OPHTHALMIC ULTRASOUND
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In 1956, Mundt and Hughes used an A-scan on an intraocular tumor, demonstrating the possible use of ultrasound in ophthalmology. Soon a body of research developed on the sound velocities of various tissues and compartments of the eye, and in the 1960s, ultrasound began to be used to measure distances in the eye. B-scan instruments developed in the 1970s, were originally used through closed eyelids. Ossoinig in the 1960s developed the standardized A-scan, which is used to differentiate tissues, particularly tumors. In the 1990s, high frequency ultrasound developed, which greatly improved the resolution and examination of anterior segment structures. Recent developments include the digitization of ultrasound so that three-dimensional imaging is possible.

Principles of ultrasound:

Ultrasound is an acoustic wave consisting of an oscillation of particles within a medium. Ultrasound waves have frequencies greater than 20 KHz (> 20,000 oscillations/second), and are inaudible to humans. In ophthalmic ultrasound, frequencies are typically between 8 MHz to 50 MHz (with higher frequencies in development). Higher frequency results in higher resolution, but at the price of tissue penetration, as shorter, more frequent wavelengths cannot penetrate tissue as far as longer wavelengths. Most standard B-scan probes for globe imaging are 10 MHz, which can penetrate about 40 mm. High resolution probes are generally around 20 MHz and penetrate 10 mm. Ultrasound biomicroscopy is usually considered to be 50 MHz or higher, but tissue penetration is only 7 mm.

An A-scan is a one-dimensional display where echoes are depicted as spikes arising from baseline. A thin, parallel sound beam passing through a small point in the eye forms an A-scan. The greater the difference between interfaces, the higher the spike will be, hence the name Amplitude-scan, or time amplitude display. A B-scan is essentially an oscillating A-scan that passes through a cross-section of tissue. The echoes in a B-scan are represented as dots rather than spikes. The configuration of the dots and the brightness of the dots creates the image. The greater the difference between interfaces or the stronger the echo, the brighter the dot will be, hence the name Brightness scan, or brightness intensity-modulated display.

Sounds waves behave essentially like light rays, and so many of the same principles of refraction and reflection apply. Just like light rays, the longitudinal sound ray can be reflected back towards its source when it strikes a tissue interface. This reflected wave is called an echo. Similar to refractive index, acoustic impedance between
two media influences the production of an echo. The greater the difference in acoustic impedance between two media, the stronger the reflected sound wave will be. The acoustic impedance is determined by sound velocity x density. For example, the difference between the anterior lens surface and the aqueous is much greater than the difference between hypopyon and the lens, so the corresponding echo would be shorter in an eye with hypopyon. Other factors that affect the formation of the echo are absorption and refraction, the angle of sound incidence, and the size, shape and texture of the acoustic interfaces.

The angle at which the sound beam strikes an interface influences the strength and formation of the echo. The angle of incidence is equal to the angle of reflection, so that when a beam strikes an interface perpendicularly, the echo is reflected directly back toward the direction of origination (i.e., the probe). Sound waves striking at an oblique fashion result in reflected sound waves diverted away from the direction of origin, resulting in a weaker echo. When doing a clinical exam, brighter structures are often perpendicular to the sound beam.

Size, shape, and texture of an interface also determine the character of the echo. A smooth surface, if the sound wave strikes perpendicularly, will reflect nearly all the wave back to its source. An ocular example of this is the retina. A smooth but convex surface will reflect some of the sound wave away from its source, so the echo will be weaker. An ocular example of this is an intraocular tumor. An irregular surface will result in scattering of the echo, which means that the returning echo will be weaker, even if the original sound wave was perpendicular. A very small interface will also produce pronounced scatter. Therefore, echoes from small or irregular surfaces are less dependent on the direction of the sound wave than echoes from larger smoother surfaces, so perpendicularity may be less crucial.

Ultrasound energy is absorbed by the tissues it passes through, and is ultimately converted to heat. The generated heat is very low and has no detrimental effects on ocular tissues. Absorption is related to frequency, sound velocity, and thickness of the tissue through which it passes. Higher frequencies are absorbed to a greater degree, hence the decreased penetration. Higher sound velocities and greater tissue thickness result in greater absorption of the sound waves. An example of absorption is the ‘shadowing’ that occurs behind calcified tissue or a metallic foreign body.

