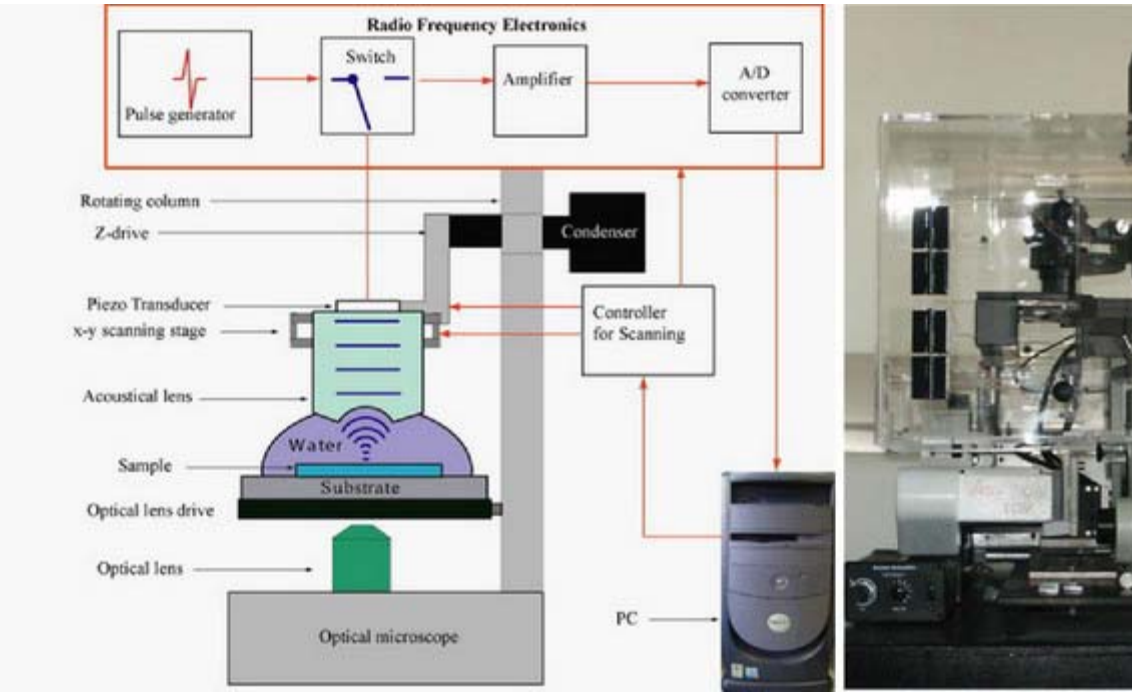
Acoustic Microscopy

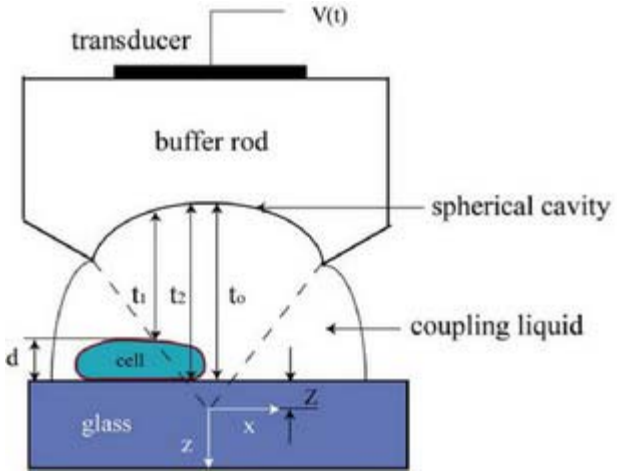
A. **History:** The acoustic microscope was developed as a tool for studying the internal microstructure of nontransparent solids or biological materials. In acoustic microscopy, a sample is imaged by ultrasound waves, and the contrast in reflection furnishes a map of the spatial distribution of the mechanical properties. Several books and handbook articles give detailed historical outlines. Briefly, the development of the first high-frequency scanning acoustic microscope was motivated by the idea of using an acoustic field to study the spatial variations of the elastic material properties with nearly optical resolution (The lateral resolution of SAM is dependent on the frequency of the acoustic waves and, at best, is about 0.75 microns). The first experiments date back to the 1940's when high-frequency acoustic images were obtained by the Leningrad scientist Sokolov (Sokolov, S., Doklady Akademia Nauk SSSR, 64, 333, 1949). He observed an acoustical image using the tube named after him, in which the acoustic picture was converted into a television display. The first scanning acoustic microscope was created by Lemons and Quate at Stanford University in 1973 (Lemons, Quate, Appl. Phys. Lett., 24, 163, 1974). It was mechanically driven and operated in the transmission mode. Since then, gradual mechanical and electronic circuit improvements have been made and image recording has been automated. In general, acoustic microscopes now work in the reflection mode.

