Acoustic Microscopy: Biomedical Applications

Variations in elastic properties within a single cell can now be visualized with the acoustic microscope.

Ross A. Lemons and Calvin F. Quate

The microscope in its various forms is one of the most powerful and widely used scientific tools. In the fields of biological and medical sciences advances of great significance have resulted from observations made with these instruments. The microscope enables us to explore the structural details beneath the limits of our direct visual perception. The variety of phenomena in this miniature world is so large that it is imperative that we examine all forms of radiation that can be used for such exploration. Optical waves in the optical microscope and electron waves in the electron microscope are the most common forms. It is the wavelength of this radiation that determines the minimum size of the object that can be distinguished. With recent technological advances it is now a straightforward matter to generate acoustic energy with a wavelength comparable to that of light. It follows that this could provide us with a new instrument for microscopy (*1*).

A scanning acoustic microscope based on acoustic radiation with this short wavelength has now been developed to the point where it can be used to resolve detail in biological material at the subcellular level (*2*). This new form of radiation in the field of microscopy should permit us to increase our understanding of that material and to gain further insight into the processes that go on within cells and microorganisms. This expectation is based upon the fundamental distinction between light and sound and upon the interaction of these different forms of radiation with the material of the specimen. In contrast to the dielectric properties of the object which determine the content of an optical micrograph, it is the elastic properties that determine the content of an acoustic micrograph. Variations in the elastic constants can be revealed by changes in the amplitude and phase of an acoustic wave which passes through the specimen. In this article we will deal primarily with the changes in amplitude. This varying attenuation can be produced either directly by absorption or by the scattering arising from the acoustic impedance mismatch between a region of the specimen and the surrounding material.

With the work to date we have learned that biological material—unstained—can exhibit high contrast when subjected to acoustic radiation. For example, hemoglobin (*3*) and connective tissue (*4*) show strong attenuation and these components can be easily identified in the acoustic micrographs. Furthermore, the instrument is sensitive to changes in viscosity and this should be of some advantage in rheological studies.

In this article we demonstrate the potential of the acoustic microscope and show that it represents an alternative technique for recording microscopic images that can complement and enhance the information that is provided by the light microscope. We hope that it will attract the interest of those who are professionally engaged in research with biological systems and stimulate them to consider the most appropriate areas for exploiting this new technology.

Instrument Design

The acoustic microscope that we will discuss is based upon a scanning principle wherein the specimen is moved through a focused acoustic beam. This instrument has been operated with acoustic frequencies as high as 1000 megahertz. In liquid water the wavelength corresponding to this frequency is 1.5 micrometers and the resolution approaches 1 μm (*5*). We are confident that further work will allow us to improve the resolution, but the present version is sufficient to reveal significant features in biological systems.

The critical components of the acoustic system are shown schematically in Fig. 1. Incoming electromagnetic energy is converted to acoustic energy by a piezoelectric film at the surface of the sapphire crystal. The resulting acoustic wave propagates as a collimated beam through the sapphire. At the opposite end of this crystal a concave spherical lens transmits the sound waves into a water cell. Since the velocity of sound in water is lower than its velocity in sapphire, the concave surface acts as a positive lens, focusing the beam in the water.

At first glance a single surface lens might seem wholly inadequate to focus a collimated beam to a waist of micron diameter. In an optical system of this design the spherical aberration inherent to such single surface lenses would limit the waist of the beam to a diameter of several wavelengths, and the resolving power would be seriously degraded. Optical intuition, however, is not directly applicable to an acoustic lens. This is because of the large velocity difference between sound waves in sapphire and in water. The ratio of velocities for sound in these two materials is 7.45 whereas a typical ratio for optical waves would be 1.5. A simple geometric calculation shows that the spherical aberration is inversely proportional to the square of this ratio and, therefore, this limitation on resolution can be neglected in the acoustic system (*6*).

The remaining limit on resolution is imposed by the effects of diffraction and this can only be extended by using the highest frequency in order to reach the shortest possible wavelength. Unfortunately, the attenuation of sound increases drastically as the frequency is increased. To give some idea of the magnitude of this effect, the attenuation in water at an acoustic frequency of 1 gigahertz is approximately 200 decibels per millimeter. This means that the power in the wave is reduced by 20 orders of magnitude each millimeter it propagates. The decibel loss per unit length will increase as the square of the frequency and it is necessary, therefore, to fabricate lenses of very small radii of curvature. In the present system the lenses have a focal length of 0.15 mm. We are not limited by spherical aberrations and these small lenses can be made with an F number of 0.65. This value is necessary if we are to maximize the resolution. It is the simplicity of single surface lens design that permits us to realize the combined requirements of small size and large aperture.

The sapphire crystal with its generating transducer on one face and a lens surface on the opposite face can be thought of as a

Mr. Lemons is a research associate at the Microwave Laboratory, Stanford University, and Dr. Quate is professor of applied physics and electrical engineering, Stanford University, Stanford, California 94305.

