Applications of high-frequency ultrasound microscopy in medicine and biology

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In this chapter, the applications of high-frequency ultrasound microscopy are reviewed. Several popular types of acoustic microscopy are included, such as scanning acoustic microscopy, ultrasound backscatter microscopy, atomic force acoustic microscopy, and resonance ultrasound microscopy. The working principle and system diagram (both hardware and signal processing) are introduced first. Currently, there are plenty of their applications in medicine and biology, such as enzymatically induced degradation of articular cartilage, three-dimensional characterization of human skin, melanoma progression, infarcted myocardium, early-stage mouse embryos, heart muscle cells, intracellular changes, and cell apoptosis. The technological development of various acoustic microscopies in the past decades is described, and their advantages and limitations are compared. The future development of this device in both technical and application aspects is discussed for both researchers and users.

Keywords: acoustic microscopy; high frequency; resolution; ultrasound transducer; backscattering; scanning; atomic force; resonance

1. Introduction

The idea of acoustic microscopy was proposed by S. Ya. Sikolov in 1936 to illustrate opaque media at the frequency of 3 GHz. The first practical instrument, an ultrasonic absorption microscopy at 12 MHz, was constructed at University of Illinois till 1959 (1). In the 1970s, advances in high-frequency ultrasound made the acoustic microscopy with high resolution and high quality feasible for biomedical applications (2, 3). The unique advantage of acoustic microscopy over the other forms of radiation is its ability to penetrate living specimens without causing damage for tomographic imaging. The high-frequency acoustic microscopy can visualize subsurface structures and has clinical applications in imaging of eye (4), skin (5), blood flow, and mouse embryonic development (brain and heart) (6) as well as intravascular and intra-articular catheter-based diagnosis. The thickness of both anterior and posterior chambers and the opening angle of the eye can be measured through the cross-sectional view. Tumor staging and boundary definition (i.e., melanoma), the progressive response of tumors to chemo- and radio-therapy, inflammatory skin disease (i.e., psoriasis), skin aging, and wound healing are also possible by visualizing the epidermis and dermis (7). Intravascular ultrasound imaging provides a morphological assessment about the vessel wall or the plaque in details only within a few millimeters. Visualizing the corneal epithelium provides deep viewing into corneal disease and the effects of photorefractive surgery, and imaging the epidermis allows the diagnosis of melanoma at its early stages. Specimens can also include cells (8), chromosomes (9) and bacteria (10) down to a resolution of 200 Å.

The imaging quality of acoustic microscopy is highly determined by the characteristics of the transducer, such as the resonant frequency and bandwidth (11). The bandwidth determines the axial resolution while the acoustic radiation from the transducer determines the lateral resolution. To obtain sufficient signal amplitude, a highly focused transducer design with low F-number was implemented. The important parameters of piezoelectric materials are the electromechanical coupling coefficient ($k_p$), the dielectric permittivity ($\varepsilon_r$), the dielectric loss tangent (tan$\delta$), and the mechanical loss tangent (tan$\delta_m$). Lead zirconate titanate ceramics (PZT), the common piezoelectric material for conventional ultrasound transducer at the low frequency, has a high dielectric constant for great conversion efficiency. PZT has a decreased piezoelectric efficiency and structural integrity with the increase of frequency when being machined from bulk samples (12). Although flat PZT plates as thin as 20 μm can be achieved by lapping, accurate machining of spherically curved radiators is challenging. The piezoelectric polymer material, such as polyvinylidene difluoride (PVDF) and copolymer polyvinylidene difluoride/trifluoroethylene (PVDF-TrFE), is flexible for easy fabrication into a spherical shape and has very broad bandwidth (i.e., -6 dB bandwidth of 60-80%), but with low dielectric constant and low sensitivity at the high frequencies. Thin piezoelectric materials can grow on a substrate to the pre-determined thickness, but with the low electroacoustic coupling. At the frequency over 100 MHz, other piezoelectric materials other than the polymers and ceramics are required. Lithium niobate, a robust single crystal structure, has good electromechanical coupling and a higher speed of sound as sensitive thickness mode resonators in the frequency range of 100-200 MHz. Its insertion loss seems not to suffer the degradation of properties as polycrystalline materials (i.e., PZT). However, lithium niobate is hard to shape and has a lower dielectric constant than PZT. The ZnO piezoelectric transducer with a sapphire lens has the working frequency of 100–210 MHz with the focal beam width from 5 μm (at 210 MHz) to 10 μm (at 100 MHz). 1-3 composite transducers, embedding the fractured crystal in epoxy, typically has an increased coupling and decreased insertion loss simultaneously compared to thickness
mode of conventional crystals and ceramics. Sol gel composites can be formed from a mixture of organics, metal alkoxides, stabilizers, and calcined PZT powder, which is spin-coated onto a substrate. Krimholtz-Leedom-Matthei (KLM) model can also be used for the analysis of piezoelectric transducer at the high frequency.

