Chapter 1

Ultrasound principles

László Oláh

Sound is a mechanical, longitudinal pressure wave that is transmitted in a medium and characterized by its amplitude, frequency and wavelength (Figure 1.1). The human ear can hear sounds at frequencies between 20 Hz and 20 000 Hz. Above 20 000 Hz, which is not audible to human ears, the sound is called ultrasound.

After investigating a piece of quartz in 1880, the Curie brothers described the piezoelectric effect. Certain crystals (e.g., quartz, ceramic and lead zirconate titanate) subjected to mechanical stress have the ability to generate an electric charge on their surface, and vice versa, electrical pulses in these special materials result in vibration [1]. Ultrasound transducers contain multiple piezoelectric crystals which vibrate in response to a rapidly alternating electric field and produce ultrasound. Sending a series of short ultrasound pulses into the body tissue results in a part of the sound wave being reflected back to the transducer (echo) and converted to an electric signal by piezoelectric crystals [2]. Therefore, ultrasound transducers have dual functions: they transmit (emit) and receive ultrasound. In pulsed-wave (PW) Doppler mode, after transmitting ultrasound, the same crystal switches to a receiving mode in order to detect the reflected echo, thus the same crystal is used to transmit and receive ultrasound. In continuous-wave (CW) Doppler mode, however, the emitting and receiving crystals are separated, that is, one crystal continuously transmits, while another crystal continuously receives ultrasound signals (Figure 1.2).

Speed of ultrasound

The speed of ultrasound propagation in a tissue depends on the compressibility and density of the material in which the sound propagates. The closer the molecules in a medium, the higher the propagation speed of the sound. Except for air (c = 330 m/s) and bone (c = 3500 m/s), the propagation speed of the sound is very similar in different biological tissues (c = 1450–1570 m/s), therefore an average value of 1540 m/s is usually used for calculations in biological samples. The speed in bone, however, is much higher, which might be of interest when ultrasound passes through thick bony structures [3]. Speed of ultrasound propagation as well as the time interval between the emission of the ultrasound pulse and the reception of the reflected echo must be known in order to calculate the distance between the transducer and the target (Figure 1.3).

Acoustic impedance

The product of the density of the medium and the speed of the ultrasound wave in the medium defines an intrinsic property of the substance, the so-called acoustic impedance [4]. Reflection of an ultrasound wave from the interface between tissues is proportional to the difference in acoustic impedance between the materials on the two sides of the interface. The acoustic impedance for water and most soft tissues (blood, fat, muscle) are very similar; however, air has a very low and bone has a very high acoustic impedance (denser material is characterized by greater impedance). The acoustic impedance of air is so low compared to the skin that practically all of the energy is reflected from the air/skin interface. This is the reason why it is impossible to image through air and why it is essential to use gel in order to exclude air from the boundary between the transducer and the skin [5].

Manual of Neurosonology, ed. László Csiba and Claudio Baracchini. Published by Cambridge University Press. © Cambridge University Press 2016.

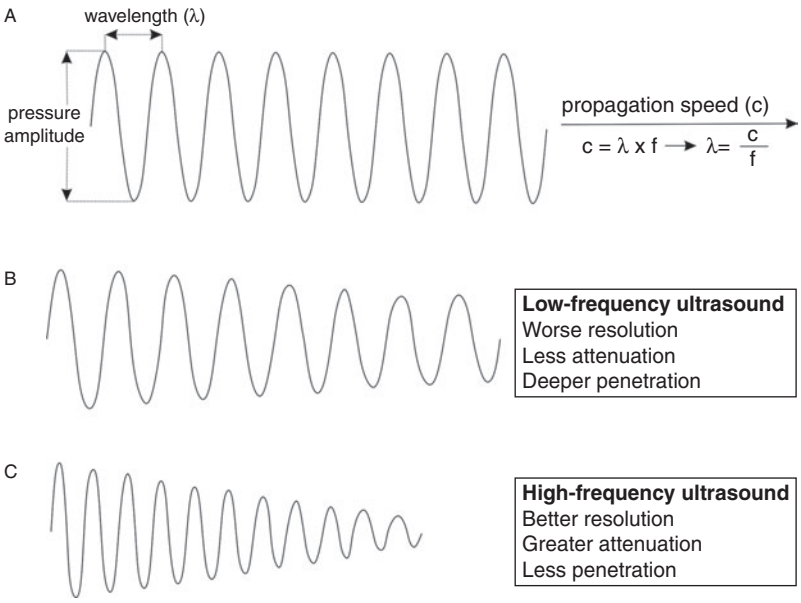


Figure 1.1 Amplitude, frequency and wavelength of ultrasound (A). Amplitude is the magnitude of the pressure changes, which is related to the energy content of ultrasound and measured in decibels (dB). Frequency is the number of times the wave is repeated in 1 second (1/s = 1 Hz). Wavelength is the distance that the wave travels in one cycle. The next equation describes the relationship between ultrasound frequency (f), wavelength (λ) and speed of ultrasound (c) in a medium: $c = \lambda \times f \rightarrow \lambda = c / f$. According to this equation, the wavelength of a 5-MHz (5 000 000-Hz) ultrasound wave in a soft tissue, in which the ultrasound speed is about 1540 m/s, is: λ = 1540 m/s / 5 000 000 Hz = 0.000308 m = 308 μm. When ultrasound travels through a medium, its amplitude decreases but its frequency remains constant (B,C). Although high-frequency ultrasound assures better resolution, its intensity in a medium decreases faster compared with low-frequency ultrasound (B,C), resulting in less penetration.