Sound waves can also be refracted due to differing sound velocities between two media. When the sound wave is directed obliquely toward an interface that divides two media with different sound velocity, the sound wave will be refracted. An oblique sound beam directed through a tissue of higher sound velocity to a tissue with lower sound velocity will result in the transmitted sound wave being refracted toward perpendicular. Refraction can occur inside the eye, which can actually help bend the sound wave so that the transmitted beam strikes another structure more perpendicularly, such as an extraocular muscle.
To create ultrasound images, an instrument must have a pulser, a transducer, a receiver, and a display screen. An ultrasound probe consists of a piezoelectric element, which converts mechanical energy to electrical energy and visa versa. The piezoelectric crystal is electrically stimulated, causing a mechanical vibration. The vibration leads to the release of a longitudinal sound wave that passes through the tissue. After each vibration, a pause of several microseconds occurs so that the transducer can receive returning echoes. The returning echo causes the piezoelectric crystal to vibrate, which then produces an electrical signal that is transmitted to the receiver and display screen. This pulse-echo process is repeated a thousand or more times per second to produce a ‘real-time’ display.

Damping material is attached to the back of the crystal, which limits the vibrations of the crystal. Damping limits the vibrations of the crystal, which shortens the pulse. Frequency and pulse length both relate directly to axial resolution, which is the minimum distance between two interfaces along the direction of the sound wave that can be displayed. Shorter pulses lead to better axial resolution.

Sound beams have near fields and far fields. Sound beam diameter decreases slightly with increasing distance from the transducer in the near field. The far field is located beyond the near field, and sound beam diameter increases with increasing distance from the transducer. Therefore, resolution is greatest when the source of the echo is within the near field. Near field length depends on both the diameter and frequency of the transducer crystal. Larger diameter and higher frequency transducers with have a longer near field.

Crystal shape also determines characteristics of the sound beam. Planar crystals produce parallel sound beams while concave crystals can produce a focused sound beam. The focal distance corresponds to the area where the sound beam is the narrowest, and this is the area of greatest resolution. A focused beam increases both the lateral and axial resolution of an ultrasound. Lateral resolution refers to the minimum separation between two echo sources perpendicular to the direction of the sound wave.

Returning echoes produce an electrical signal that is received by the ultrasound as a weak radio frequency signal. This signal undergoes processing that can include amplification, compensation, demodulation, and rejection. Amplification is most important in ophthalmic ultrasound instruments. Three types of amplification are used; they are linear, logarithmic or S-shaped. Amplification type affects the range of echo intensities that can be displayed by the system, which is termed the dynamic range and described in decibels. A linear amplifier has as small dynamic range, so it can display minor differences in strength between echo sources, but the range of echo intensities is very limited. Logarithmic amplifiers display a wide range of intensities, but cannot distinguish slight differences between echo signals. The S-shaped curve is used to combine the wide range of the logarithmic with the greater sensitivity of the linear amplifier. This is used specifically in diagnostic A-scans, which are not commonly used in veterinary medicine.
Gain is another important factor in ultrasound display. Gain is essentially turning up the volume, or increasing the amplification of the displayed signal. Gain is also measured in decibels (db). Decibels are relative units of ultrasound intensity. Adjusting the gain does not alter the amount of energy coming from the transducer; rather, it only changes the intensity of the returning echo that is displayed on the screen. Increasing gain increases sensitivity of the screen and allows for display of weaker signals, but decreases resolution. Increasing the gain allows the display of weaker signals like vitreous opacities. As the gain is lowered, the sensitivity decreases, but the resolution increases both axially and laterally. Weak vitreal opacities will go away, but stronger echoes (retina, sclera, masses) will remain. Lowering gain effectively narrows the sound beam, because the strongest echoes are in the central axis of the returning sound wave. Since weaker echoes from deeper tissue are not amplified enough to be displayed, lowering gain also effectively decreases the depth of penetration. Many instruments have an automatic time gain compensation, which enhances weak echoes displayed from deeper tissues. This allows greater amplification of distant, weaker tissues compared to closer, stronger echoes. This equalizes echoes from similar tissues located at varying distances from the transducer.

**Instruments:**

A-Scan: A-scans are one-dimensional displays in which returning echoes are reflected as vertical spikes from a baseline. Spacing between the spikes depends on the time it takes for the sound beam to reach the interface and for the echo to return to the transducer. The time between spikes can be converted to distance by knowing the sound velocity of the medium through which the echoes are traveling, which is expressed through the formula:

\[
\text{Distance} = \text{velocity} \times \text{time}
\]

Height of the spikes indicates the strength, or amplitude, of the echoes.

There are different types of A-scans in ophthalmology. A-scans can be used for axial eye length measurements. This is known as biometry, and typically uses linear amplification, a focused transducer and a frequency between 10 and 15 MHz. A vector A-scan is used simultaneously with B-scan echograms and has similar characteristics to the B-scan, usually logarithmic amplification, a focused transducer, and a frequency of 10 MHz.

