Acoustic Microscopy: A New Window to the World of the Small

A new window to the world of the microscopically small has been opened by the development of microscopes that provide images with sound waves rather than light waves. Acoustic microscopes reveal a far greater wealth of detail than that available with optical microscopes because their images are based on physical characteristics other than refractive index. They are also capable of revealing details hidden below the surface of an object—details that cannot be seen at all with light microscopes and only poorly with x-rays. The new instruments show strong signs of becoming an important tool for quality control in the electronics industry, but they also show promise for applications in biology, materials science, and other fields.

The idea of the acoustic microscope was apparently first suggested in 1949 by the Soviet scientist S. Y. Sokolov, but the technology for generating high-frequency sound and processing the signals did not become available until the 1960's. The first practical acoustic microscope was developed in 1974, and the first commercial instrument was sold the following year. Subsequent work has been aimed at increasing the resolving power, increasing the depth of field, and refining the technique. Some of this work culminated this summer when Calvin F. Quate and Victor Jipson of Stanford University announced that they had developed an acoustic microscope that operates at the same wavelength, and thus has the same resolution, as optical microscopes.

Acoustic microscopy is related to the now-familiar ultrasonic imaging in much the same manner that electron microscopy is related to optical microscopy—the basic principle is the same, but resolution is increased with the use of radiation of a shorter wavelength. Ultrasonic imaging is generally performed with sound at frequencies ranging from 2 to 15 million cycles per second (2 to 15 MHz), but acoustic microscopes operate at frequencies ranging from 100 to 3000 MHz or 3 GHz. At 3 GHz, the acoustic wavelength in water is 520 nm, slightly shorter than the optical wavelength of 550 nm (green light) which corresponds to the center of the visible spectrum.

There are two complementary types of acoustic microscopes that have somewhat different modes of operation and different, but overlapping, applications. One is called a scanning acoustic microscope or SAM; the second is called a scanning laser acoustic microscope or SLAM.

The first SAM was developed by Quate and Ross A. Lemons, now at the Bell Telephone Laboratories. The heart of current instruments (Fig. 1) is a short sapphire rod that has a flat surface on one end and a polished hemispheric depression on the other. The flat end is coated with a thin, piezoelectric film of zinc oxide that converts radio-frequency current into sound waves and vice versa. The hemispherical surface is coated with a very thin layer of glass that serves as an impedance transformer or mechanical link between the stiff sapphire and the water that transmits sound waves to the specimen.

Current is applied to the piezoelectric transducer in pulses 20 to 100 nsec long. The electrical energy is converted into acoustic energy, transmitted the length of the rod, and focused by the hemispherical lens. Because the velocity of sound is much greater in sapphire than in water, an ideal lens with a single spherical surface can be used without spherical aberration—the blurring of an optical image that results when light rays passing through the center of a lens are not focused on precisely the same point as rays passing through the edge. Reflected sound waves are captured by the same lens and transmitted to the piezoelectric, which converts them back to an electric signal. Electronics separate the returning pulse from the original pulse and spurious reflections, and display the image on a cathode-ray tube. Alternatively, a second sapphire rod identical to the first can be placed on the opposite side of the sample and focused on the same point. In this case, the instrument becomes a scanning transmission acoustic microscope.

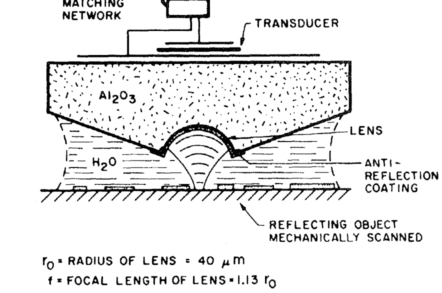

Fig. 1. The lens configuration of Calvin F. Quate's 3-GHz scanning acoustic microscope. The radius of curvature would be larger in instruments operating at lower frequencies.

At the sonic frequencies employed in microscopy, the impingent sound can be focused on a spot only a fraction of a wavelength in diameter. To obtain an image of the entire specimen, it is necessary to move either the lens or the specimen; it is easier to move the specimen. Quate's original instrument moved the specimen in what is known as a raster scanning pattern of 256 lines. These were recorded and displayed on the cathode-ray tube at the rate of one frame in 5 seconds. A newer version divides the specimen into 200 lines, but scans at the rate of 12 frames per second.

There are now four SAM's in use in this country (and at least three in Europe), operating at frequencies ranging from 150 MHz to 3 GHz. Each was built in the laboratory where it is used at a cost of about $80,000. Many investigators have expressed hope that some company will begin to manufacture SAM's commercially.