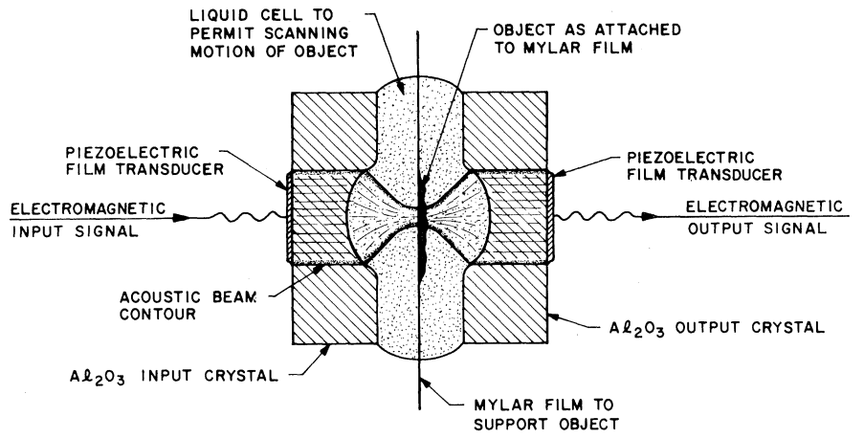

Fig. 1. Schematic diagram of the acoustic system showing the lens configuration.

transmitter which generates an acoustic probe of micrometer size. The remainder of the acoustic system consists of a receiving crystal of exactly the same design. This second crystal has a lens surface which faces the transmitting lens. The two lenses are confocal and in this configuration the energy which diverges from the acoustic focus will be collected and collimated in the receiver crystal. At the output end of this crystal a piezoelectric film reconverts the oscillatory strain of the incident acoustic wave into an electromagnetic signal.

The symmetry of this confocal geometry gives us an additional advantage. When the receiver has its focus coincident with that of the transmitter, the effective resolution of the complete system is enhanced. The basis for this enhancement lies with the directional sensitivity of the detecting transducer. The piezoelectric film is sensitive both to the amplitude and to the phase distribution of the wave incident on its surface. This produces a pattern of sensitivity at the focus which is of the same form as the energy distribution generated by the transmitter. The product of these two functions gives an effectively narrowed focal beam diameter. A detailed analysis shows that with a large signal-to-noise ratio a confocal system can resolve spatial frequencies twice as high as can a single lens alone (*7*). Another advantage of the confocal geometry is that the signal-to-noise ratio of the system is improved. There is no background signal: the information that is detected is limited to that coming from the focus.

The acoustic properties of a specimen are measured by first affixing the sample to a 2-μm-thick Mylar membrane. In turn this membrane is mechanically translated through the focal plane of the instrument. Within the limited working space between the lenses the Mylar membrane provides a thin, strong support for the sample. Mylar has an additional advantage in that it has an acoustic velocity only slightly greater than the water which surrounds it. This helps minimize the diffraction spreading and reduces the acoustic reflections at the interface.

To obtain the two-dimensional image of the object, the object itself is scanned through the beam waist in a raster pattern. The use of mechanical scanning has been nearly abandoned in the field of macroscopic acoustic imaging, but on a microscopic scale it proves to be a highly practical technique. By definition, a microscopic object will be not only of small size but of small mass as well. Because of this, scanning can be done quite rapidly. A typical image covers a square 0.25 mm on a side and contains 5×10^4 elements of information. This area can be scanned in approximately 1 second.

The mechanism that provides the mechanical scanning of the object is shown schematically in Fig. 2. For clarity, the two lenses have been drawn apart as they are when a new specimen is mounted. The Mylar membrane that supports the object is stretched across a thin metal ring. This ring is then connected to the movement of a loudspeaker cone by a rod running through a closely machined sleeve. The loudspeaker is driven sinusoidally in time and thus provides the fast, or x-directed, scan. The y motion is brought about by displacing the whole assembly of loudspeaker and object holder in a vertical direction in a slow and uniform manner. This assembly is displaced by a hydraulic piston driven with a small pump.

The acoustic image is formed by displaying the output signal from the receiver element on a cathode-ray display. There the electron beam of the display is moved in synchronism with the mechanical motion of the object, and the signal arising from the transmitted acoustic beam is used to modulate its intensity. In this way the acoustic transmission of each point on the object will be mapped onto the display screen. An advantage of this display technique is that any desired magnification can be realized by simply adjusting the voltages that deflect the electron beam on the display. Focusing of the acoustic image is accomplished by displacing the entire object assembly with a differential micrometer in the axial or z direction. When the object plane coincides with the narrowest cross section of the acoustic beam the sharpest focus is achieved.

When the lenses are brought together in the viewing position the liquid that surrounds the object is held in place purely by surface tension. This liquid serves to maintain a continuous acoustic path as the object is scanned relative to the focused beam. The choice of water was made not only because it is compatible with biological systems. Water also has one of the lowest attenuations known to occur in those liquids with small acoustic velocities. Small acoustic velocity is, of course, desirable because this improves the resolution that can be achieved for a given input frequency. The large absorption of liquids, although limiting the highest obtainable resolution, does have one desirable feature. At the acoustic frequencies normally used in our instrument the attenuation in the water cell is high enough so that stray acoustic radiation caused by multiple reflections does not cause degradation in the image, provided care is taken to shield the electrical input and output connections from each other.

The remaining mechanical components of the acoustic microscope serve to align one lens with respect to the other. A coincidence of the lens foci is necessary and, for a beam of micrometer diameter, this requires highly precise alignment. To achieve this alignment our system employs an orthogonal pair of elastic levers to position in the x–y plane. The separation in the z direction is adjusted with a differential micrometer mounted on a precision translation stage. This translation stage also allows the lenses to be widely separated when the sample is changed. Adjustment of the relative lens position is made to give a maximum signal through the acoustic system.

It is the basic simplicity of the construction for this kind of scanning microscope that makes it possible to approach the theoretical limit in resolution. Other more elegant but more complicated schemes have been tried but their performance is inferior to that permitted by diffraction. Mechanical scanning seems to be a small price to pay for such a large benefit.

Work with Mammalian Cells and Tissue Sections

The acoustic microscope in the form as described above has been used to examine a variety of specimens. We have accumulated a number of acoustic images and are beginning to catalog the response of various biological materials to acoustic waves. In this initial survey we have seen clear distinctions between the corresponding acoustic and optical micrographs.