In this chapter, several popular types of acoustic microscopy, such as scanning acoustic microscopy, acoustic impedance microscopy, ultrasound backscatter microscopy, ultrasound assisted atomic force microscopy, resonance ultrasound microscopy, and intravascular ultrasound, are introduced. The working principles of different systems are explained briefly, and their latest applications in medicine and biology are reviewed. Finally, the technical advantages of current modalities and the future development trends are reviewed for both microscopy operators and researchers.

2. Acoustic microscopy and its application

2.1 Scanning acoustic microscopy (SAM)

Scanning acoustic microscopy (SAM) is the common acoustic microscopy in the applications and can be used for pathological examinations with no requirement of special staining. An ultrasound transducer is usually attached to the flat posterior surface of a buffer rod to excite a plane acoustic beam, which propagates into the buffer rod, is focused at the anterior surface by a spherical or cylindrical lens, and is then transmitted into the coupling fluid to a diffraction-limited spot. The reflected ultrasonic beam by the specimen propagates back through the lens and is picked up by the transducer. The transmitted ultrasound beam through the specimen reflects from the glass base and returns to the transducer later (see Fig. 1). Variations in the localized mechanical properties of a specimen or the location of the surface with respect to the beam focus change the amplitude, phase, and arrival time of the reflected signal. By scanning either the ultrasonic transducer or the specimen, a 2D topography of the specimen could be constructed. Typical scanning speeds of each frame are 20-30 s at the frequency of 0.1-2 GHz for a reviewing field of up to 0.5×0.5 mm. SAM is basically a transmission imaging method. The biomechanical properties (i.e., elastic bulk modulus) of the specimen could be demonstrated by acoustic parameters, such as the speed of sound (SOS), attenuation, and acoustic impedance (see Fig. 2). The acoustic attenuation and SOS of the specimen can be determined from the amplitude and phase of the received signals, respectively. The acoustic attenuation depends on the molecular weight of the sample. SOS is closely correlated to the density and elastic bulk modulus of the specimen. Thus, acoustic properties can be used to determine the characteristics of biological samples. Scattering is related to structural features of the material and spatial distribution of acoustic impedance while absorption is intimately related to the macromolecular content of the material. Thus, for structural media in high density and large molecular weight, such as collagenous tissue, both scattering and absorption lead to high attenuation. The signal processing could be carried out in either time domain for burst wave or frequency domain for a single ultrasound pulse. Since the 1980s, SAM has been used to diagnose myocardial infarction (13), renal diseases (14), aortic atherosclerosis (15), ligaments (16), and lungs (17).