The second type of instrument, the SLAM, was developed by Lawrence W. Kessler and his colleagues at the Zenith Radio Corporation and is now manufactured by Sonoscan, Inc., of Bensenville, Illinois. The most important difference between SAM and SLAM is that the latter does not focus the sound. Instead, the SLAM has a flat piezoelectric transducer that distributes acoustic waves uniformly over the surface of the sample.

The side of the specimen opposite the impingent sound waves is covered with a partially mirrored cover slip (Fig. 2). Transmitted sound waves that have been modulated by passage through the specimen induce vibrations in the cover slip to produce what is termed a "shadow image." This shadow image is converted into an electrical signal by a highly focused laser beam that scans the cover slip and detects minute displacements produced by the acoustic waves. The process is analogous to the familiar espionage technique in which a laser beam is focused on a window to detect conversations on the opposite side. If the specimen is a highly polished, nonbiological sample, the cover slip is not required.

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The electrical signal from the optical detector is displayed on a cathode-ray tube at a rate of 30 frames per second.

The laser beam serves a dual purpose. Because the cover slip is only partially mirrored, part of the laser beam is either reflected from the surface of the specimen, or transmitted through it, to a second photodetector that generates an optical image simultaneously with the acoustic image. Thus landmarks on the optical image can be correlated with features revealed by the acoustic image.

The SLAM can also be operated in an interference mode to produce patterns analogous to optical interference fringes. This is achieved by mixing the acoustic signal with an electronically generated reference signal that is coherent with the sound wave. Lateral shifts in the fringes (Fig. 3) indicate localized changes in the velocity of sound through the specimen and thus may be indicative of the presence of voids or other defects or of subtle changes in elastic properties.

The resolution of a SAM is limited by the wavelength of the impingent sound, but the resolution of a SLAM is limited by the diameter of the scanning laser beam. Theoretically, that limit is about 0.5 micrometer, but in practice the limit is higher. Three of the four SLAM's that Sonoscan has sold operate at 100 MHz, and they can resolve objects as small as 10 μm in diameter; the fourth instrument, installed at the U.S. Department of Agriculture's Western Regional Laboratory in San Francisco, operates at 100 and 500 MHz and has a resolution of about 3 μm. Quate's 3-GHz SAM, in comparison, has a resolution of less than 1 μm and a 375-MHz SAM built by Rolf D. Weglein of Hughes Research Laboratories has a measured resolution of 1.67 μm. A typical SLAM costs $97,000.

The principal limitation in operation of a SAM is attenuation of the sound wave as it passes from the lens to the specimen and through the specimen. Water is used as the medium between the lens and the specimen because it transmits sound waves much more efficiently than air does, but water also has limitations. The distance that sound will travel in water is inversely proportional to the square of its frequency. Thus, for example, if the frequency of the impingent sound is increased from 1 GHz to 2 GHz to achieve a doubling in the resolution, the distance the sound can be transmitted through the medium decreases by a factor of 4. At high frequencies, the distance sound can be transmitted is incredibly small. The radius of the lens in Quate's 3-GHz instrument is only 40 μm, and even then the water must be heated (sound travels farther in hot water) to 60°C for adequate transmission.

One alternative is to use liquids that have a lower coefficient of attenuation than that of water. Carbon disulfide and mercury, for example, are significantly better than heated water, Quate says, but there are severe problems of incompatibility with the specimens. Another alternative is the use of cryogenic fluids.

Joseph Heiserman of Quate's laboratory has developed a SAM designed to operate at temperatures as low as 2°K. Sound travels much more slowly in cryogenic fluids than in water. In liquid argon, the speed of sound is about half its speed in water at room temperature; in liquid helium, its speed is about one-quarter that in liquid argon. The wavelenght of sound at any given frequency is thus reduced by a proportional amount, with an attendant increase in resolution. With cryogenic fluids, Quate says, it should be possible to achieve a resolution at least twice that of optical microscopes.