The standardized A-scan was developed by Ossoinig to enhance tissue differentiation. Standardized A-scans use S-shaped amplification, a nonfocused 8 MHz transducer, and a parallel sound beam. Each probe/instrument combination is externally standardized using a Tissue Model, which determines the decibel setting. This decibel setting is referred to as Tissue Sensitivity, and is unique to every probe. Standardized A-scans depend on using that particular decibel level. Images are then compared to known patterns to make a diagnosis. Standardized A-scans have been used extensively in physician-based ophthalmology, and experienced users can make very definitive diagnoses of tumor and exudate type based on recognition of these patterns. These studies
have not been repeated to any great extent in veterinary medicine. One reason for this is that diagnostic A-scans are used to diagnose choroidal tumors, which are much more common in humans than in the typical veterinary patient. The anterior uveal tumors more common in veterinary medicine are harder to image with A-scans.

B-scan: The B-scan probe emits an oscillating sound beam that ‘slices’ through a tissue, producing a two-dimensional acoustic section. Most B-scans used in ophthalmology use logarithmic amplification and a focused, narrow sound beam to produce a sector image. Most B-scans cover approximately 6 clock hours in each slice. Most B-scan probes have a frequency of 10 MHz. Each echo is represented by a dot, and the brightness of the dot represents the strength of the echo. B-scans are essentially a combination of multiple A-scans. Probes have a marker that indicates the direction of the slice, and corresponds to the upper portion of the echogram.

The angle of the scanning section, the speed of transducer oscillation and the gray scale all affect the B-scan image. The angle of the transducer influences which area of the eye and orbit that can be imaged at any given time. The rate of transducer oscillation also influences the B-scan image. In order to obtain a ‘real time’ image, the B-scan must produce 10-60 ‘slices,’ or frames, per second through a tissue. Lastly, the range of gray scale that an instrument can detect plays an important role in image generation. The greater the range of gray scales that an instrument can detect, the greater its ability to distinguish differences in echo intensity.

Artifacts in ultrasonography:

Multiple signals: When sound waves reverberate between interfaces, multiple artifactual echo signals are produced. These signals must be distinguished between true echoes. External and internal multiple signals occur.

External multiple signals results from reverberations between the probe tip and an acoustic interface. Examples of objects that produce external multiple signals are the surface of the crystalline lens, an artificial intraocular lens, an air bubble, the sclera or bone (such as the orbital bone). The multiple reverberations are the result of the reflection of a sound wave of significant magnitude back to the probe. When this true echo is strong enough, a portion of it will be reflected off the probe surface and will go back to the original interface. The second wave will then produce a reverberation echo that appears distal to the true echo in the echogram. In the image, the first reverberation echo will appear twice the distance between the probe tip and the originating acoustic interface. Additional reverberation echoes may be produced by additional round trips of the sound wave. The true echo and the reverberation echoes are equidistant, and the reverberation echoes usually decrease in strength with increasing distance from the true echo. Furthermore, multiple signals move to a far greater degree than true echoes do when the probe is pressed against the tissues.

Internal multiple signals occur by reverberations within certain types of foreign bodies. They are associated with the sound beam striking a spherical foreign body, such
as a BB, gunshot pellet, or a small bubble of air or gas. Flat foreign bodies with closely spaced, parallel surface, such as slivers of glass, can also cause internal multiple signals. Internal multiple signals appear as a chain of closely spaced signals emanating from the foreign body echo, or a ‘comet tail’ artifact. It is generated by some of the energy from the sound wave being trapped in the object. The trapped energy bounces back and forth, and portions will escape and return to the probe. This results in a chain of echoes of decreasing amplitude extending from the foreign body. A comet tail artifact can be useful in identifying the type of foreign body.

Shadowing: Strong sound attenuation causes shadowing. An absence of echoes posterior to an extremely dense interface such as bone, calcium, or a large foreign body is due to complete shadowing. Partial shadowing leads to reduction of the echoes posterior to a lesion, which occurs in the case of large tumors, for example. Shadowing may make evaluation of structures behind the source of shadowing difficult, but can also aid in diagnosis. Shadowing can also occur due to refraction at the edges of a smooth curved interface rather than solely by sound attenuation. This occurs at the edge of the globe or from a cystic lesion.

Enhancement: Echoes are increased in amplitude posterior to a very weakly attenuating structure or lesion. When examined through the very low reflective vitreous cavity, the soft tissue and bone can be well demonstrated due to enhancement.

Perpendicular sound beam incidence: When the sound beam is directed perpendicularly in B-scan echograms, it can strike a very smooth highly reflective interface such as the surface of the retina, producing a bright, focal signal in the center of the echogram, particularly when a medium or low gain setting is used. To differentiate this signal from a foreign body or a localized area of calcification, the probe should be re-oriented to display the area in question at the periphery rather than the center. Re-positioning engages the area obliquely rather than perpendicularly, and should cause the bright area to disappear if it is indeed an artifact.

Baum’s bumps: Baum’s bumps appear as elevation of the peripheral fundus, but are B-scan artifacts. These artifacts are thought to be created by refraction of the sound beam as it sweeps through the peripheral aspect of the lens in an axial probe position. Re-positioning the probe peripheral to the limbus to avoid the lens should eliminate these artifacts.