The samples that we have worked with can be conveniently divided into three groups. First we have results with simple cell systems containing isolated cells of only a few types. We next deal with living cells grown in culture. The last and largest group are tissue sections which contain a complex collection of cell types. We include here a selection of acoustic micrographs from each of these categories. The majority of these images were obtained with an acoustic frequency of 600 Mhz ($\lambda = 2.5$ μm). The more recent images, however, were made at a frequency of 900 Mhz ($\lambda = 1.7$ μm) with a resulting resolution which approaches 1 μm. In presenting these images a convention was chosen such that points on the sample with greater acoustic transmission are lighter in the corresponding image. Thus, dark areas correspond to regions of large acoustic attenuation.

Simple cell systems. As an illustration of this category we show in Fig. 3 a comparison between the acoustic and optical images of a human bone marrow smear (*8*). This sample was prepared by smearing the fresh material onto the 2-μm-thick Mylar support membrane. The smear was then fixed in formalin. The most numerous components in this smear are the erythrocytes and they show the largest acoustic attenuation. In addition, a number of granulocytes can be seen. These cells are the developmental elements of the leukocyte family. In the acoustic micrograph they appear larger than the erythrocytes and they have a markedly lower acoustic attenuation. In some instances, details within the granulocytes can be seen. The 900-Mhz acoustic image corresponds closely with its optical counterpart. That the shapes and outlines of individual cells are so clear is impressive to us because the red corpuscles average 7.5 μm in diameter and this is less than 4.5 acoustic wavelengths. The most striking feature, however, is the superb contrast seen in the acoustic image. For the optical image, Wright's-Giemsa's stain was applied to enhance the contrast. Thus the nuclei stained blue, allowing them to be easily distinguished from the cytoplasm; without the stain the cellular detail in the light micrograph would be poorly defined.

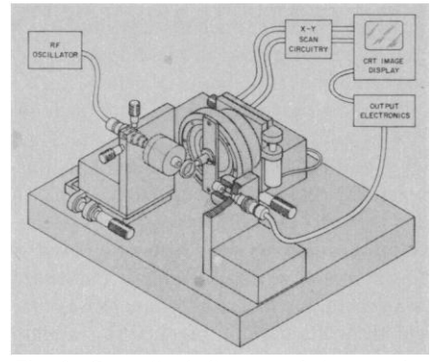

Fig. 2. Generalized diagram of the scanning acoustic microscope.

In the acoustic image, the contrast that highlights the red blood corpuscles is due primarily to the increased absorption of the hemoglobin within the cell. This absorption has been the subject of previous studies with acoustic waves at low frequencies (*3*, *4*). These studies show the absorption to be related both to the viscosity and to the molecular structure of the material. The acoustic microscope is therefore sensitive to these parameters, and can map them on a microscopic scale. Accordingly, the acoustic microscope may be useful in studying cells which exhibit abnormalities in these two properties.

Living normal human diploid cells. Cell cultures represent an important component in experimental work on living cells, normal and abnormal. We have only begun to explore the application of the acoustic microscope to this field of work, but the micrograph shown in Fig. 4 demonstrates the compatibility of the acoustic microscope with a culture of living human cells. To date we have demonstrated that cells can be cultured on the Mylar support membrane used in the acoustic microscope and that they stick to the membrane with sufficient tenacity to withstand the forces that are encountered in a mechanical scanning system of this type. In addition, a growth medium can be substituted for the water between the acoustic lenses in order to sustain the life of these cells.

Even though the lung fibroblast cells shown in Fig. 4 are nearly confluent, the extented spindle shape of individual cells can be seen. The nuclear region of an individual cell is acoustically more attenuating than are the processes which extend from it. In some instances, small areas of re-

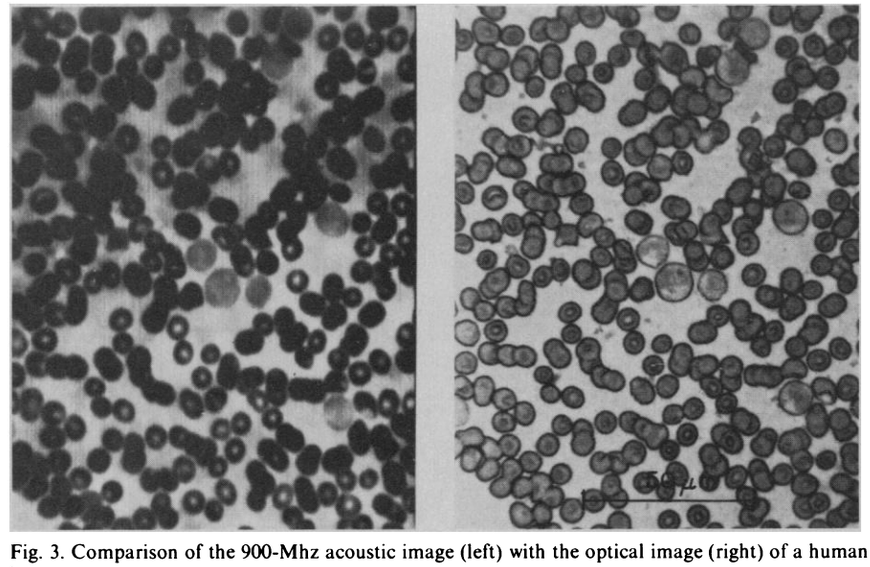

Fig. 3. Comparison of the 900-Mhz acoustic image (left) with the optical image (right) of a human bone marrow smear.