Behavior of ultrasound at acoustic boundaries

When ultrasound enters the body, it travels through different tissues. Ultrasound waves may be transmitted to deeper structures (transmission), reflected back to the transducer (reflection), scattered, or partly absorbed and converted to heat (Figure 1.4). Certainly, reflected waves (echoes) are the most important, since these waves provide information for imaging purposes. Behavior of ultrasound at acoustic boundaries depends on the relative dimensions of the ultrasound wavelength and the target [3,5,6].

When an ultrasound wave reaches a large target, part of the ultrasound wave continues to penetrate deeper into the structure, while the remaining part is reflected back from the boundary between tissues with different acoustic properties (Figure 1.4A, B). The fraction of the reflected and transmitted ultrasound waves depends on the acoustic impedance of the materials on the two sides of the boundary. The greater the difference in impedance between the two materials, the more sound will be reflected rather than transmitted. That part of the ultrasound which passes on and crosses the tissue interface becomes slightly bent away from its original direction (refraction). The degree of refraction depends on the difference between the speed of sound before and after the boundary. A large difference in the propagation speed of the ultrasound on the two sides of the boundary results in more significant refraction (Figure 1.4B). As the propagation speed of the sound is much higher in bone than in soft tissues, refraction effects should be considered at soft tissue/bone interfaces (e.g., in transcranial investigations). Refraction is one of the important causes of incorrect localization of a structure in ultrasound imaging; moreover, it may lead to a duplication artifact.

If the ultrasound pulse encounters targets whose dimensions are smaller than the ultrasound wavelength (e.g., red blood cells), or if the surface of the reflector is rough and irregular, scattering occurs (Figure 1.4C). In this case, echoes are reflected through a wide range of angles. Although scattering results in the loss of a huge amount of sound energy for imaging, some echoes will invariably reach the transducer regardless of the angle of the incident pulse. This means that echoes due to scattering are relatively weak, but they depend much less on the insonation angle. Since most biological tissues are filled with small scattering structures, scattering allows visualization of the blood flow and parenchyma of body organs.

Attenuation of ultrasound

The energy of ultrasound decreases as it travels through body tissues. This attenuation results partly from reflection and scattering of ultrasound waves, but in most cases, the main cause of attenuation is the conversion

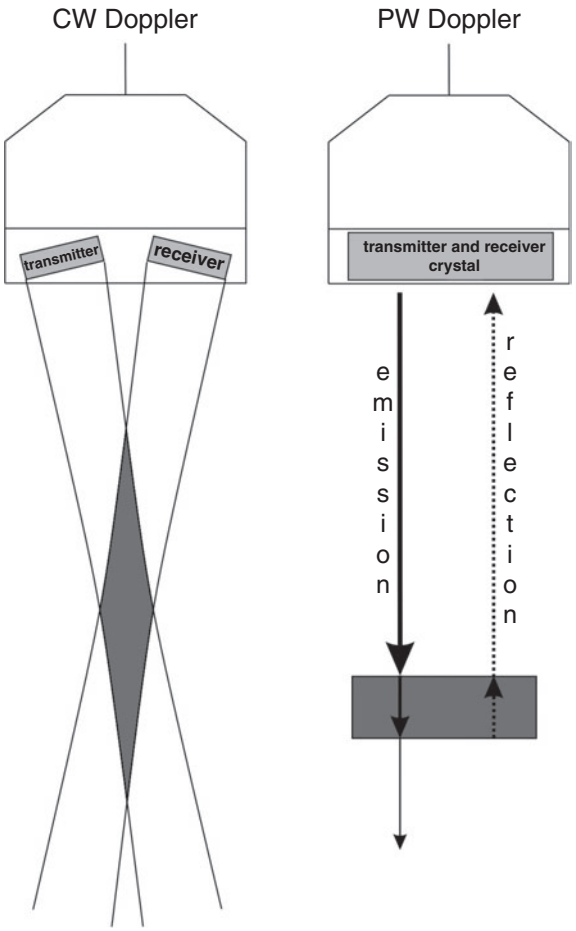
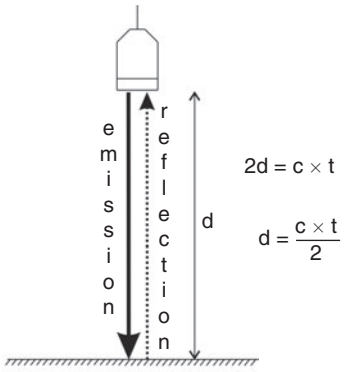


Figure 1.2 Continuous-wave (CW) and pulsed-wave (PW) Doppler. In CW mode, the transmitter and receiver crystals are different (i.e., the transmitter crystal only emits ultrasound, while the receiver crystal only detects echoes). In PW mode, the same crystal emits and detects ultrasound. CW Doppler detects each object along the course of the beam, while PW Doppler is able to differentiate signals from a specific depth (see Figure 1.6).



If:
c=1540 m/s, time delay=52 ms (=0.000052 s)
→ d=1540 m/s × 0.000052 s / 2=0.04 m (4 cm)

Figure 1.3 Calculation of the distance /d/ between the transducer and the target with knowledge of the speed of ultrasound propagation /c/ and the time interval /t/ between ultrasound pulse emission and echo reception: $2d = t \times c \rightarrow d = t \times c / 2$. Note that ultrasound has to reach the target and the echo has to return back to the transducer, therefore the distance has to be considered twice.

of the mechanical sound energy into heat (absorption). Attenuation of ultrasound energy depends on several factors including ultrasound frequency, path length and attenuation coefficient of the medium.