**Clinical use of A-scans:**

A-scans in veterinary medicine are typically used for biometry, or measurement of ocular structures. Exams can be performed in awake, sedated or anesthetized animals. The probe can be placed directly on the cornea, or used with a scleral shell and waterbath (immersion technique). The probe should always be placed axially, and a good scan is one in which the height of the spikes from baseline are equal. Each spike should start at a perpendicular, not sloping angle from baseline. In dogs, particularly older dogs, good A-
scans can be difficult to obtain because the posterior lens capsule is very thin and seems to not provide a very good interface for acoustic reflection.

Some debate about the acoustic wave velocity in dogs exists. One study (Schiffer SP, AJVR, 1982) found a mean velocity of 1710 m/s using 2 canine lenses. Recently, Gorig et al (AJVR, 2006) used 40 canine lenses and established a mean sound wave velocity of 1707 m/s. This study also established a mean sound wave velocity in the vitreous of 1535 m/s. In people, the velocity through the crystalline lens is reported as 1641 m/s, and 1532 m/s in the vitreous. Although it may seem that a difference of a few meters/s when measuring millimeters is inconsequential, using the human values for IOL calculation using biometry measurements from a dog would result in a mean calculated IOL power of 43 diopters, rather than 41.8 diopters.

Biometry is sometimes used for determining changes in ocular distances during disease, such as increased axial length in buphthalmic globes in glaucoma. Biometry can also be used to attempt to elucidate underlying pathophysiologies of diseases, such as documenting shallow anterior chambers due to anterior shifting of the lens-iris diaphragm in cats with aqueous humor misdirection syndrome in cats (Czederpiltz JM, JAVMA, 2005). Ekesten in 1995 (AJVR) found that lens thickness increased with age in Samoyeds, leading to a shallow anterior chamber. These age related changes may precipitate pupillary block, leading to primary angle closure.

The main use of A-scans, however, are to determine optical characteristics of the globe to calculate IOL strengths and the source of refractive errors. Schiffer et al first described anterior chamber, lens thickness, vitreal chamber, and overall axial length measurements in 32 dogs in 1982. Cottrill et al (AJVR, 1989) described A-scan characteristics of mesocephalic and dolichocephalic dogs, and found that dolichocephalic dogs had longer axial globe lengths. In 1992, Murphy et al (IOVS) investigated a population of myopic German shepherd dogs used as guide dogs for the blind, and found no difference in axial length in myopic versus non-myopic eyes. Gilger et al in 1998 (AJVR) used biometry, combined with keratometry, to predict an IOL strength of 53-55 diopters in cats. Follow up studies of IOL implantation in research cats by Gilger et al (AJVR, 1998) demonstrated that a 52-53 diopter IOL is required to achieve emmetropia in cats. McMullen and Gilger (Vet Ophth, 2006, AJVR 2010) used biometry and keratometry to predict an IOL strength (although the large equine eye may result in positioning difference that require a substantially different power IOL). Murphy et al (IOVS, 1999) determined that myopia in Labrador retrievers is due to an elongated vitreous chamber, analogous to human myopia.

As discussed above, standardized A-scans have not been well worked out in veterinary medicine. One study by Baptista et al in 2006 (Vet J) demonstrated that anterior uveal melanomas had low to medium internal reflectivity, and internal vascularity. Future work may establish guidelines for more specific tumor diagnosis through standardized A-scans.

**Clinical use of B-scans:**
B-scans are typically used for evaluation of intraocular structures that cannot be seen through opaque media, such as corneal opacities, hemorrhage or hypopyon in the anterior chamber, cataracts, or vitreal opacities.

B-scans are typically used by applying the probe directly to the globe after application of topical anesthetic. A coupling medium, such as methylcellulose or a thick artificial tear, such as Genteal gel, is applied to the probe face prior to application. Examination through closed lids can be more difficult due to Bell’s phenomenon (rotation of the globe with the eyes closed). On the screen, the initial line that appears on the left side of the echogram represents the probe face. The upper part of the echogram corresponds to the probe marker. Since the best resolution is in the central portion of the probe face, lesions should be centered within the echogram whenever possible.

Probe positioning: In humans, certified ultrasound technicians use a standardized approach to ocular ultrasonography. This ensures a complete examination of the globe, and standardized exams and labeling facilitate communication between echographers.

Remember that each ‘slice’ of the B-scan only scans approximately six clock hours of the globe. In humans, this is overcome by three main probe positions: transverse, longitudinal, and axial. In transverse and longitudinal, the probe is placed on the conjunctiva posterior to the cornea. These positions bypass the lens, which allows better sound penetration as there is no absorption of the sound wave, as occurs in the axial position. In humans, the patients are directed to look away from the probe, which allows excellent exposure for probe placement. Clearly, this is not an option in a conscious veterinary patient. The transverse probe position means that the probe marker is parallel to the limbus. Longitudinal means the probe marker is perpendicular to the limbus. Images are labeled by the probe position, and what quadrant is being examined. The optic nerve is used as a reference point.