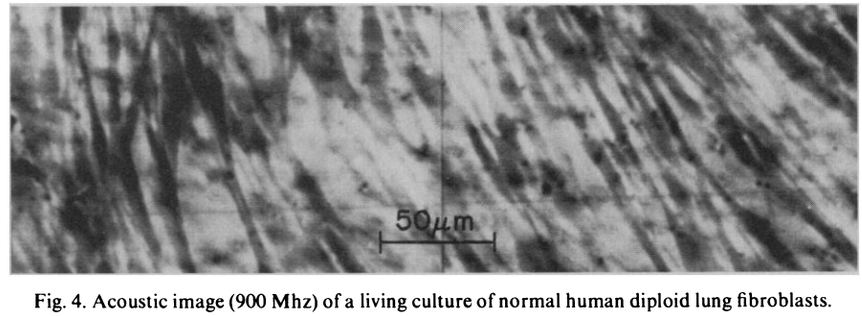

Fig. 4. Acoustic image (900 Mhz) of a living culture of normal human diploid lung fibroblasts.

duced transmission can be seen within the nuclear region; these probably correspond to the nucleoli, which often occur in pairs. The acoustic contrast of these cells is not great. In an optical microscope, however, the cells would be so nearly transparent that phase contrast techniques would have to be used to visualize them. By using acoustic phase contrast techniques it might also be possible to enhance the contrast of cells examined with the acoustic microscope.

The prime advantage of the acoustic microscope lies in the different sources of acoustic and optical contrast. For example, in liquids the acoustic absorption increases with the viscosity. Therefore, changes in viscosity should appear as changes in the absorption of the specimen. With the acoustic microscope it should be a direct and simple matter to observe those viscosity changes. Such a study might well give us new insight into the differences between normal and abnormal cells.

Normal tissue sections. In the next series of micrographs we present the images of a number of tissue sections that illustrate the power of the acoustic microscope in viewing complicated cell systems. Samples of tissue were originally fixed in formalin and were subsequently embedded in paraffin. Each of the specimens was then cut from the paraffin block with standard microtome techniques to a nominal 5-μm thickness. These sections were then mounted onto the Mylar supporting membrane and the paraffin was removed. All of the tissues were unstained and, therefore, the acoustic response is typical of what can be expected from the tissue as altered only by the procedures of fixation and embedding.

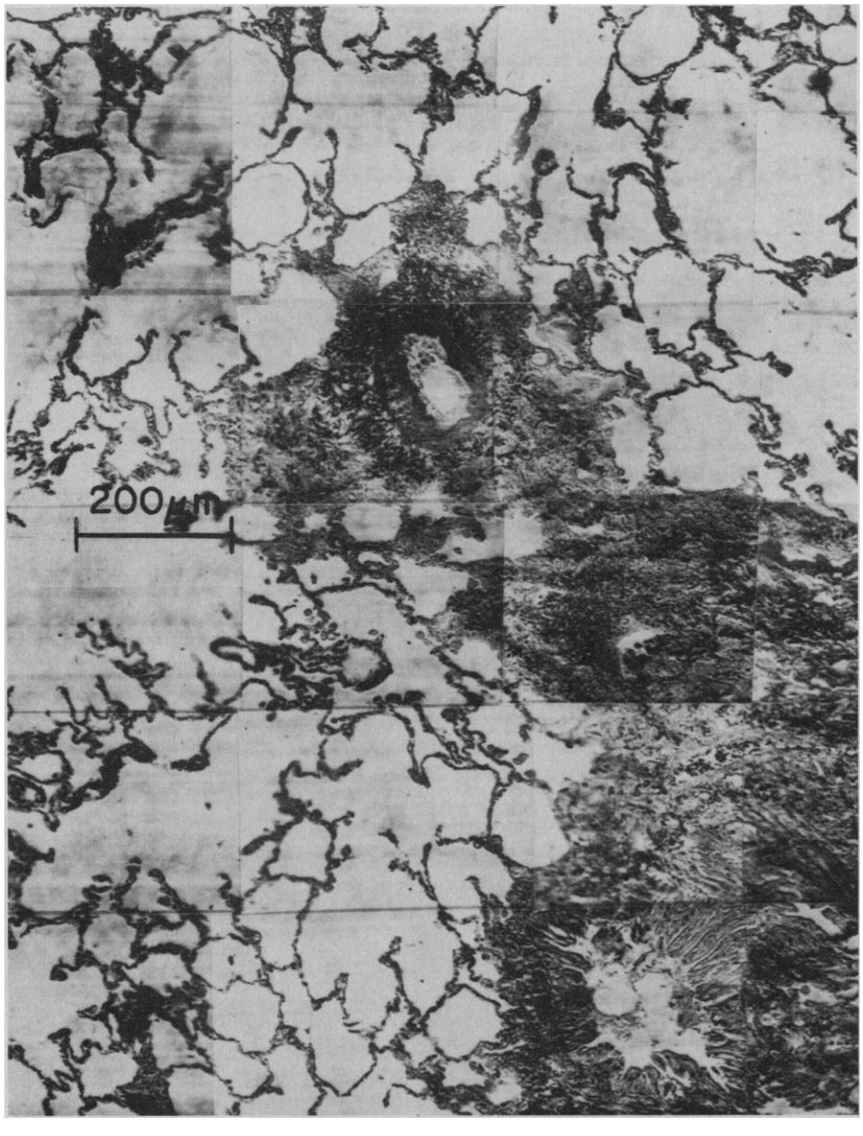

Fig. 5. Acoustic image (600 Mhz) of an unstained section of human lung tissue.

In Fig. 5 a number of the characteristic features of human lung tissue are evident. In the lower left corner is an alveolar duct with a number of adjacent alveoli. The individual cells which comprise the walls of the alveolar sacs can be seen along with several capillaries. At the lower right is the folded epithelium of a bronchiole. A small vessel is also evident just above the center of the image.