Attenuation coefficient, expressed as a decrease of ultrasound pressure amplitude in decibels over a distance of 1 cm, varies from tissue to tissue. Among biological materials, bone has the highest attenuation coefficient, which explains why examination behind bony structures is so difficult. Since ultrasound energy is attenuated with path length, and energy of high-frequency ultrasound decreases more than low-frequency ultrasound (Figure 1.1B,C),

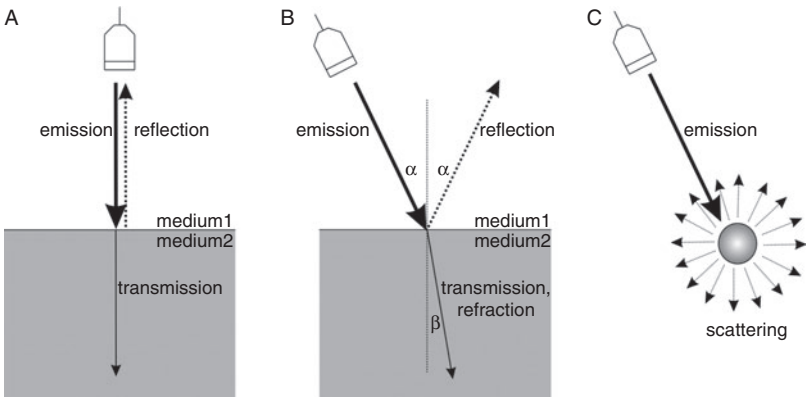


Figure 1.4 Behavior of ultrasound at acoustic boundaries: reflection (A,B), transmission (A,B), refraction (B) and scattering (C). When an incident ultrasound pulse reaches a large, plain interface of tissues with different acoustic impedance, a significant amount of sound energy is reflected. This type of reflection is called mirror-like or specular reflection. If the emitted ultrasound is perpendicular to the boundary (A), the reflected wave returns directly to the source (transducer). However, if the angle between the transmitted ultrasound and the reflector surface is not 90°, the echo will miss the transducer since it will be reflected at an angle equal to the angle of incidence (B). This is the reason why it is difficult to image large surfaces which are not perpendicular to the emitted ultrasound.

attenuation results in worse image quality in deeper regions, especially when high-frequency ultrasound is used. In order to brighten the deeper and thus the more attenuated area, the so-called time-gain compensation can be used. Time-gain compensation allows selective amplification of the attenuated waves by increasing the gain in the deeper body regions.

Although high-frequency ultrasound assures better resolution, it is attenuated significantly within a short distance, thus limiting the effective penetration depth. The practical consequence of this phenomenon is that deeper structures are imaged by using low-frequency ultrasound, which is less attenuated, and which therefore penetrates deeper into the body (Figure 1.1B).

Axial and lateral resolution

Axial resolution refers to the ability to discriminate between two points located in the same direction as the ultrasound beam, while lateral resolution refers to the ability to distinguish between two reflectors which are perpendicular to the beam. Since shorter wavelengths give better axial resolution, application of high-frequency ultrasound is suggested for better image quality. Concerning the lateral resolution, it should be noted that the emitted ultrasound does not maintain its linear shape, but becomes centrally narrowed with converging (near-field) and diverging (far-field) parts of the ultrasound beam. The narrowest point between these converging and diverging parts is called the focal zone, and this is where the lateral resolution is the best. Lateral resolution can be improved by enlarging the transducer surface, as well as dynamic electronic focusing and multiple ultrasound beams, generating multiple focal zones [7].

In clinical practice, excellent resolution and deep penetration would be desired. However, because of the physics of ultrasound, both requirements cannot be fully met at the same time. For good axial resolution, high-frequency ultrasound is needed, while for better penetration, low-frequency ultrasound is required. Therefore, the optimum ultrasound frequency chosen should allow visualization of the structure of interest as well as good resolution.

Doppler ultrasound

In 1842, an Austrian professor of mathematics and physics, Christian Andreas Doppler, described that stars appear in different colors when observed from a constant position on earth, suggesting that the motion