Although the number, contour, adjacent soft-tissue edema, and internal architectures of surface lymph nodes swelling can be diagnosed by ultrasonic echography easily, the resolution is limited in revealing the precise anatomical structure. The echogenic hilum in lymphomatous nodes is hard to find, and metastatic nodes with extracapsular spread demonstrate ill-defined borders. However, SAM could show normal structures and localized/diffuse lesions of the lymph node clearly and accurately (see Fig. 3). Soft tissues with poor cell structures (i.e., cystic necrosis, medullary cord, and sinus) have smaller SOS than harder ones (i.e., coagulative necrosis, granulomas, collagen, fibrosis, muscle fibers, follicles, and paracortex) with the cell-rich structures. SOS increases because of stromal desmoplastic reactions and cellular concentration. In neoplastic lesions, SOS is significantly different among scirrhous carcinomas, lymphomas, and medullary carcinomas. In comparison to light/optical microscopy, SAM has inferior resolution and a limited visualization field, but with several advantages: the tissue elasticity could be imaged; quantified SOS data could be used for statistical comparison; image reconstruction is quick (a few minutes) without special staining; SAM images
have higher resolution than echographic images for clinical diagnosis to discriminate normal structures of lymph node components.

**Fig. 2** Presentative images of acoustic intensity, speed of sound, acoustic attenuation, and thickness of the specimen reconstructed by scanning acoustic microscopy, with courtesy of (Miura, Nasu 2013).

**Fig. 3** Comparison of lymphoma cells in H&E stain (top) and SOS images in scanning acoustic microscopy (bottom) of diffuse large B-cell lymphoma (left), Hodgkin lymphoma (middle), and follicular lymphoma extending into the extracapsular adipose tissue (right), with courtesy of (18).
Ultrasound is important in assessing musculoskeletal soft tissues (19), articular cartilage and subchondral bone (20) because of its high frame rate, low cost, and high resolution compared to magnetic resonance imaging (MRI). High-frequency acoustic microscopy also provides a sensitive and quantitative analysis of cartilage structure and properties, such as the diagnosis of early osteoarthritis (OA) during arthroscopy. Cartilage exposed to high stresses during joint motion has higher acoustic attenuation than that exposed to low stresses. High-frequency ultrasound has already illustrated heterogeneous echo characteristics in normal articular cartilage (21). The acoustic scattering from the surface of the normal and degenerated cartilage is different, which is used to detect the surface roughness in OA (22). SOS in cartilage is related to collagen fibril orientation (23), but no significant correlations are found between the SOS and the biochemical composition of human cartilage (21). The acoustic reflection coefficient at the interface of bovine cartilage and saline and SOS decrease (-96.4% and -6.2%, respectively) after the collagenase digestion. Collagenase and chondroitinase ABC, enzymes that selectively degrade collagen fibril network and digest proteoglycans, respectively, significantly decrease Young’s modulus of cartilage (24). High-frequency acoustic microscopy produces the images of the articular cartilage in high resolution with its intensity strongly and positively correlated with SOS in SAM in the superficial and middle layers (25). It has several operating modes: C-mode acoustic imaging of excited thin tissue slice, acoustic impedance imaging of the tissue surface, and 3D tissue visualization reconstructed from B-mode images. Its spatial resolution is 15 μm working at the central frequency of 120 MHz so that it can clearly distinguish three layers and visualize the non-homogeneous middle layer of articular cartilage as the conventional high-frequency B-mode sonography. Although SAM can evaluate the mechanical property (i.e., elasticity) and distribution of tissue, it is not adaptable for in vivo real-time and sequential evaluation because of the requirement of sliced tissue.

2.2 Acoustic impedance microscopy

As the specimens for conventional scanning acoustic microscopy need to be flat and thin, only excised sample or cultured cells can be used. Acoustic impedance microscopy can assess the specimen surface without slicing in high resolution for both in vivo and intra-operative examination (26). The specimen is usually on the plastic plate, and ultrasonic wave penetrates through the plastic plate and reflects at the tissue surface. 2D profile of acoustic impedance can be measured by scanning the focal point of the ultrasound transducer on the rear surface of the substrate either mechanically using the x-y translational stage or electrically using the phased array design. As most of the substrates have higher acoustic impedance than biological samples, strong reflection and the phase change of the transmitted signal occurs at the interface. However, too high acoustic impedance of the substrate may reduce the transmitted signal to the specimen and subsequently the signal-to-noise ratio (27).