Figure 6A shows a section of the human fallopian tube with its characteristic deep branching folds of the mucous membrane. The most striking feature of the image is the sharp contrast between the internal matrix of connective tissue and the epithelial layer. As will be seen in the following figures, the large acoustic attenuation of connective tissues holds for a variety of specimens. In this instance the connective tissue is loose with numerous fibroblasts. The columnar structure of the epithelium is evident in some regions of the image, and some of the subtle detail within cells can also be seen. Of particular interest are the highly attenuating points at the outer boundary of certain epithelial cells. The epithelial layer is composed of both ciliated columnar cells and nonciliated secretory cells. The points of high attenuation seen in the acoustic image probably indicate the ciliated components.

The most prominent feature of the section of human spleen shown in Fig. 6B is the oblique view of a small artery. Inside the vessel a great number of red blood cells can be seen. Within the wall of the vessel the internal elastic membrane is the most distinctive structure. This membrane strongly attenuates the acoustic beam and thus appears as a folded black line around the inside of the artery. In contrast, optical micrographs show the elastic membrane as a translucent structure unless it has been specifically stained. The band of muscle tissue surrounding the artery is also clearly distinguished in the acoustic image. Outside of the vessel the highly absorbing erythrocytes are contrasted with the much lower average attenuation of the spleen tissue.

As a final illustration of the acoustic appearance of normal human tissue, Fig. 6C shows a section of mammary gland. At the top of the figure a small group of secretory tubules can be seen surrounded by a highly attenuating mass of connective tissue. The attenuation of this intralobular connective tissue is on the average considerably larger than that of the interlobular connective tissue as seen at the lower right. Using this normal tissue as a point of reference we will now proceed to discuss the acoustic appearance of breast tissue afflicted with cancer.

Tissue Sections Showing Pathology

Whenever a cell is modified by disease or other agents it is likely that some of its elastic properties are changed as well. This makes an investigation of the acoustic response of such material interesting and important. Our preliminary work in this area indicates that there may be significant diagnostic applications of the acoustic microscope.

This potential can be illustrated with some comparative acoustic and optical images of a sample from a malignant tumor of the human breast. A panoramic acoustic view of a section of this material is presented in Fig. 7A. After the acoustic image of this specimen had been recorded, the section was stained with hematoxylin-eosin (HE) so that a comparative optical micrograph could be made. Thus, Fig. 7B shows an optical image of the same area. This micrograph was made by means of a Zeiss Photo-Microscope II with a × 10, numerical aperture 0.32, planapo objective. A close inspection of Fig. 7 will show that the basic structural features seen in the acoustic micrograph correlate on a one-to-one basis with the optical image. This carcinoma is of the infiltrating ductile variety, as evidenced by the abnormal duct-like structure seen in the upper right corner of each image. In order to facilitate a comparison of the detail in the acoustic image with that seen optically, one area of interest has been selected for enlargement. The acoustic and optical images of this enlarged area are shown in Fig. 8, A and B, respectively. Each individual cell can be compared in these two images.

The emphasis of particular details is, however, very different in the two images. In the optical image the HE stain provides a clear distinction between the cell nucleus and the cytoplasm, while distinctions between cell types are less marked. In contrast, differences in acoustic attenuation tend to discriminate between cell types. In some instances a difference between two areas can be quite subtle in the optical image while being obvious in the acoustic micrograph. An example of this can be seen in Fig. 8A. In the central area of this image several small localized regions show a very large acoustic attenuation compared to the surrounding material. The same regions in the optical image, Fig. 8B, appear little different from the bulk of the tissue. These distinctive areas are most probably compact bundles of connective tissue with an increased collagen content. For some soft tumors the recognition of increases in collagen content and connective tissue can be an important diagnostic criterion.

We are aware that connective tissue and collagen deposits can be accentuated in a light micrograph by applying a specialized stain such as Masson's trichrome; however, many of these stains require as much as 12 hours to apply. There are important situations, such as biopsies on patients in the operating room, where this time interval is prohibitive. For those cases it has been necessary to work with frozen sections that are stained with HE. A rapid evaluation of a frozen section with the acoustic microscope should assist in making a more accurate diagnosis.

As a second example of the acoustic appearance of diseased tissue, Fig. 9 shows a section of lymph node afflicted with Hodgkin's disease. Several reasonably normal-looking lymphatic nodules can be seen in this image as circular areas of high acoustic transmission. The individual cells of these nodules are not well defined. In contrast, a highly attenuating matrix of material can be seen between the nodules. Evidently these are strands of collagenous material which have formed in response to the disease. Since these strands have a vastly greater attenuation than the normal tissue, the acoustic microscope can easily show minute amounts of this material. In some instances this might prove useful as a diagnostic technique.

In the final illustration, Fig. 10, we present a comparison between the acoustic images of cancer cells within a lymph node and the lymph node discussed in connection with Fig. 9. The neoplastic cells originated with a breast tumor which metastasized and spread to the lymph node. This comparison is made to point out an interesting difference in the acoustic response of the two cell types. As mentioned before, the cells of the lymph nodule shown in Fig. 10B are not well distinguished. The