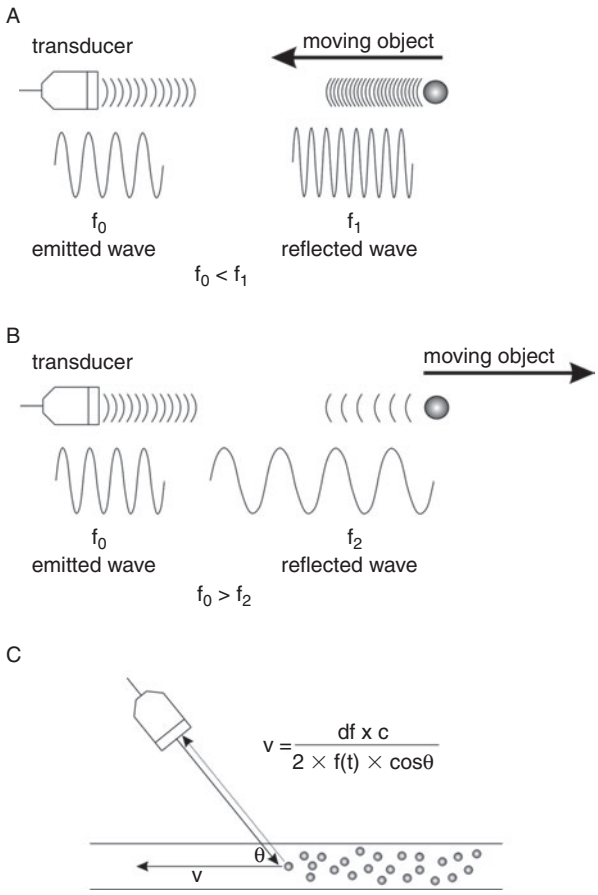


Figure 1.5 The Doppler effect. Ultrasound frequency increases if reflected from an object moving toward the source of ultrasound (A) and decreases when reflected from a target moving away from the source of sound (B). According to the Doppler effect, flow velocity /v/ can be calculated in the knowledge of the frequency shift /df/, the transmitted ultrasound frequency /f(t)/, the speed of ultrasound propagation /c/, and the angle between the ultrasound beam and the flow vector /θ/ (C).

of the star causes a change in the emitted frequency [8]. This frequency change can be observed in any wave whose source is moving relative to the observer. If the source of the wave moves away from the observer, the frequency will be lower; however, if the source of the ultrasound and the observer approach, the frequency will be higher than the emitted frequency (Figure 1.5A,B). This phenomenon was named after Christian Doppler and it is known as the Doppler effect.

The difference between the frequencies of the transmitted and detected waves (frequency shift or Doppler shift) is directly proportional to the velocity of the source relative to the observer. According to the Doppler effect, moving blood cells change the

ultrasound frequency. Since this frequency shift (i.e., the frequency difference of the transmitted and reflected ultrasound) is proportional to the velocity of moving erythrocytes, the Doppler effect can be used in clinical practice to measure blood flow velocity. Frequency shift (df) and flow velocity can be calculated by the following formula:

$$df = 2 \times f(t) \times v \times \cos\theta / c \rightarrow v = df \times c / 2 \times f(t) \times \cos\theta$$

where df is the frequency shift, f(t) is the transmitted ultrasound frequency, v is the flow velocity of the target, θ (Doppler angle, or insonation angle) is the angle between the ultrasound beam and the flow vector, and c is the speed of the ultrasound propagation (Figure 1.5C).

This equation indicates that if the ultrasound beam is perpendicular to the direction of blood flow, the frequency shift will be zero ($\cos 90^\circ = 0$), therefore flow velocity cannot be measured in this case. For proper velocity measurement the angle between the sound beam and blood flow should be considered and kept less than 60°. The reason of this is that cosθ changes rapidly at angles greater than 60° and even small errors in angle correction may lead to significant measurement errors [9].

Time-based gating in pulsed-wave Doppler mode

In PW mode, as previously mentioned, a piezoelectric crystal both sends and receives ultrasound signals. After the ultrasound pulse is emitted, the piezoelectric crystal switches to receiving mode in order to detect the reflected signals. However, the receiver is opened only after a controlled delay, and only for a specific duration (Figure 1.6). Since the time interval between ultrasound emission and signal reception determines the insonation depth, and the duration of reception defines the sample volume, time-gating of the returning echo allows adjustment of the depth and size of the sample volume from where the reflection is expected [9].

Relationship between pulse repetition frequency, insonation depth and flow velocity

Ultrasound waves are generated in pulses that usually consist of two or three cycles of the same frequency. The number of pulses transmitted in 1 second is known as the pulse repetition frequency (PRF). Since the ultrasound wave must reach the target of interest, and the reflected echo should return to the transducer before the next ultrasound pulse is generated, the PRF value cannot be increased above a certain value (Figure 1.6A). It is easy to understand that the maximum PRF varies inversely with the depth of the sample volume, since the return of the signal takes a longer time from a deeper structure, requiring a longer delay between pulse transmissions.

When PW Doppler is used, not only the PRF but also the detectable highest flow velocity value is limited. This limitation can be understood by the so-called Nyquist limit. According to the Doppler equation, a higher flow velocity results in a higher frequency shift that is practically a sound wave and can be described by a sinus curve. In order to detect the frequency of a sinus curve, at least two measurements are necessary during a complete sinus period. This means that if the flow velocity, and thus the frequency shift (df) is higher, the sampling frequency, which corresponds to the PRF in ultrasound systems, should also be increased to at least double the frequency shift for correct measurement:

$$PRF \geq 2 \times df \rightarrow PRF/2 \geq df \text{ (Nyquist limit).}$$

However, as discussed earlier, the PRF is limited in PW mode, because the next pulse cannot be sent out until the echo signal has returned. Therefore, if the frequency shift, which is proportional to the flow velocity, is higher than half of the maximum PRF (the Nyquist frequency), the frequency shift cannot be measured correctly and aliasing occurs. In this case, the velocity and the direction of flow are inaccurately displayed and part of the spectrum above the Nyquist limit shows a change in direction without crossing the zero line.

Examination of a tissue sample in the deeper region is associated with a decrease of the maximum PRF and thus the Nyquist limit, leading to an increased probability of aliasing artifact [10]. Aliasing can be controlled by decreasing the depth (if possible), decreasing the baseline or increasing the PRF (PRF corresponds to scale in ultrasound equipment).