2.3 Ultrasound backscatter microscopy

Backscatter ultrasound systems operating at the frequency higher than 40 MHz could provide microscopic resolution, and thus is termed as ultrasound biomicroscopy (UBM). The acoustic burst at the frequency of 40-100 MHz is transmitted into the tissue, and the backscattered signal is received by the same transducer and amplified using a time-gain circuit to compensate the attenuation of acoustic beam in the tissue. After logarithmic compression, the backscattered signals from a 4-mm depth around the focal plane are digitized and then displayed as B-mode images. Quantification of backscatter, attenuation, and SOS of both tissue layers and blood as well as fiber orientation, skin tension, and water content are necessary for proper interpretation of superficial skin under acoustic microscopy in the frequency range of the 100-200 MHz. The current UBM has a lateral resolution of 14 μm, an axial resolution of 12 μm, and an insertion loss of 18 dB (28). Despite a great progress, high-frequency ultrasound microscopy has limited penetration depth due to the signal losses in the tissue. Recent breakthroughs in the transgenic mouse technology allow mouse skin models of melanoma to evaluate the performance of UBM. However, translation to clinics still needs some efforts.

Furthermore, UBM can also be used for in utero analysis of early brain and heart development and manipulation of mouse embryos (29). The mouse is the primary model of mammalian development and has been used to develop a large number of human disease models by using the transgenic and gene targeting methods. Initially, the investigation of embryogenesis in the mouse was limited to histological analysis of fixed specimens in high resolution using scanning electron microscopy (SEM) (30) and magnetic resonance microscopy (MRM) (31). In comparison, UBM is the noninvasive real-time diagnostic modality for mouse embryos, and can easily provide accessible image guidance for manipulation of mouse embryos in utero. The use of ultrasound contrast agents under the guidance of UBM can allow to observe the effectiveness and precision of injections into parenchymal tissues of embryo easily and simply. However, the most significant image artifacts are caused by respiratory, which may be solved by the ultrafast sonography using the technology of planar wave excitation. Overall, application of high-resolution UBM in the field of genetics provides a powerful tool to investigate and understand the mammalian developmental processes.

Rapid and quantitative measurement of the structure and organization of tissue is in a great need not only for research but also in the clinic. Crypts and cells in polyps are arranged irregularly, causing the increased backscatter and acoustic impedance for their ultrasound signals. Heterozygosity in adenomatous polyposis coli (Apc), a precancerous situation,
appears normal in the examined cross-sections, but the significantly increased variations in the tissue organization by ultrasound microscopy. Measuring these premalignant changes (backscatter and acoustic impedance) provides a potential approach to detect microscopically abnormal tissue regions, independent of visual examination or biopsies, especially for endoscopy screening of the intestinal tract in the clinic. Although the resolution of ultrasound microscopy (about 100 μm at the frequency of 45.0 MHz with the aperture of 1.5 mm and F number of 4) is not sufficient to measure the properties of individual crypts (≥ 50 μm), changes in their packing and overall structure can be detected (32). Optical microscopy obviously illustrates an increased density of epithelial cells and myofibroblasts in polyps (see Fig. 4). The higher acoustic impedance allows the diagnosis of polyps much earlier (no larger than 300 μm) than current possible methods while backscatter shows their concentration and size and relates to their spatial distribution. In isolated cells, the most effective scatterers are nuclei due to the differences between their density and compressibility and those of other intracellular structures. In cell aggregates, increased backscatter is because of the distribution of nuclei with more irregular spacing.