In veterinary medicine, the most common probe position is axial. The scan is performed with the eye in primary gaze and the probe face centered on the cornea. The image is intersected by the optic nerve as the sound beam is directed through the center of the lens, and the beam is swept along the two opposing meridians. This image is usually easiest to understand because the lens and optic nerve are in the center of the lesion, but there is decreased resolution of the posterior segment due to sound attenuation and refraction from the lens. In veterinary medicine, however, this is the easiest probe position to use in conscious animals.

No standardized labeling or exam system exists in veterinary ophthalmology that compares to the system in physician-based ophthalmology. The conventions used in human patients, however, can be easily adapted to veterinary patients. Typically, scans are labeled vertical axial when the probe marker is at 12:00. Horizontal axial scans are always done with the marker facing towards the patient’s nose. Oblique scans are labeled according to the clock hour the probe marker is facing.
Lens: The lens should exhibit a reflection at the anterior and posterior lens capsule, but the normal cortex and nucleus should be anechoic. Sometimes a very strong echo from the lens capsule can cause multiple external reverberations.

Cataracts are echogenic. An incipient cataract will only have a small echogenic area corresponding to the area of opacity. Cortical and nuclear opacities may be differentiated. Lens thickness will increase with some types of cataracts, such as the osmotic cataracts seen in diabetes. Lens thickness will decrease as the lens becomes hypermature and begins to resorb. Luxated lens are easily identifiable in an abnormal position. Congenital defects such as persistent hyaloid arteries, PHPV and associated lenticonus can be identified with ultrasound. These lesions are important to planning cataract surgery and often cannot be seen through a cataractous lens.

Vitreous: Normal vitreous is anechoic. Posterior vitreal detachments are often identified on cataract screening evaluations. Vitreal opacities can be differentiated from retinal detachments by turning the gain down. The more echogenic retina will continue to be visible, while most vitreal opacities will rapidly disappear. Floaters can often be seen in the vitreous from a variety of causes. Typically, vitreal degeneration appears as dots to linear strands that are freely floating in the vitreous. Asteroid hyalosis appears as multiple bright white dots in the vitreous. In synchysis scintallins, the cholesterol crystals sink to the ventral aspect of the vitreous. Posterior vitreal detachments can be identified. They can appear very similar to retinal detachments, but tend to be thinner, and disappear as gain is decreased.

Vitreal hemorrhage can be difficult to differentiate from other lesions such as tumors or inflammatory exudates. Vitreal hemorrhage varies in appearance depending on the location, amount and duration. With severe hemorrhage, the entire vitreous may be opaque, while with mild hemorrhage opacities of varying size can occur. Hemorrhage tends to settle ventrally, and if pseudomembranes occur (making hemorrhage appear to be a retinal detachment), they must be followed carefully to determine their endpoint. Pseudomembranes from hemorrhage will end in the vitreous while retinal detachments will still be attached at the optic nerve at least, if not the ora serrata. Hemorrhage is more likely to disappear as gain is decreased. Fresh hemorrhage is usually less dense, but older hemorrhage can be denser and therefore harder to diagnosis. Older hemorrhage may also not disappear with decreased gain. Carefully monitoring over time with consistent probe positioning and gain settings can help to distinguish hemorrhage from other lesions.

Other lesions that can be noted in the vitreous are foreign bodies. These are typically highly reflective and may or may not cause shadowing or multiple echoes, depending on their composition.

Retina: Retinal detachments are probably the most common indication for ophthalmic ultrasound. The classic appearance of a retinal detachment is the ‘gull’ wing appearance, which occurs when the retina remains attached at the ora serrata and the optic nerve. However, a detached retina can have many different ultrasonographic appearances, depending on if it is partial or complete, exudative, or disinsertional. Following a
membrane located on an ultrasound examination to the optic nerve will generally establish that is a retinal detachment. Again, when the gain is turned down, the reflective retina will remain on the image while vitreal opacities will often disappear.

Choroid: Chorioretinitis typically appears as a thickening of the choroid and/or retina, which may be hyperechoic or hypoechoic. Choroidal detachments generally have a sharper angle at the area of detachment than seen in a retinal detachment.

Sclera: Scleral ruptures can be identified in trauma with careful examination. Often a hemorrhagic track will lead to the area of scleral rupture. Posterior scleritis has a typical ultrasonographic appearance. There is diffuse thickening of the posterior sclera, often associated with diffuse retina and choroidal thickening. Episcleral inflammation causes distention of sub-Tenon’s space, producing a hypoechoic space outside the globe. When this occurs around the optic nerve, it is referred to as the “T-sign.”