Continuous-wave Doppler

CW Doppler uses a transducer which contains two piezoelectric crystals: one of the two crystals continuously emits ultrasound while the other one continuously receives the reflected and scattered signals. Since the PRF is not limited (the next ultrasound pulse can be

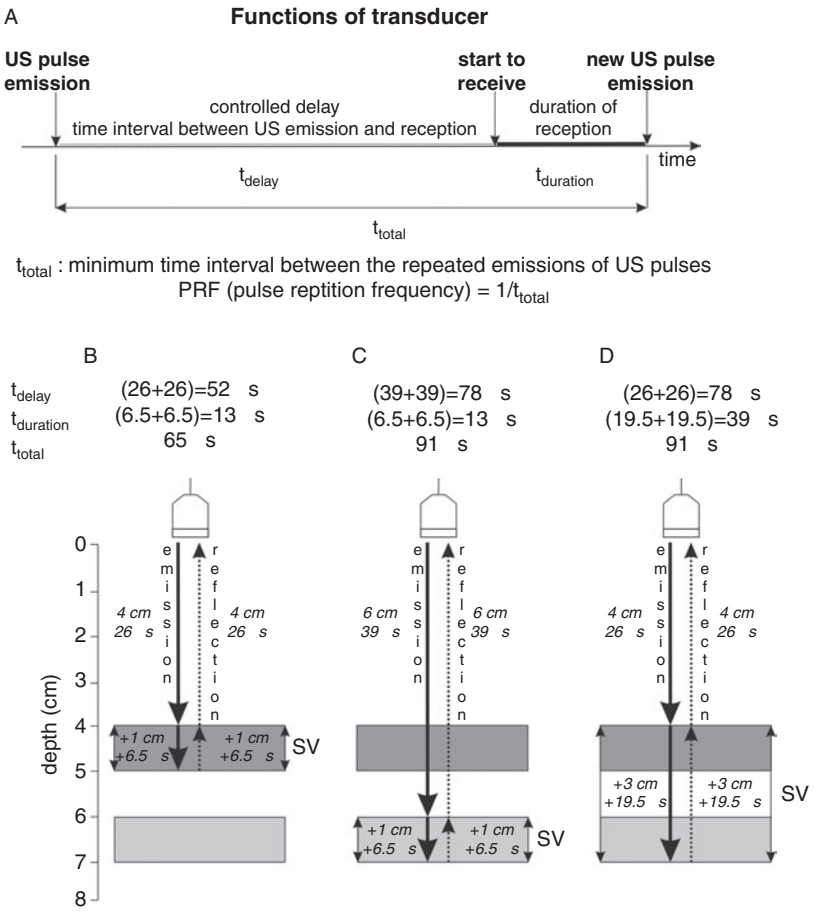


Figure 1.6 Time-based gating in PW Doppler mode. In PW mode, time-based gating (A) means that after ultrasound (US) pulse emission the receiver function of a crystal is opened only after a controlled delay (t_{delay}) and only for a specific duration ($t_{duration}$). Since the signal needs to return before the transmission of a new ultrasound pulse, the maximum rate of pulse transmission (pulse repetition frequency, PRF) is inversely related to the time that is necessary for an ultrasound wave to reach the target at a certain depth and to return back to the transducer (t_{total}) (A). Using the speed of ultrasound propagation in soft tissues (c = 1540 m/s) and the time interval between ultrasound emission and echo reception, a specific depth from where the reflection is expected can be calculated. It is known that ultrasound travels 1 cm (0.01 m) distance in a soft tissue in 6.5 μs (0.01 m/1540 m/s = 0.0000065 s = 6.5 μs). If the investigated sample volume is in a deeper region, the piezoelectric crystal starts to receive the reflected ultrasound signal after a longer delay (t_{delay}) (B,C). If the sample volume (SV) is adjusted to be larger, the duration of echo reception ($t_{duration}$) is increased (B,D).

emitted before returning of the echo), and thus the CW mode is not affected by the Nyquist limit, this method allows detection of high blood flow velocities without aliasing artifact. However, the main disadvantages of this modality are that, in the absence of the time-gating technique, CW Doppler cannot localize the depth of the target along the sound beam and the size of the sample volume cannot be adjusted. Therefore, CW Doppler detects the velocities of all the blood cells moving along the course of the sound beam. Since CW Doppler cannot localize the targets which are at different depths, this modality is rarely used today.

Ultrasound imaging

Today, modern medical ultrasound equipment uses the pulse-echo approach, the name indicating that after transmission of a short ultrasound pulse, the transducer switches to receiving mode in order to gather the reflected and scattered echoes from the tissue. If ultrasound speed and time interval between pulse transmission and echo reception are known, the position of the target producing an echo can be localized (Figure 1.3).

For ultrasound imaging, different modes are used (Figure 1.7).

A-mode (amplitude mode) is the simplest type of ultrasound imaging. In A-mode ultrasound, a single transducer emits an ultrasound pulse and then switches to receiving mode to detect echoes from interfaces between different tissues. The returned waves are displayed on a screen as spikes on the time (x-) axis. The stronger the returned wave, the larger the amplitude of the spike on the y-axis. Depending on the ultrasound speed in biological tissues, the time difference between the different spikes determines the distance that the ultrasound travels. The main function of A-mode ultrasound is to measure the size, or distance, in different biological samples.