Fig. 4 Comparison of superficial polyps in the intestinal tissue from ApcMin/+ mice viewed (A) at 10× in the optical microscopy and (C) three-dimensional image reconstructed from 580 individual 45 MHz B-mode sonographs, and the corresponding cross section (B) and (D) along the line between i and ii showing the region of resected and sectioned polyp, white asterisks corresponds to the muscle layer, scale bars of 1 mm, with courtesy of (32).

2.4 Ultrasound assisted atomic force microscopy

Atomic force microscopy (AFM) has the resolution on the order of sub-nanometer, more than 1000 times better than the optical diffraction limit. In AFM the cantilever contacts with the specimen and is vibrated at or near the resonant frequencies and then quantitative information of specimen, such as Young’s modulus, is obtained through these dynamic approaches. The high lateral resolution is given by the contact area between the AFM tip and the surface of the specimen. AFMs can determine the forces between the cantilever and the specimen as a function of the mutual separation, form topography of the specimen surface by raster scanning, and manipulate the sample and change its
properties in a controlled way. The AFM has been applied widely in solid state physics, semiconductor science and technology, molecular engineering, polymer chemistry and physics, surface chemistry, molecular biology, cell biology and medicine. Applications of ultrasound in AFM can be categorized into two types (see Fig. 5): atomic force acoustic microscopy (AFAM) (33–35) and ultrasonic atomic force microscopy (UAFM) (36–39).

AFAM combines the traditional AFM with acoustic vibration to map the elastic modulus distribution of specimen surface in higher resolution and more information than the topography. The tip of AFM cantilever is in contact with the specimen, whose bottom is attached to a piezoelectric transducer, causing out-of-plane surface vibrations in the specimen and vibration coupling between the AFM cantilever beam and the specimen surface. The amplitude of cantilever vibration at the transducer excitation frequency (on the order of MHz) is measured by the AFM photodiode using a lock-in amplifier. A spectrum of the cantilever response can be acquired by sweeping the excitation frequency, from which the first two flexural resonance (contact-resonance) frequencies are determined and quantitative measurements and imaging of specimen are exploited (33). The contact-resonance frequencies are higher than those in the free space (the free-resonance frequencies). Interaction forces between the cantilever tip and specimen are related to the increased stiffness of the system and the shift of the resonance frequencies. The static loads applied to the cantilever in AFAM are typically much greater than the adhesion forces, but still lower than the threshold of plastic deformation of the surface (40). AFAM needs a reference material with known elastic properties. In comparison, dynamic AFM in the mode of force modulation, the contact resonance technique needs only relative cantilever vibration amplitudes for quantitative measurements and avoids direct measurement of the contact radius. AFAM can make the measurement in good agreement with more conventional techniques (i.e., nano-indentation) and successfully measure films as thin as 50 nm, which is the major challenge for nano-indentation. The small tip diameter (10–100 nm) enables in situ elastic property information with nanoscale spatial resolution (34). AFAM is useful for determining the topology, structure and elasticity property of vascular smooth muscle cells (VSMCs) with short scanning time and negligible harm or damage to the cell (41). Increased vascular stiffness with aging is attributable not only to changes in extracellular matrix (ECM) but also to intrinsic changes in VSMCs. However, AFM could not illustrate the intrinsic changes and intracellular structure in VSMCs. AFAM showed clearly the unsmooth and weak integrity of the nucleus membrane surface of VSMCs, elasticity heterogeneities of nucleus, nuclear membrane and nuclear pores across the cell surface, and the distribution of the nucleus, nuclear membrane, cytoskeleton, cytoplasm, cell membrane and contradiction among cells. In comparison to the fluorescence microscopy, electron microscopy, and the laser confocal microscopy, the sample preparation for AFAM is extremely simple with negligible damage to cells. In addition, AFAM can analyse the surface, internal structure and elasticity of cells at the nanometer level in a short scanning time (only a few minutes).