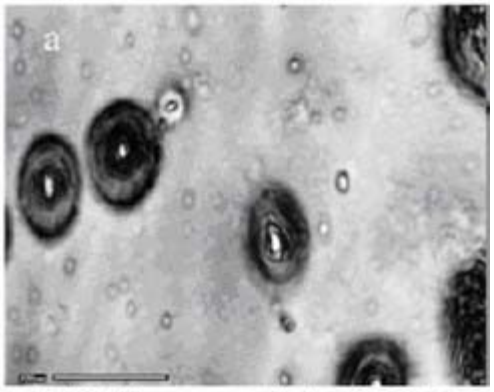


Left: The schematic diagram of the combined optical and acoustic microscope (Weiss, Lemor et al., IEEE Trans. Ultrason. Ferroelectr. Freq. Contr., 54 2257, 2007).Right: A photograph of the combined optical (Olympus IX81) and time-resolved scanning acoustic microscope, SASAM, Fraunhofer-Institute for Biomedical Technology, St. Ingbert, Germany.

B. **Principles:** The scanning acoustic microscope (SAM) can be characterized by a combination of operating principles distinguishing it from other microscope types (Zinin Weise, “Theory and applications of acoustic microscopy”. in T. Kundu ed., Ultrasonic Nondestructive Evaluation: Engineering and Biological Material Characterization. CRC Press, 654-724, 2004). These principles are (a) image generation by scanning, (b) far-field wave imaging, and (c) the use of acoustic waves. Image generation by scanning is basically different from the functionality of a conventional optical microscope which is the oldest microscope type. The conventional microscope can be considered a parallel processing system in which we can see all points of the object at the same time. In contrast to this, the scanning acoustic microscope is a sequential imaging system in which a piezoelectric transducer emits a focussed ultrasound beam that propagates through a water, to the sample. The beam is scattered by the sample, and the scattered ultrasound wave is detected piezoelectrically. The output signal is just one single voltage. As the sample is scanned, the voltage is recorded in each scanning position of the focus and a grey-scale image is generated. The use of a focussed beam leads to the second operating principle. As the focus is formed by converging propagating waves, the size of the focal spot (or focal area) is limited by diffraction. Imaging with ultrasound is the third operating principle. The operating frequencies of SAMs are between 100 MHz and 2 GHz; the high frequency provides the opportunity to obtain accurate measurement results for crack and void distributions with a resolution of up to 1 µm at a depth of 10 µm. Images made by the SAM are called C-scans. They are obtained when the acoustic microscope mechanically scans sample in a plane parallel to the sample surface Variation of the mechanical properties with depth

can be studied by scanning at various defocus values. Collecting images obtained at various defocus positions allows a three-dimensional image to be constructed, representing the volume of the entire microstructure of the investigated sample.