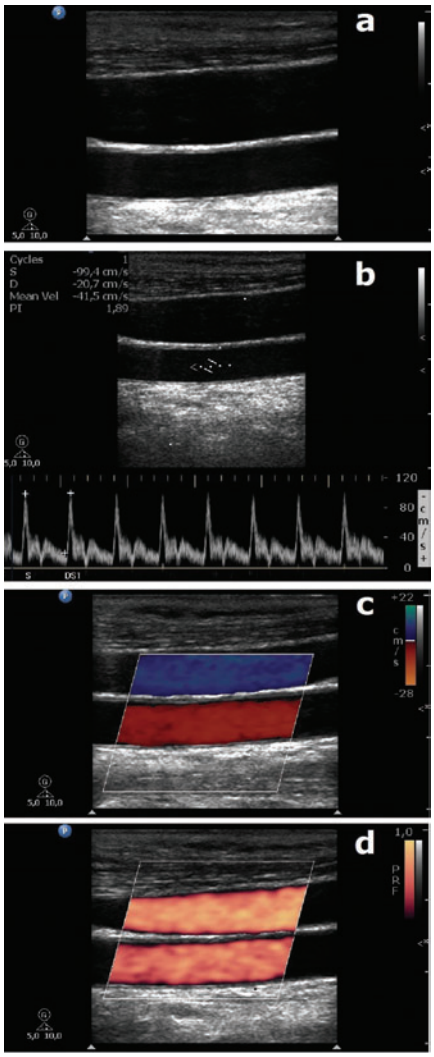


Figure 1.7 Different modes for ultrasound imaging. B-mode (A), duplex mode (B), color Doppler flow imaging (C), and power Doppler imaging (D) are shown. Note that B-mode picture (A) shows anatomy of the investigated region, while duplex mode (B) allows additional measurement of flow velocity in a region of interest selected in the B-mode image. Flow direction relative to the transducer is shown by color Doppler flow imaging (C; red color in common carotid artery, blue color in jugular vein, in this image); however, this information about flow direction is missing in the case of power Doppler imaging (D; both vessels are shown by the same color).

B-mode (brightness mode) is the most common form of ultrasound imaging. In B-mode imaging, the strength of the returning wave is recorded as a bright dot instead of a spike. The brightness of the dot on a gray scale indicates the intensity of the returning echo. Certainly, those echoes which return from a target localized deeper in the body return back to the transducer later and are represented farther from the surface of the ultrasound transducer. Thus, the location of different gray dots relates to the depth of the target, while the brightness of the dot corresponds to the intensity of the echo (Figure 1.7A). By sequential activation of different ultrasound-emitting crystals, multiple scan lines are created, which are put together to construct a two-dimensional image (frame). Since the ultrasound speed is very high in biological samples, the imaging process happens very quickly, allowing scanning of the region of interest and redrawing of the image many times per second. Generation of multiple images (frames) in 1 second creates an illusion of a real-time picture (e.g., heart beating, or pulsation of the carotid artery can be seen). The frame rate, which shows how many times the image is updated in a second, determines the temporal resolution potential of the system and is important when assessing moving interfaces. For slow-moving organs, the frame rate is not very important, but to capture the motion of the heart or blood flow, it can be essential. Frame rate is limited by depth, width of color box and low PRF [4,11].

The **duplex image** is derived from the combination of a B-mode gray-scale image and PW Doppler flow velocity measurements. The B-mode image provides the anatomical localization of the vessels, showing the region of interest where a Doppler sample volume should be placed and where the flow velocity is measured (Figure 1.7B). Assuming that the blood flow is parallel to the direction of the vessel, the Doppler angle can also be measured, and thus the frequency shift can be corrected with the use of cosine of the insonation angle.

Color Doppler flow imaging modality is based on PW Doppler technology by measuring mean frequency shift in each sample volume. Color Doppler flow imaging represents color-coded velocity information, which is superimposed as a color flow map on a B-mode image (Figure 1.7C). In each sample volume, the color reflects the blood flow velocity in a semiquantitative manner, as well as the flow direction relative to the transducer. Blood flowing toward or away from the transducer is shown by different colors (red and blue). Moreover, fast flow is indicated by a lighter hue and slow flow by a deeper one. The color flow map shows the position and orientation of the vessels, as well as the site of turbulent flow or stenosis.

Since color flow mapping is based on flow velocity measured by PW technology, aliasing occurs if the

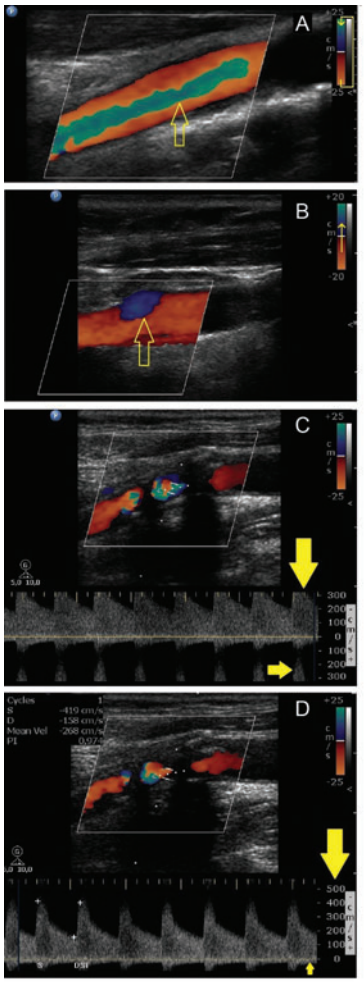


Figure 1.8 Aliasing phenomenon. Note the conversion of the red-orange color into the green-blue color in the middle of the common carotid artery (CCA) without crossing the zero line (A). This color aliasing phenomenon, showing apparent reversal of flow direction, is due to the low pulse repetition frequency settings (low color scale settings), leading to incorrect measurement of higher flow velocity values in the middle of the CCA. Since the flow velocity is lower close to the vessel wall, aliasing cannot be detected in this region of the artery. In the next image (B), real reversal of flow direction can be seen at the origin of the internal carotid artery due to helical flow in this region. Conversion of red into blue color and crossing of the zero line indicates real change of flow direction. Spectral aliasing is demonstrated in the image (C); the artifact, in this case, can be avoided by the proper settings of scale (PRF) and baseline level (D).

frequency shift is higher than half of the PRF. Low PRF value and high flow velocity, especially in the deeper regions, increase the probability of aliasing which can be recognized in a color flow map as an apparent flow reversal: color changes from the maximum velocity in one flow direction to the maximum velocity in the opposite direction without crossing the zero line (Figure 1.8) [7].

