrected flow velocity. Anatomy, however, is more easily recognized in 2-D planes that can be derived from the 3-D data.
requirement of pulmonary passage (to allow intravenous injection), potential toxicity, and their brief span of action. In addition, it becomes difficult to resolve individual vessels after application of a contrast agent. Therefore, addition of a contrast agent may not facilitate studies of blood velocity and volume flow within individual microvessels.

An alternative to Doppler measurement of blood flow is the use of time-domain speckle tracking.\(^{38-41}\) In B-mode ultrasonic imaging systems, the display is refreshed periodically at the system frame rate. If the frame rate is sufficiently high, flowing scatterers can be tracked from frame to frame, allowing visualization and measurement of flow. The advantage of this approach over Doppler is that it can be implemented without sacrifice of spatial resolution (using a highly damped pulse). Disadvantages include the requirement of a sufficiently high frame rate, reduced sensitivity, and the complexity of extracting flow information from rapidly received successive frames, especially under conditions in which tissue movement can occur. Achieving high frame-rates and compensating for tissue motion are especially onerous problems for VHF imaging of the eye.

Although time-domain measurement of blood flow has advantages over Doppler, it remains a curious fact that Doppler is by far the dominant acoustic flow measurement technique.\(^{42}\) This situation exists partly because of the high computational costs of cross correlating successive image frames. The method proposed in this report, while retaining the advantages of time-domain techniques, requires far less computational power than does frame-to-frame correlation. Because correlations are between successive vectors within each frame, motion artifacts are also reduced. The continuous nature of the acquisition process and image structure, mixing spatial and temporal dimensions at different scales, make both the acquisition process and wall filtering efficient. In addition, the method’s sensitivity to flow may be enhanced by use of contrast agents.

We showed that, at least in anesthetized experimental animals in which eye motion is insignificant, it is feasible to localize a vessel with swept-mode scanning, direct the line of sight to the vessel, and then use a pure M-scan to interrogate pulsatility. Peak systolic and minimal diastolic velocities can then be reliably estimated. Using this method, we observed a 27% change in velocity over the cardiac cycle and a 72% change in peak velocity after atropine administration.

A number of clinical applications suggest themselves, including evaluation of diabetic neovascularization of the iris, iris and ciliary body blood flow in glaucoma and hypotony, and evaluation of tumors of the iris and ciliary body. The latter is of particular interest, given recent evidence linking microvascular patterns with survival risk in uveal melanoma.\(^{43-45}\) The technique is by no means limited to frequencies in the 50 MHz range and can easily be used with either conventional (10 MHz) or intermediate frequencies, albeit with lower resolution, to assess blood flow in more posterior structures of the eye and orbit. The relatively high resolution and low energy emission of this technique may offer advantages over Doppler-based techniques even in these anatomic locations.

Our new techniques allowed evaluation of the effects of glaucoma treatments on ocular blood flow. Current drug therapies include \(\alpha\)-agonists, \(\beta\)-adrenergic receptor blocking agents, and prostaglandin-based agents.\(^{46-50}\) The effects of these drugs on blood flow within the eye are expected to vary greatly, but assessment of their impact has been difficult because of the lack of imaging tools with sufficient spatial and velocity resolution. Development of the capability to assess flow quantitatively in each region of the eye could have significant impact on current clinical management and on the integration of the many new glaucoma drugs under development. The only current method for evaluation of changes in ciliary body arterioles is microvascular corrosion casting. Using this difficult method, it has been shown\(^{51}\) that timolol produces a 20% constriction in ciliary body arterioles, and betaxolol produces a 30% constriction, although changes in blood velocity and volume flow clearly could not be assessed.

In this report we have described a new ultrasound methodology for measurement and imaging of blood flow. When implemented with VHF transducers as described here, the technique allows assessment of the microvasculature of the anterior segment. The ability to assess changes in blood flow will be helpful in elucidating disease and treatment mechanisms in glaucoma and other conditions affecting anterior segment hemodynamics.

References