Orbit: Orbital disease can cause deformities of the posterior aspect of the globe, blunting of the posterior aspect of the globe, diffuse increased echogenicity of the retrobulbar space prohibiting delineation of the optic nerve, and discrete masses in the retrobulbar space. Tumors tend to be hyperechoic and cause deformities of the posterior aspect of the globe and/or appear as discrete masses. Inflammatory retrobulbar processes can be diffusely hyperechoic to distinct hypoechoic masses. Diffuse, non-deforming lesions are most consistent with cellulitis. A retrobulbar abscess can mimic a mass, but if fluid is identified, it is more likely to be an abscess. These lesions can be differentiated by ultrasound guided aspirates.

**Correlating ultrasonography with histology:** Gallhoefer NS, Bentley E, Ruetten M et al, JAVMA, 2013. In this study we correlated histopathologic diagnoses with ultrasonographic diagnoses, and found overall acceptable correlation for most categories (neoplasia, hemorrhage, retinal detachment, subretinal exudate). Some findings, such as retinal tears, were not often detected via ultrasonography, and the ability to diagnosis other categories, such as subretinal exudate, varied by location. This study confirmed that it can be difficult to differentiate hemorrhage from neoplasia, and that clinicians should be cautious when trying to determine ultrasonographic diagnoses in eyes with extensive hemorrhage. Additionally, several cases with vitreous membranes that looked remarkably similar to retinal detachments on ultrasonography were identified. Contrast-enhanced ultrasonography with stabilized microbubbles has been shown to confirm the presence of retinal detachments in dogs (Labruyere, JSAP, 2011) and further studies in eyes with hyphema will likely demonstrate its utility in differentiating intraocular hemorrhage from neoplasia.

**High resolution ultrasound/ultrasound biomicroscopy**

Non-invasive visualization of the relationship between living tissues in vivo at a microscopic level is a goal of many imaging modalities. High frequency ultrasound probes with frequencies ranging from 20MHz (high resolution ultrasound, HRUS) to 60MHz (ultrasound biomicroscopy, UBM) have been developed which allow imaging at
resolutions which are comparable to low power microscopic views (20-80 µm). This represents a substantial improvement compared to conventional 10MHz probes, which have resolutions of 300 to 400 µm. With improved image detail afforded by the higher frequency probes, however, tissue penetration is limited to 5-10 mm. This depth is especially well suited to ophthalmologic applications because the critically important but optically occult structures of the anterior segment of the eye and peripheral retina are within this range. High resolution ultrasound (HRUS) fills a niche in ophthalmology in that it allows evaluation of ocular structures that cannot be fully examined by slit-lamp biomicroscopy or are obscured by opacities in the ocular media.

The probe frequency can be confusing on commercially available machines in veterinary ophthalmology. Probe labeling is not regulated by the FDA, and most probes emit a bandwidth of sound. Companies can label a probe at whatever frequency is in the bandwidth, which may lead to some variety in what kinds of images can be obtained by different frequency probes between machines, though the accepted standard to use the middle of the emitted bandwidth. To compare machines and probes, use the axial and lateral resolution which should be provided by the company, as well as the penetration distance to get a truer comparison.

Clinical ophthalmic applications of high resolution ultrasound in physician-based ophthalmology include evaluation of anterior segment tumors and cysts, scleral disease, intraocular lens assessment, trauma, and differentiation of the various forms of glaucoma. Several in vivo study involving ultrasound biomicroscopy (UBM) in dogs have been published; although these studies showed the promise of UBM, originally UBM often required heavy sedation or general anesthesia in small animal patients, and patient positioning is critical in obtaining an image. These limitations have gradually been decreasing as higher and higher resolution handheld probes have been developed. Imaging with currently available handheld high resolution probes (20-50MHz) does not typically require anesthesia or sedation, and can be performed with the animal in almost any position.

**Technique:**

Most animals can be examined with manual restraint in sternal recumbency following topical administration of 0.5% proparacaine hydrochloride. Some animals may require light sedation and/or need to be examined in lateral recumbency. The probe can be easily positioned to examine the superior and temporal quadrants of the eye, but light sedation may be required in some cases to examine the ventral and nasal quadrants in some animals.

The eyelids are manually held open and the probe is placed directly over the cornea or sclera. To examine the peripheral cornea and sclera, iridocorneal drainage angle, iris, ciliary body and lens, scans are obtained with the probe held perpendicular to the globe with the scan plane perpendicular to the limbus (longitudinal probe position). To examine the ciliary processes and iris, the probe is held perpendicular to the globe with the scan plane parallel to the limbus (transverse probe position). Images are
generally labeled with the clock hour and probe position. Structures can be measured using the internal calipers on the ultrasound machine.