Power Doppler mode uses the signal intensity of the returning Doppler signal instead of frequency shift. Power (intensity) of the signal is displayed as a color map superimposed on a B-mode image. Since the Doppler power is determined mainly by the volume rather than the velocity of moving blood, power Doppler imaging is free from aliasing artifacts and much more sensitive to detect flow, especially in the low-flow regions [3]. However, this imaging modality does not contain information about the flow direction or flow velocity (Figure 1.7D).

Tissue harmonic imaging is based on the harmonic echo signals produced as the ultrasound beam passes through tissues. In conventional ultrasound imaging, when tissue is insonated at a certain frequency, most of the echo signals returning to the transducer will be related to this fundamental, emitted frequency. However, the so-called harmonics, which are multiples of the fundamental ultrasound frequency, are also generated as the ultrasound propagates in tissues. Since harmonic waves are characterized by much lower amplitude, they are masked by the stronger fundamental echoes. Therefore, echoes of the fundamental frequency are eliminated and only the harmonic echo signals are used when tissue harmonic imaging is performed. The main advantage of tissue harmonic imaging over conventional pulse-echo imaging is the artifact reduction. This is due to the fact that most disturbing ultrasound artifacts usually result from weak waves that produce little or no harmonics. Further advantages of tissue harmonic imaging is the better contrast resolution, improved lateral resolution, reduced noise and improved signal-to-noise ratio [12].

Ultrasound contrast agents

Intravascular ultrasound contrast agents were originally designed to improve conventional ultrasound imaging; however, their therapeutic use over the last decades has become as, or even more, important than their diagnostic application [13].

Microbubble characteristics

Ultrasound contrast agents can be divided into microbubble-based and non-microbubble-based contrast agents (Figure 1.9) [13].

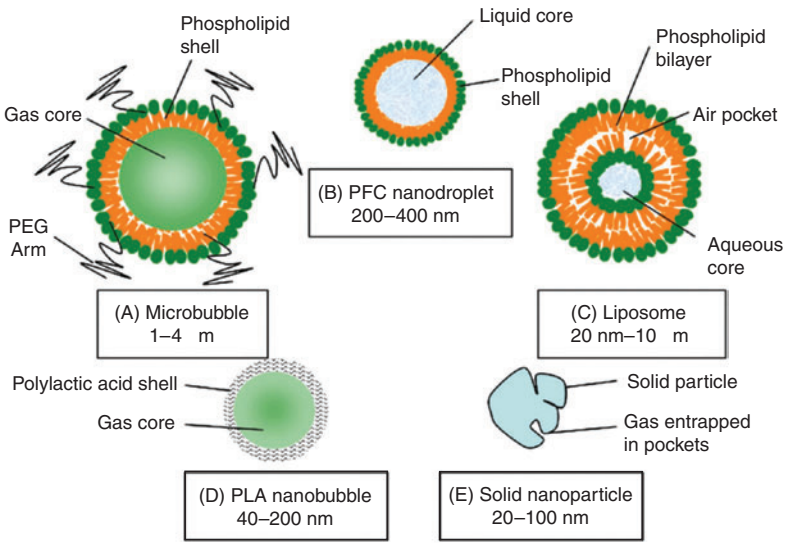


Figure 1.9 Different types of ultrasound contrast agents. (A) Microbubbles are gas–liquid emulsions with a polyethylene glycol (PEG) polymer on the surface to prevent aggregation. Microbubbles are highly echogenic and the most commonly used contrast agents for molecular ultrasound imaging. (B) Perfluorocarbon emulsion (PFC) nanodroplets are liquid–liquid emulsions that can be vaporized into echogenic gas-bubbles following administration of acoustic energy. (C) Liposomes are phospholipid bilayers that can enclose air pockets for ultrasound imaging. (D) Nanobubbles are gas–liquid emulsions that can fuse into echogenic microbubbles at the target site. (E) Solid nanoparticles are solid amorphous substances with gas entrapped in their pores or fissures increasing echogenicity. Reprinted from reference [13] with permission from Elsevier.

Microbubbles consist of a gaseous core surrounded by a liquid shell that prevents gas leakage as well as aggregation of microbubbles (Figure 1.9A). The gaseous core can be air, or it may consist of heavy gases such as perfluorocarbon, sulfur hexafluoride or nitrogen. Since heavy gases are less water-soluble, the half-life time of the heavy gas-containing microbubbles is much longer than air-containing ones [14]. The microbubble shell is usually composed of albumin, galactose, lipids, phospholipids or polymers [15].

The contrast effect of microbubbles depends on the difference in acoustic impedance between gas-containing microbubbles and the surrounding tissue. Since gas is less dense by several magnitudes than blood, the microbubble/blood interface is enormously echogenic. Enhancing the backscatter of ultrasound signals, contrast agents are used to increase weak signals to a detectable level [16], improving the detection of flow and identification of vessels.