UAFM combines high spatial resolution and nanomechanical contrast with the non-destructive nature of tapping mode AFM. Ultrasonic vibration is applied to the AFM cantilever (42) and leads to reliable measurement of stored and loss moduli by measuring the spectra of deflection vibration of a cantilever (36). The cantilever is excited by a voltage-controlled oscillator (VCO) whose input voltage is swept to produce the resonance of tip in contact with the specimen. The deflection signal from the cantilever is measured by the photodiode and split into two parts: one being low-pass filtered to control the vertical position of the sample and the other being band-pass filtered and compared with the phase of VCO output signal. The output of the error amplifier caused by the phase change is added to the input of VCO as the negative feedback control to stabilize and determine the resonance. Thus, the cantilever is always vibrated at the resonance frequency, and the vibration amplitude represents the $Q$ factor (the ratio of the peak frequency to its width). Because the vibration amplitude at the resonance frequency is linearly proportional to the $Q$ factor, the resonance frequency and $Q$ factor are determined by scanning the sample in the constant force mode and tracking the corresponding resonance spectra, which allows mapping of the resonance frequency and $Q$ factor more quickly (38). While diagnosing the amyloid-$\beta$ aggregate morphology in vitro is critical in understanding the pathology of Alzheimer’s disease (AD) and the development of aggregation inhibitors, the assessment is highly toxic. Early aggregates are difficult to observe by electron microscopy (EM) and AFM due to low contrast and variation of peptide attachment to the substrate. The requirement of heavy metal staining in EM imaging of biological samples leads to cross-linking between residues in the protein, distortion of the substructure, and mask of nanostructural features.

Therefore, diagnosing the unstained samples is limited to proteins with the molecular weight of more than 100 kDa, which is problematic for very small structures. In contrast, AFM imaging is relatively slow, does not permit rapid alteration of the magnification and viewing field, and needs nanoscale flat rigid substrates. The nanomechanical imaging by UAFM reveals a periodic twist to the $\beta\beta$ amyloid fibrils, a frequently observed criterion of $\beta\beta$ fiber morphology (see Fig. 6). However, the topography does not show the variation in the elastic properties of the fiber, but only a slight decrease in height (43). Although UAFM is less susceptible to adhesion artifacts than AFM, its increased normal forces may change the vertical features more than in AFM, and irreversibly change nanostructures if they have a low threshold for plastic deformation.
2.5 Resonance ultrasound microscopy (RUM)

The internal friction of material shows mechanical loss caused by the anelastic motion of dislocations, thermally activated diffusion of defects, interactions between magnetic domains and mechanical vibrations. Local measurement of internal friction is important in nondestructive evaluation and materials science. A rectangular-parallel-piped monocrystal-langasite (LGS) oscillator touches a specimen through a monocrystal diamond tip. Driving the solenoid coil which surrounds the probe without touching it by high-power radio-frequency bursts produces an oscillating electric field and excites free probe vibrations through the converse piezoelectric effect. After that, the same coil picks up the vibration through the piezoelectric effect (see Fig. 7). The surface of the specimen is scanned to measure the resonance frequency and attenuation coefficient using the generalized Hertzian-contact model. The RUM images can illustrate the distribution of the elastic and damping properties (44). In comparison to the observations with optical microscopy, RUM can show clearly the modulus at the interfaces between the matrix foils and fiber foils (see Fig. 7). It would be easy to construct a mobile probe for in situ applications. However, AFAM approach is too sensitive to the environmental effects (i.e., temperature and sound noises) for a stable measurement of the resonance frequency.