Cornea/Sclera: Corneal thickness may be assessed with a high resolution probe. Both tumors and sequestrums are hyperechoic. High resolution ultrasound can be used to assess lesion depth, although our experience suggests that it is more accurate for tumor depth than sequestrum depth. The most pigmented portion of the sequestrum corresponds with hyperechogenicity on the ultrasound image, however, we have found that deeper tissue can be very slightly stained but appear normal on ultrasound. The faint pigment seen clinically probably does not cause enough alteration of the corneal stroma to alter the passage of sound waves, which is correlated by the minimal changes seen histopathologically on such lesions. Nevertheless, useful information regarding depth can be obtained pre-operatively with a high resolution probe.

Uvea: Differentiation between iris cysts and iris masses as well as evaluation of tumor extent is facilitated by a high resolution probe. Cysts can appear as thin walled, fluid filled structures, or anterior displacement of the iris due to the cysts can be noted. Difficult cases are those in which the cysts are peripheral and posterior to the iris, causing an apparent mass lesion. Careful assessment of the posterior aspect of the iris for anterior bowing on ultrasound is usually diagnostic even if the cysts cannot be easily imaged due to their thin walls. Tumors are easily recognized as hyperechoic masses, and judging the extent of the tumor is helpful to differentiate good candidates for laser photoablation. The high resolution probe also provides the means to quantitatively follow uveal tumors which have undergone laser photoablation. After laser treatment, the tumor is less homogeneous, with a mottled appearance of both hyper- and hypoechoic areas. High resolution ultrasound can also be used to locate the ciliary processes for more accurate trans-scleral cyclophotocoagulation.

Lens: The high resolution probe can be useful to evaluate the lens for peripheral lens capsule ruptures, which cannot be easily seen with slit lamp biomicroscopy.

Iridocorneal drainage angle: In the longitudinal position, the iridocorneal drainage angle and the ciliary cleft can be assessed and followed. This can be useful in evaluating glaucoma patients or potential glaucoma patients, as the ciliary cleft can be assessed as being open or closed. Evaluation of the iridocorneal drainage angle and ciliary cleft is also a tool to investigate the mechanism by which various anti-glaucoma drugs lower intraocular pressure in domestic animals.

Contrast-enhanced ultrasonography/Doppler

In 1968, investigators reported visualizing cardiac shunts with ultrasound using air bubbles via injection, and contrast ultrasound began. Free air bubbles were too large to pass in the pulmonary circulation, which limited their use. Over time, specific contrast ultrasound agents were developed for intravenous use, typically in a bolus. These agents are essentially high molecular weight/low solubility gas micro-bubbles (perfluorocarbons or sulfur hexafluoride) stabilized by a lipid polymer or albumin shell. These bubbles can
pass through the pulmonary circulation given their small size (1-10µm), and therefore have a longer half life than normal air bubbles. Ultrasound contrast agents are strictly intravascular, making them ideal for differentiating vascular versus avascular structures, such as intraocular tumors versus hemorrhage. The agents were initially developed for blood pool imaging (ie passive circulation), however, newer agents are being developed that can have specific ligands on their shells, allowing them to interact with target molecules. This would enable targeting specific sites, drug delivery, etc.

The other common way to see blood vessels via ultrasound is through Doppler modalities. Doppler can see movement of blood and contrast agents, but movement (such as small eye movements) results in artifacts, limiting its usefulness for intraocular structures, although it can still be useful in some orbital cases. Doppler is based on an apparent shift in sound frequency when reflected off a moving target (e.g. red blood cells in vessels). Motion away from the transducer results in a lower frequency than the original and motion toward the transducer results in a higher frequency. This change in frequency is detected and displayed as color map or graph.

Unfortunately, B-mode, gray scale imaging results in poor detectability of ultrasound contrast agents in tissue. The microbubbles of ultrasound contrast agents are very small and behave as scattering reflectors, and the microbubbles expand and contract when insonified, which means they produces echoes that are at multiples of the fundamental (probe) frequency (ie harmonics). At the fundamental frequency, contrast agents are echogenic only at high doses and are severely attenuating, which means that signal strength is lost deep to enhanced structures. In order to better image the contrast agents, contrast harmonic imaging is used. Contrast harmonic imaging utilizes the nonlinear oscillation of the contrast agents, which occurs due to the expansion and contraction of the contrast agent microbubbles as sound passes through them, which then generates harmonic frequencies. Harmonic imaging is a technique in which the beam is sent at one frequency (fundamental or probe frequency) while the machine listens for a different returned frequency. An example would be using a transducer that is 4MHz (the fundamental frequency) but is capable of listening for a second harmonic (8MHz) or a subharmonic (2MHz) frequency. The returning harmonic frequencies are of lower amplitude; therefore, the fundamental frequency is filtered to better detect the harmonic frequency. This increases the contrast-to-tissue ratio. The second harmonic (twice the fundamental frequency) image when using ultrasound contrast agents will have decreased intensity but less artifacts and noise than the fundamental B-mode images we are used to seeing. A second way to enhance contrast is phase inversion imaging. In phase, or pulse inversion, 2 pulses are emitted, but the second pulse is inverted in relation to the first. A linear reflection sends the waves back unchanged so that they cancel each other and no signal is received. Nonlinear reflectors will change the transmitted pulses enough that they no longer cancel each other out. Contrast-enhanced phase inversion can be used in combination with harmonic imaging.