Since the contrast agents are usually administered intravenously, microbubbles must pass through the lungs for arterial investigations. This fact limits their size and characteristics, because microbubbles larger than 5–8 μm or unstable microbubbles will be filtered in the lungs. Microbubbles are usually 1–8 μm in size, therefore they circulate in the vessels and do not leave the intravascular space within physiological conditions. They are metabolically and hemodynamically inert, that is, they do not cause an immune response of the host and do not affect blood flow. Microbubbles are cleared by the reticuloendothelial system (RES) within several minutes (the imaging time in the case of commercially available microbubbles is between 2 and 10 minutes), thus a constant concentration of contrast agents can only be maintained by continuous administration through an infusion pump, prolonging the diagnostically useful time of elevated signal intensity [13].

Acoustic cavitation

Before discussing the behavior of microbubbles in different acoustic fields, the cavitation phenomenon has to be briefly explained. Acoustic cavitation is the formation of gaseous and vapor bubbles in a liquid subjected to rapid changes of acoustic pressure. Ultrasound, existing from negative and positive pressure waves, induces the formation of cavities in low-pressure regions. Cavitation is generally classified into two types. Stable cavitation is the process in which a bubble in a fluid is forced to oscillate in size due to an acoustic field, while inertial cavitation describes the phenomenon when a bubble in a liquid rapidly collapses, producing a shock wave. Although ultrasound alone may cause cavitation, microbubbles significantly lower the threshold for cavity production [17,18].

Behavior of microbubbles in acoustic field

Microbubbles, being compressible, undergo volumetric oscillation during the alternate pressure cycles of ultrasound, that is, microbubbles repeatedly contract during the compression and expand during the

rarefaction phases of ultrasound wave. This oscillation of microbubbles produces strong echoes and leads to contrast enhancement [19,20,21].

Microbubbles can act in different ways depending on the mechanical index (MI) which is calculated as the quotient of the acoustic pressure amplitude (P) and the square root of frequency of the ultrasound signal (f): MI = Pf[22].

- At a very low MI, microbubbles show linear or sinusoidal oscillation (the speed of expansion and compression is similar) at the same frequency of the transmitted ultrasound, therefore the frequency of the backscattered signal will be equal to or very close to the fundamental (emitted) ultrasound frequency.
- After increasing the acoustic pressure, and thus the MI, the oscillation of the microbubbles will be not sinusoidal, but will show a nonlinear relationship to the pressure changes of the ultrasound wave (faster compression compared to expansion). Nonlinear microbubble oscillation generates harmonic ultrasound waves at frequencies that are multiples or submultiples of the emitted ultrasound frequency. Since these harmonic waves generated by microbubbles differ from ultrasound waves reflected from tissues, the harmonic echo components make the separation of microbubble signals from tissue noise possible, providing that the ultrasound system is equipped for detection of harmonic frequencies [20,21,23,24].
- Further increases of the acoustic pressure result in unstable microbubble oscillation. In this case, the emitted ultrasound wave and the oscillation of microbubbles are incoherent and after some cycles of unstable and irregular expansion and compression the microbubbles collapse.
- When short pulses of high ultrasound energy are used, microbubbles may suddenly be fragmented into small pieces, leading to immediate destruction of microbubbles. Microbubble destruction is associated with emission of a strong detectable signal. Increasing acoustic pressure, decreasing ultrasound frequency, and decreasing resting microbubble diameter increase the probability of microbubble destruction [18,20,24].

In summary, a low-to-intermediate MI ultrasound pulse results in linear and nonlinear microbubble oscillations, without causing collapse or destruction of microbubbles. Under these conditions, microbubbles oscillate coherently with the emitted ultrasound pulses causing stable cavitation. At higher acoustic pressure, however, microbubble oscillation becomes unstable and finally microbubbles collapse (inertial cavitation), generating shock waves and liquid jets. Similar processes at higher energy level can be observed when ultrasound energy is further increased, leading to immediate microbubble destruction [18,20,24].

Bioeffects of microbubbles on vascular permeability

The physical changes of microbubbles in an ultrasound field can cause strong mechanical stress to the adjacent endothelial cells and vascular wall, increasing the vascular permeability to circulating macromolecules. These bioeffects of microbubbles can be spatially and temporally controlled by application of ultrasound energy. Increase of vascular permeability can be explained by different mechanisms [25].

- At high acoustic pressure, inertial cavitation of microbubbles as well as microbubble destruction can transiently (for 20–30 s) permeabilize cell membranes (sonoporation) due to shock waves and liquid jets produced by the collapse of microbubbles [26]. (Cell membrane permeability can be increased by high-intensity ultrasound on its own as well; however, microbubbles significantly increase this effect in the presence of ultrasound with high acoustic pressure [18].)
- Volumetric changes of oscillating microbubbles (stable cavitation) increase the gap-junction distances between endothelial cells simply because of physical expansion of microbubbles leading to distension of vessel walls.
- Mechanical perturbation of cell membranes by microbubble cavitation alters the cell membrane potential and stimulates endocytosis of circulating macromolecules.

Non-microbubble-based contrast agents

The other types of ultrasound contrast materials are the so-called non-microbubble-based contrast agents (Figure 1.9B–E) [13]. These contrast materials can be:

- perfluorocarbon emulsion nanodroplets (liquid–liquid emulsions with a liquid perfluorocarbon core encapsulated by a phospholipid monolayer)