SAM image made by OXSAM
at 300 MHz showing subsurface

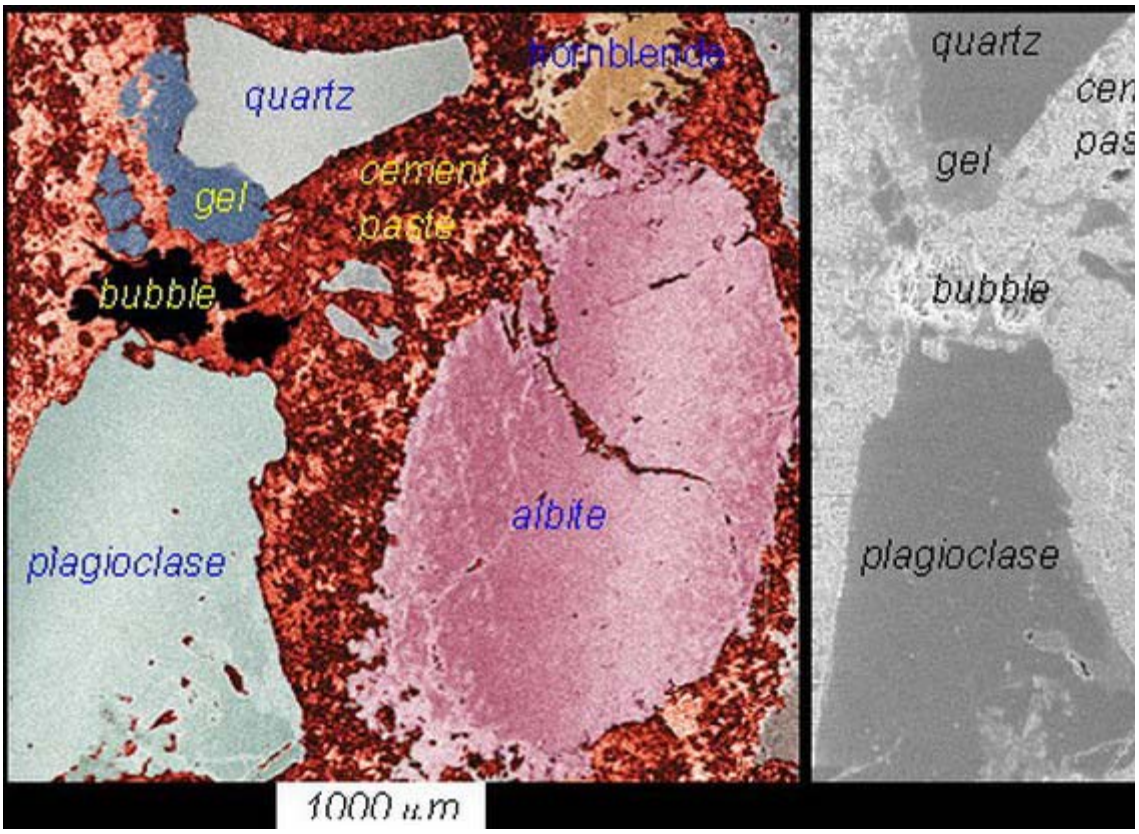
Time-resolved acoustic microscopy adds an additional degree of freedom for quantitative measurement, namely time. In time-resolved acoustic microscopy a short sound pulse is sent toward a sample (for instance biological cell). The setup for quantitative time-resolved acoustic microscopy: t_0 is the arrival time of the echo reflected from the glass substrate outside the cell ("reference echo"), t_1 is the arrival time of the echo reflected from the surface of the sample (surface echo), t_2 is the arrival time of the echo reflected off the sample/substrate interface (bottom echo), and d is the sample thickness. For layered materials the reflected signal represents a train of pulses (A-scan). The time delay of the pulses and their amplitudes provides information about the elastic properties and attenuation of sound in the layer. The velocity of the wave can be determined by measuring the time delay of the corresponding pulse. Time resolved images obtained by mechanical scanning along a line are called B-scans.

C. **Development:** Considerable progress in the acoustic microscopy of solid structures has been made since then (Briggs, A. Acoustic Microscopy, 1992, Zinin, "Quantitative Acoustic Microscopy of Solids", in Handbook of Elastic Properties of Solids, Liquids, and Gases. Volume I, Levy et al., eds. 2001, 187). Considerable progress in the acoustic microscopy of solid structures has been made since then. Developments in the theory of the image formation of subsurface defects (Lobkis et al. 1995) and three-dimensional objects (Zinin, Weise et al., Wave Motion, 25, 213, 1997) allow size and location of objects inside solids to be determined. Conventionally, SAM images show variations of

the amplitude of the acoustical signal. Reinholdtsen and Khuri-Yakub (Reinholdtsen, Khuri-Yakub, IEEE Trans. Ultrason. Ferroelect. Freq. Contr. 38, 141, 1991) measured amplitude and phase of the SAM signal at low frequency (3 to 10 MHz) to improve subsurface images. Grill extended this technique to high (1.2 GHz) frequency . This technique permits reconstruction of the surface relief of the sample with submicron resolution (Grill, Hillmann, et al., Advances in Acoustic Microscopy. Briggs, Arnold, eds. vol. II: 167, 1996). Combining the time-of-flight technique with acoustic microscopy provides a powerful tool for investigating adhesion problems as well as the microstructure of small superhard samples. An important step has been made in the direction of imaging subsurface structures at high temperatures. Ihara et al.(Ihara, Jen, France, Rev. Sci. Instrum., 71, 3579, 2000) developed a sound imaging technique to see a small steel object immerged in molten zinc at 600^{o}C. With the development of the ultrasonic force microscope (Kolosov, Yamanaka, Jap. J. Appl. Phys. 32, L1095, 1993) and the atomic force acoustic microscope (Rabe, Arnold, Annalen Der Physik 3, 589, 1994) the capability of the conventional acoustic microscope has been expanded to nanometer resolution.

Application of SAM in Materials (Natural and Artificial) Science

- Measuring the elastic properties solids and thin films
- Measurement and visualization of adhesion in layered structures.
- Subsurface imaging: the most common application of the acoustic microscope is the detection of subsurface defects in coatings.
- Visualization of stress inside solid materials (Drescherkrasicka, Willis. *Nature* 384, 52, 1996).
- Characterization of carbon-fiber-reinforced composites (Manghnani, Zinin, *et al*., Acoustical Imaging, Vol. 27, 83, 2004).