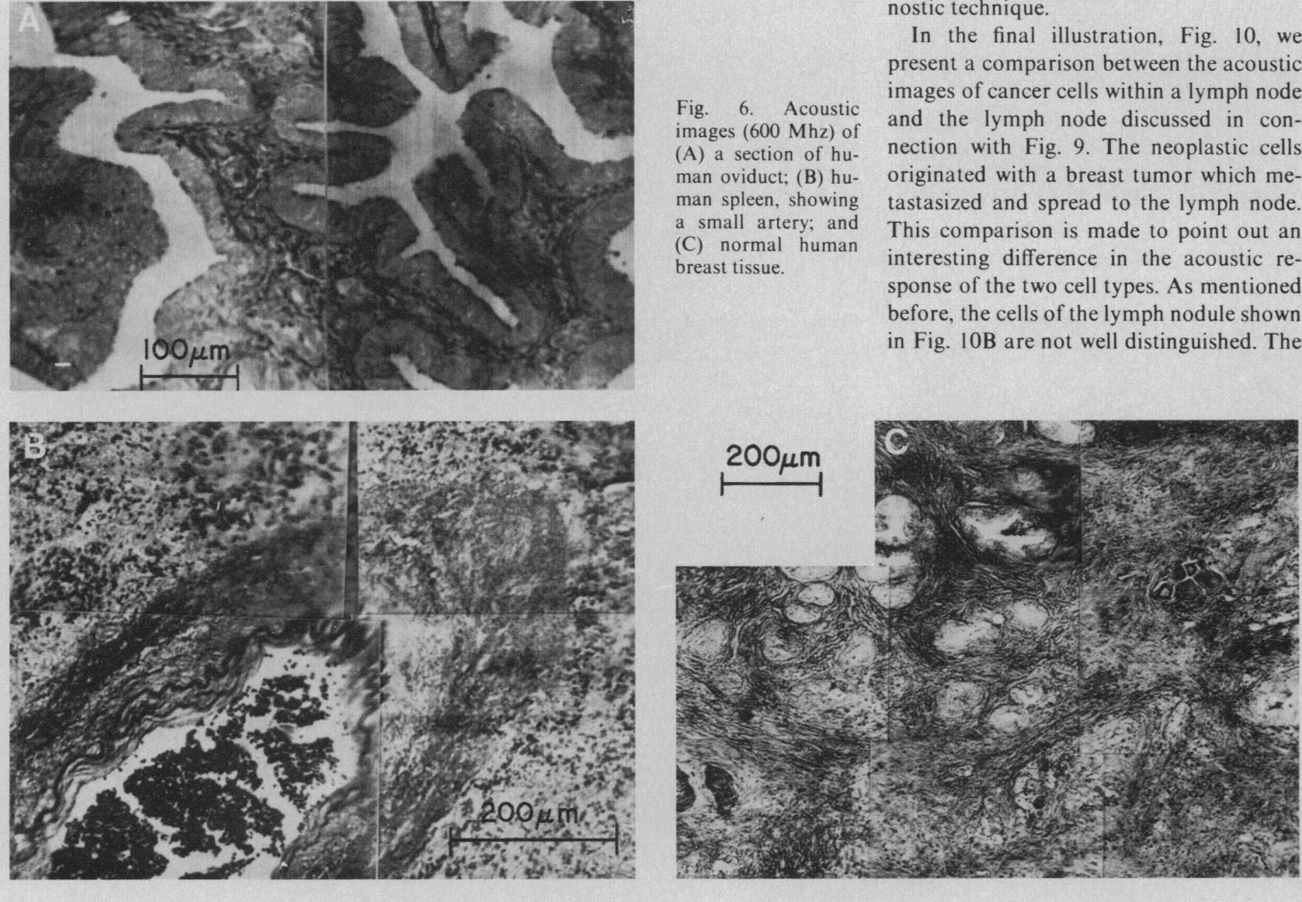

Fig. 6. Acoustic images (600 Mhz) of (A) a section of human oviduct; (B) human spleen, showing a small artery; and (C) normal human breast tissue.

cancerous cells seen in Fig. 10A can, however, be readily distinguished by the clearly outlined nuclei. These nuclei are particularly conspicuous because points of large attenuation can be seen.

Future Developments and Applications

The transmission mode as illustrated in this article is but one of several modes of operation. In an alternative mode we can image those areas which exhibit uniform transmission by recording the acoustic phase shift through the specimen. We do this by comparing the phase shift of the signal through the instrument with a reference waveform of fixed phase as derived directly from the signal generator. Further than this we have studied the reflection mode as a method for viewing the minute detail of integrated circuits (*9*). This can be readily adapted to biological specimens mounted on a glass microscope slide. The large discontinuity of acoustic impedance at the glass interface will provide the reflected signal for this method of examination. There are still other possibilities based on the interference patterns of multiple beams—each displaced in frequency—passing simultaneously through the instrument.

The resolution can be further improved by going to higher frequencies in liquid cells of smaller dimensions and by using fluids such as liquid nitrogen and argon. These cooled liquids have an attenuation per unit length that is lower than that of water and a sound velocity that is nearly half that of water. Therefore, we will in principle realize a wavelength that is one-third that of what we are now using and the resolution should be improved by a corresponding factor.

In another study (*10*) we have found that certain biological specimens have a large nonlinear response to acoustic waves. This response is rather easily measured by tuning the output circuit to the second harmonic of the input signal and recording the scanned image at this double frequency. This information is distinct from that which we obtain in the linear imaging process and it provides a method for further increasing the informational content of the images of a given specimen.