The FDA in the US has only approved contrast agents for cardiac imaging, and in 2007, announced a black box warning for all ultrasound contrast agents following the death of 4 patients. Subsequent studies and post marketing studies led to the FDA downgrading the
warnings. An initial small study in veterinary patients noted an anaphylactoid reaction to Optison (perflutren protein type A) in two dogs (Yamaya Y J Vet Med Sci 2004). Optison has a human albumin shell, which was thought to cause the immunogenic response in these two dogs. A later study (Seiler et al JAVMA 2013) evaluated the safety of ultrasound contrast agents in 488 dogs and cats that received an ultrasound contrast agent compared to 262 patients that had ultrasonography alone. In this study, Definity, Levovist, Optison, Sonovue, and Targestar-P were the contrast agents used. Only dogs were noted to have adverse effects, and those were vomiting and syncope, occurring in 0.2% of dogs. None of the four dogs with reactions were given Optison, however the authors advised caution when using that contrast agent.

The main disadvantage of contrast enhanced ultrasound is the requirement for expensive, specialized equipment. Ophthalmic ultrasounds are not equipped for contrast harmonic imaging, and ultrasound machines that can do this are typically hundreds of thousands of dollars. However, more and more veterinary radiologists have access to machines with this capability. Another disadvantage is the cost of the contrast agent, typically at least $120/bottle. Each bottle is only stable for a few hours, so realistically one bottle is needed per patient.

In an experimental setting, visualization of the normal rabbit choroid was possible, and artificially created avascular lesions could also be demonstrated (Hirokawa T, J Ultrasound Med 2002). High frequency ultrasound was used to assess experimentally induced choroidal melanomas in mice and rabbits, and finding correlated well with histology (Zhang Q, IOVS 2011; Kang SJ Br J Ophthalmol 2013). Interestingly, in this study they did not use harmonic imaging but used fundamental high frequency.

Early studies in physician-based ophthalmology demonstrated the utility of contrast-enhanced ultrasound in evaluation of tumors, mostly choroidal melanomas. Prior to treatment, choroidal melanomas were well perfused on contrast harmonic imaging, while Doppler failed to highlight some of the same tumors (Schlottmann K, AJR 2005). Contrast ultrasound also highlighted changes in vascularization of choroidal melanomas after treatment, demonstrating its possible use as a monitoring tool (Forte, Acta Ophthalmologica Scand 2005). A later study used contrast ultrasound to evaluate a newer treatment for choroidal melanomas in humans (Venturini M, J Clin US, 2015).

While there are multiple publications investigating the use of contrast-enhanced ultrasound in various abdominal organs in veterinary patients, only Lubruyere et al (JSAP 2011) have published on the use of contrast-enhanced ultrasound in veterinary ophthalmology. In this paper, the authors used both pulse inversion and contrast-tuned (harmonic) imaging and Sonovue was the contrast agent. The peak intensity was somewhat higher using contrast-tuned imaging, however the authors noted the spatial resolution was subjectively less. This study also compared color Doppler ultrasonography, and found that in most eyes, the color assessment was unsuccessful due to eye movement artifacts, which has been this author’s experience as well. Contrast-enhanced ultrasound, however, enabled visualization of blood flow in detached retinas in all cases and did not falsely show blood flow in vitreous membranes.
The next step for ultrasound contrast agents will be their use in targeted therapy. Drugs or vector DNA for gene therapy could be attached to the microbubbles to allow for specific delivery, particularly if specific ligands are attached. High intensity ultrasound can rupture the bubbles, thereby delivering agent to a specific area while simultaneously imaging that area.

**3D ultrasound**

Three dimensional ultrasonography is most well known through its use for prenatal imaging in humans. In this technique, sequential 2D scans are combined with computerized reconstruction software. This allows surfaces of structures and lesions to be rendered and the volume of lesions to be calculated. Its use in ophthalmology has remained relatively limited, and is mostly reported for the use of posterior segment and optic nerve tumors, including extraocular extension. At the moment, the need for specialized equipment limits its use in veterinary medicine, although the utility of 3D imaging and printing diagnosis and surgical planning is obvious (see Dorbandt et al, Vet Ophthal, 2016).

Further reading:


McMullen RJ Jr, Davidson MG, Campbell NB, Salmon JH, Gilger BC. Evaluation of 30- and 25-diopter intraocular lens implants in equine eyes after surgical extraction of the lens. AJVR 2010;71:809-816.


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