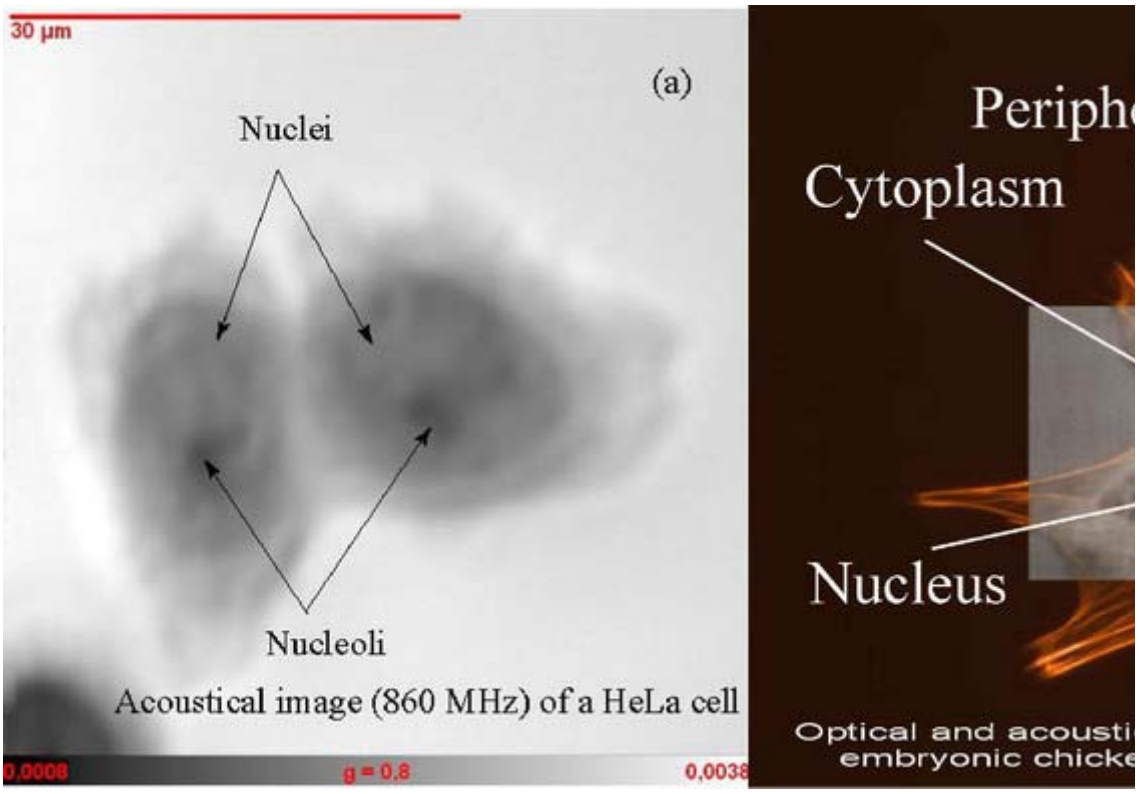


Acoustic (left) and SEM (right) images of concrete sample made with granitic aggregate grains and Portland cement paste. The acoustic image was made at 400 MHz.

Application of SAM for Elastic Characterization of Biological Cells

Mechanical characterization of biological cells and tissue cytoplasm by a conventional acoustic microscope was discussed thoroughly in the following review: Bereiter-Hahn, Blase, Ultrasonic Characterization of Biological Cells, in T. Kundu ed., Ultrasonic Nondestructive Evaluation: 722, 2004. Recently, a new high-frequency (1 GHz) time-resolved acoustic microscope was developed at the Fraunhofer-Institute for Biomedical Technology, St. Ingbert, Germany (Weiss, Lemor et al., IEEE Trans. Ultrason. Ferroelectr. Freq. Contr., 54 2257, 2007). It is based on an optical microscope from Olympus and it operates in a reflection mode. The design of the new microscope is different from that of conventional acoustical microscopes in that it has a modular structure. The microscope consists of four main modules: acoustical lens; optical module; scanning unit; and high-frequency electronics. This new microscope can be characterized by a combination of operating principles and design features distinguishing it from other high-frequency acoustic microscopes. These principles are: (a) it operates in time-resolved mode; (b) it is designed as an attachment to an inverse

optical microscope; (c) it is fully automated ; (d) measurements can be done at 37oC. Such a combination is of importance for studying dynamical processes in biological cells and temperature sensitive materials. This microscope enables us to measure acoustical properties of a single HeLa cell in vivo and to derive elastic parameters of subcellular structures.



From *IEEE Trans. Ultrason. Ferroelectr. Freq. Contr.*, **54** 2257, 2007, *Ultrasound Med. Biol.* **33**, 1320, 2007.

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