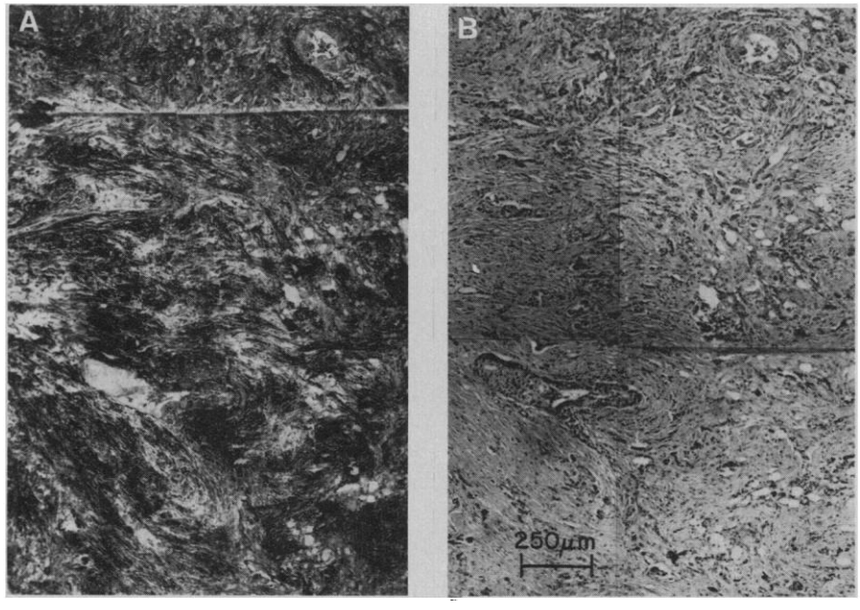

Fig. 7. (A) Acoustic image (600 Mhz) of a malignant tumor of the human breast, shown under low magnification. (B) Comparative optical micrograph (stain: hematoxylin-eosin).

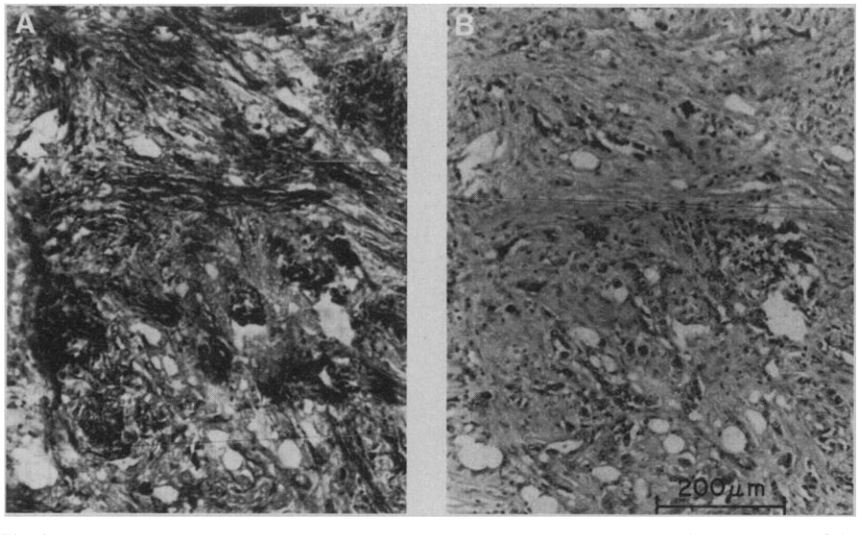

Fig. 8. (A) Acoustic image of a small region of the tumor shown in Fig. 7. (B) Optical image of the same area.

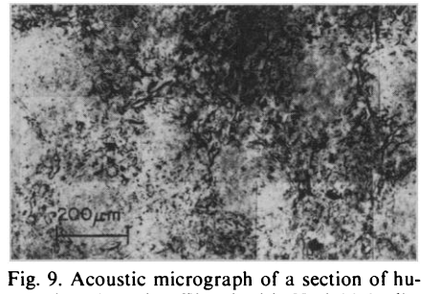

Fig. 9. Acoustic micrograph of a section of human lymph node afflicted with Hodgkin's disease.

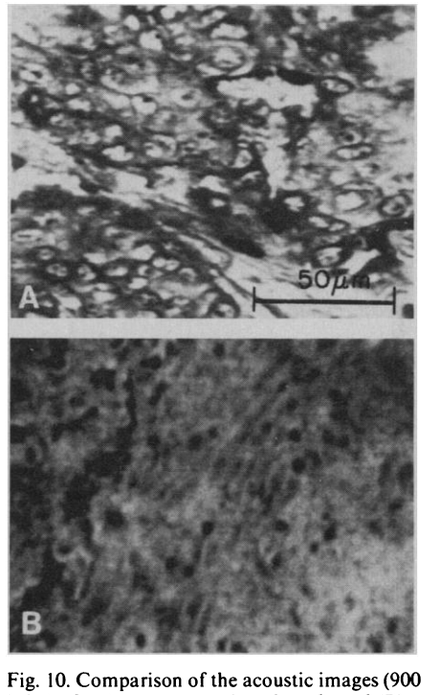

Fig. 10. Comparison of the acoustic images (900 Mhz) of (A) a cancerous lymph node and (B) a lymph nodule of the sample shown in Fig. 9. Both are shown under high magnification.

Conclusions

The technology of acoustic microscopy has now been advanced to the point where it can be used to record micrographs of cells and of cell complexes. The acoustic microscope responds to the elastic properties of the specimen and it therefore provides information which is distinct from that of an optical microscope. We believe that this instrument can be used in the fields of biological and medical research to obtain new information or to obtain some information more rapidly than is possible with present techniques.

The micrographs we have presented show that unstained biological material can be acoustically imaged with good contrast. Certain materials such as collagen and connective tissue have a particularly marked attenuation. As a result, the acoustic microscope can reveal information that is now only available through time-consuming staining techniques. Recently it has been shown that the acoustic microscope is compatible with frozen sectioning techniques and, with proper development, this may provide improved diagnostic techniques.

That the absorption of acoustic energy is proportional to the viscosity of the medium can be of particular importance in investigating living systems (*11*). It might be possible to use the acoustic microscope for monitoring the viscosity within the cytoplasm of cells undergoing mitosis. The acoustic microscope might also be useful for monitoring the various rheological states of the contractile systems that are responsible for movement in several cellular systems. An increased understanding of the rheology of protoplasm or of cell division is a goal worthy of a large and extended effort.