2.6 Intravascular ultrasound (IVUS)

Intravascular ultrasound (IVUS) has been developed to diagnose atherosclerosis which may cause the ischemia in the downstream organs for inferior limb arteriopathy, coronary ischemia, myocardial infarction, and brain stroke and is the major cause of morbidity and death in the developed countries (46, 47). After cannulation of the artery and passage of a standard interventional guiding catheter, an ultrasound catheter can be introduced and positioned in the region-of-interest generally under a radioscopic control. A radial 360° scan can be performed in different ways either by rotating a side-looking single piezoelectric transducer or rotation of a deflecting mirror in front of a fixed transducer or by the use of an electronic cylindrical phased array (up to 64 elements). The mechanical rotation speed can be at 1800 rpm, resulting in 30 images per second for real-time diagnosis. The radial scanning leads to a bird’s eye representation of the vessel slice so that the operator can immediately observe the available lumen for blood circulation, the layered structure of the artery wall, and the depth of the atherosclerotic lesions. Phased array IVUS catheter is easy to set up and use and can achieve almost the same image quality as that by the mechanical probes. The current IVUS catheter has a diameter of 0.87-1.17 mm (from 2.6 to 3.5 French) mounted on a 6-French guiding catheter and the driving frequency of 20-50 MHz. It is possible with high-frequency ultrasound imaging to diagnose fine details of the vessel wall structure. IVUS...
has the axial spatial resolution of approximately 150–200 μm at the driving frequency of 30 MHz and wavelength of 50 μm for the diagnosis of the arterial wall. Changes in acoustic impedance between different tissues produce the strong acoustic reflection as an interface between anatomical compartments of the vessel wall, for example between the lumen and endothelium, and between media and external elastic lamina. Lipid-laden lesions appear hypoechogenic, fibromuscular lesions generate low-intensity echoes, and fibrous or calcified tissues are echogenic in IVUS. Calcium obscures the underlying wall as acoustic shadowing. Thus, IVUS can determine the thickness and echogenicity of vessel wall structures accurately. Diagnosis of left main coronary artery diseases is one of the most important applications of IVUS, quantifying the degree of lumen restriction and characterizing the type and the geometry of the plaques. It also emerges as the optimal method for the detection of transplant vasculopathy, identifying atheromas at risk of rupture. As a real-time diagnostic technique, IVUS has the unique potential to provide assistance and feedback to the intraluminal operation or surgery. The safety of intracoronary ultrasound is satisfactory with the rate of minor complication (i.e., transient spasm) varying from 1% to 3% and major complication (i.e., dissection or vessel closure) < 0.5%. It is noted that all complications are related to intervention instead of ultrasound diagnosis. Vessels examined by IVUS shows no accelerated progression of atheroma at 1 year of follow-up. There are also some artifacts involved in IVUS sonography, such as “ring-down” artifacts produced by acoustic oscillations of the ultrasound transducer that obscure the near acoustic field, resulting in an acoustic catheter size larger than its physical size; geometric distortion in an oblique plane; “non-uniform rotational distortion” due to uneven drag on the drive cable of the mechanical catheters, resulting in cyclical oscillations in rotational speed. The primary disadvantages of IVUS are its expense, the increased time in the interventional procedure time, and only being performed by angiographers trained in interventional cardiology techniques. One of the current developments in IVUS is the introduction of the forward-looking probe to identify the lumen in front of a total occlusion and the location of lesions. In comparison to the optical coherent tomography (OCT) with an axial and transverse resolution of 20 μm and 30 μm, respectively, IVUS has an inferior delineation of vessel layers, leading to less clear visualization of the near-occlusive coronary plaques and intima (see Fig. 8).

Fig. 8 Comparison of stenosis in the right posterior descending artery before (A, arrow) and after (B) with cutting balloon angioplasty, by intravascular ultrasound (C), and multiple tears in the intima after dilatation with a cutting balloon found on the optical coherence tomographic image (D), with courtesy of (48).
3. Discussion and conclusion