With the work that has been done to date it is not possible to pinpoint the possible areas of application with great accuracy, but we do claim to have demonstrated that this is a new method for viewing the microscopic world. With this in mind it is important to examine carefully the features and limitations of this new tool and to search for areas where it can be used to extend our knowledge.

References and Notes

1. A. Korpel, in *Ultrasonic Imaging and Holography*, G. W. Stroke, W. E. Kock, Y. Kikuchi, J. Tsojivchi, Eds. (Plenum, New York, 1974), pp. 345–
→ L. W. Kessler, *J. Acoust. Soc. Am.* **55**, 909 (1974); R. K. Mueller (*Inst. Electr. Electron. Eng.*) *Proc.* **59**, 1319 (1971). These reviews cover the state of the art of acoustic microscopy up to the spring of 1973. The series entitled *Acoustical Holography* (Plenum, New York, 1970–1974), vols. 1 to 5, contains several articles on the various ideas that have been put forth for an acoustic instrument.
→ R. A. Lemons and C. F. Quate, *Appl. Phys. Lett.* **24**, 165 (1974).
→ E. L. Carstensen and H. P. Schwan, *J. Acoust. Soc. Am.* **51**, 305 (1959).
4. A clear demonstration of this effect for low frequency sound has been given by Anderson where he compares the attenuation in liquid blood with the absorption in an organized blood clot [R. E. Anderson, in *Acoustical Holography*, P. S. Green, Ed. (Plenum, New York, 1974), vol. 5, p. 508, fig. 3].
5. This has been pointed out by F. Dunn and W. J. Fry in *The Encyclopedia of Microscopy*, G. L. Clark, Ed. (Reinhold, New York, 1961), p. 544; and even earlier S. Sokolov recognized this potential for an acoustic imaging system [see G. Devey, *Radio-Electron. Eng.* (a translation) (February 1953), p. 8].
6. R. A. Lemons and C. F. Quate, *1973 Ultrasonic Symposium Proceedings*, IEEE Cat. 73CHO807-8 SU (January 1974), paper E-6, pp. 18–21.
7. E. M. Slayter, *Optical Methods in Biology* (Wiley Interscience, New York, 1970), p. 241.
8. W. Bloom and D. W. Fawcett, *A Textbook of Histology* (Saunders, Philadelphia, ed. 9, 1968).
→ R. A. Lemons and C. F. Quate, *Appl. Phys. Lett.* **25**, 251 (1974).
10. R. Kompfner and R. A. Lemons, in preparation.
11. A. D. Keith and W. Snipes, *Science* **183**, 666 (1974); R. D. Allen, *Acta Protozool.* **11**, 75 (1972). These authors include a discussion of the various techniques that have been used in the past to measure the rheological properties of cells.

12. The research was funded by a grant from the John A. Hartford Foundation, Inc., and we are grateful for that support. Drs. N. K. Wessells, L. Hayflick, R. Dorfman, R. Kempson, and M. Billingham gave us great encouragement and pointed to areas of possible utility. Drs. E. Farber and F. Ebaugh guided us in the work with cell complexes and tissue sections. The cell cultures of Fig. 6A were prepared in Dr. Hayflick's laboratory (Department of Medical Microbiology) and the tissue sections were prepared in Dr. R. Lawson's laboratory (Department of Pathology). Dr. W. Bond taught us how to fabricate and accurately assemble the components of the prototype. Dr. R. Kompfner originally suggested this project to us, and he and Dr. M. Chodorow were of great help in discussions concerning the physics of imaging.

NEWS AND COMMENT

Nuclear Proliferation: India, Germany May Accelerate the Process

As representatives of 69 nations meet in Geneva this month to review the status and ponder the future of the 1970 Non-Proliferation Treaty (NPT), awareness is growing that further restraint on the spread of nuclear weapons may depend as much on controlling technology as on guarding world commerce in the fissionable fuels—uranium and plutonium. Certainly this was a cardinal lesson of India's first nuclear explosion a year ago. India, after all, got its plutonium not by theft but openly from a Canadian-supplied reactor and is building more reactors based on Canadian technology.

While the Geneva meeting continues, two imminent events promise to lend a grim new aura of urgency to controls on nuclear technology. For one, knowledgeable U.S. officials believe that India is prepared to detonate its second nuclear explosion. At the same time, West Germany is on the verge of concluding an agreement with Brazil that would provide Latin America's largest nation not only with the technology it needs to become self-sufficient in nuclear energy but would also endow Brazil with the technological base from which, if it saw fit, to build nuclear weapons.

Like India, Brazil has refused to sign the NPT, under which it would be obliged to foreswear development of nuclear explosives and submit to international inspection of its nuclear facilities. Brazil's government has maintained the position that the NPT discriminates against nonnuclear powers. Various Brazilian government officials since the late 1960's also have asserted a right to build and detonate "peaceful" nuclear explosives.

Informed U.S. officials believe that both the Indian nuclear test and the signing of the West German–Brazilian technology agreement are being delayed now only for diplomatic reasons, possibly in deference to the NPT review conference, which ends on 30 May. West Germany and Brazil are participating in the conference, but India is not. India is believed to recognize that it has little to gain by an act that would inevitably be interpreted as a calculated insult to conference participants and a blow to the treaty itself. Also, India is just now reaching an understanding with Canada that could lead to a resumption of nuclear cooperation between the two countries. Even so, the second Indian explosion is regarded as imminent. "It could be days, it could be weeks," one U.S. official told *Science*. "It's just a matter of when they decide to push the button."

Similarly, or so it seems, "all systems are go" with the West German–Brazilian deal, worth by some estimates as much as $8 billion over the next 10 to 15 years.

Of the two developments, the pending agreement between West Germany and Brazil may have the greater significance for the proliferation problem. Details are sketchy, but the agreement apparently grew out of talks that began last Novem-