The noninvasive visualization of living biological sample at the microscopic level is a long cherished requirement in the science of medicine and biology. Optical microscopy has the advantage of the intrinsically high resolution, even for subsurface detail by on the development of confocal microscopy and optic coherent tomography (OCT). Another popular modality is magnetic resonance microscopy (MRM) with the ability to directly determine the density of hydrogen atoms in the tissues and to trace the early motion of cells in embryology using contrast tags (49). The development of acoustic microscopy could provide novel and critical information for histological analysis of tissue sections. The applications of acoustic microscopy are in ophthalmology, intravascular, dermatology, cartilage assessment, tumor biology and mouse embryonic development (50). There are many trade-offs between resolution, penetration, and system dynamic range in system construction and application. The use of high frequency results in better resolution and conspicuity changes in the acoustic backscattering from particles, but limited penetration depth of field and challenges in the design and manufacture of transducers. Understanding the imaging quality in the acoustic microscopy requires the deep and detailed knowledge of the acoustic properties of human tissues and characteristics of acoustic field produced by the microscopy. The essential components and considerations of acoustic microscopy are similar to those of a conventional sonography system at the low frequency. The eye is an ideal target for commercially available acoustic microscopy in detecting anterior segment tumors, glaucoma, scleral disease, corneal disease and pachymetric mapping, intraocular lens assessment and trauma. Diagnosing angle recession, iridodialysis, cyclodialysis, hyphaema, intraocular foreign bodies, and scleral laceration in ocular trauma are approved effective (4). Doppler could also examine the microcirculatory system of the eye in both 2D and 3D viewing. Blood velocities as slow as 0.2 mm/s were detected in ciliary vessels of a rabbit eye as small as 40 μm (51). Ocular acoustic microscopy could provide the assessment of multiple injuries, characterization of the vascular status of anterior segment tumors, and insight into the pathophysiology of diseases, such as glaucoma, which has limited blood or corneal opacity. However, intraocular penetration depth is only 3–6 mm. Intravascular ultrasound (IVUS) imaging has been developed well with novel interventional instrument, such as balloon angioplasty and stenting for the treatment of coronary artery disease. Intracoronary blood velocity measurement at the amplitude of tens of cm/s, chronic and acute measuring of blood flow in small arteries in the diameter of 0.5–3 mm, and the intraoperative assessment of microvascular anastomosis are possible using IVUS. So it has been used in clinical coronary care in the diagnosis of calcium content, residual plaque burden, tissue trauma, and final lumen dimensions because of high sensitivity than angiography, evaluation of short- and long-term outcome following intervention, prediction of restenosis, and determination of vasculopathy related to heart transplantation, where angiography is inaccurate (52). Combined morphological and functional imaging has also been examined in renal artery stenosis. High-frequency ultrasound has also been tried in the diagnosis of cutaneous lesion. Although physical measurements agree reasonably well with histology (53), it cannot differentiate benign and malignant skin lesions (54, 55). Thus, its clinical acceptance is still limited now. Further development of acoustic microscopy relies on two aspects (56). One is the technical advances in the transducer development, beam forming and signal-processing at the high frequency, such as piezoelectric material, phased-array beamforming, color-flow Doppler, and power Doppler (57, 58). Optimization of transducer sensitivity and focusing properties are critical factors in the continuing evolution. The resolution is usually improved by increasing the driving frequency or decreasing the F-number of the transducer. Time reversal mirrors used in the acoustics, such as medical therapy and nondestructive evaluation of materials, can improve the focusing ability of high-frequency ultrasound through the heterogenous media. In the time reversal process, the pressure field detected by a set of transducer elements is digitized and stored during an evaluation of materials, can improve the focusing ability of high-frequency ultrasound through the heterogenous media. In the time reversal process, the pressure field detected by a set of transducer elements is digitized and stored during a time interval. The pressure field is then resynthesized by the same transducer in a reversed temporal chronology. As a result, conversion of a divergent wave from an acoustic source into a convergent wave focusing on the source is possible (59). The other is the new imaging modality, such as contrast enhanced imaging (60, 61), second harmonic imaging (62, 63), photoacoustic imaging (64, 65), elastography (66). Extending the driving frequency to over GHz, it is also possible to imaging the cellular structure and evolution (i.e., intracellular changes), and the response to different stimuli (i.e., cell apoptosis).

References