Because acoustic microscopy is still a

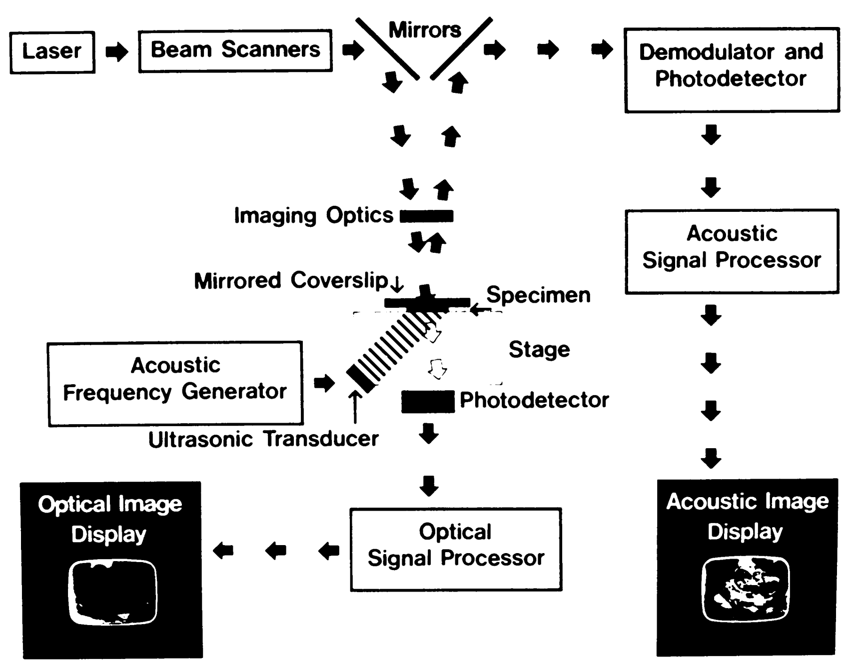
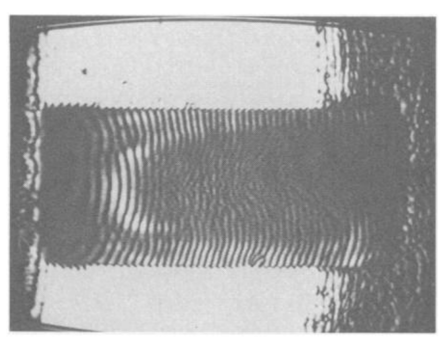


Fig. 2. A block diagram of a scanning laser acoustic microscope. [Source: Lawrence C. Kessler, Sonoscan, Inc.]



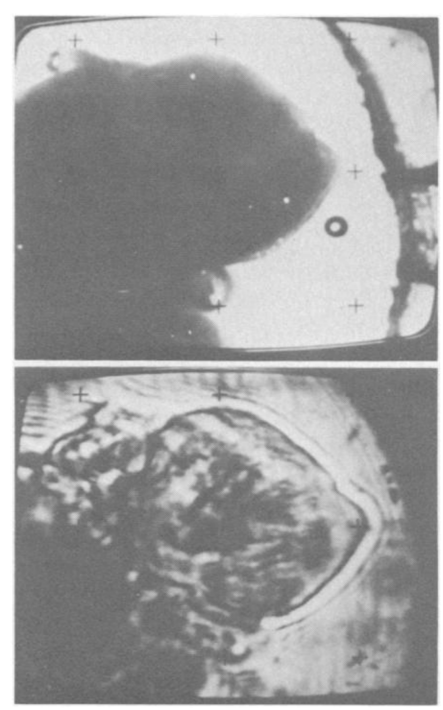


Fig. 3 (left). Interferogram of a ceramic chip capacitor. The discontinuities in the fringes indicate an area of delamination between layers. [Source: Lawrence C. Kessler, Sonoscan, Inc.] Fig. 4 (right). Optical (top) and acoustic (bottom) images of a fetal mouse heart in culture. [Source: Lawrence C. Kessler, Sonoscan, Inc.]

new science, much of the work with it so far has been exploration for potential applications—what Robert C. Waag of the University of Rochester Medical School characterizes as "turning over rocks and looking for diamonds." The search has been fruitful for biological samples because they can be viewed in a natural state: they do not need to be exposed to a vacuum, as is necessary for electron microscopy, or stained to increase contrast, as is necessary in optical microscopy. Differences in tissue elasticity and density generally provide more than enough contrast.

Investigators such as Waag and William D. O'Brien, Jr., of the University of Illinois at Urbana-Champaign have been looking at various types of organs with a 100-MHz SLAM to determine what factors are responsible for image production. They have found that the greatest attenuation of sound is produced by collagen, a major structural component of many kinds of tissue. O'Brien has been able to measure the velocity of sound in a single strand of collagen—a feat not possible with other techniques—and thinks his results will provide useful information both for modeling ultrasonic imaging and for better understanding structural tissues. Waag has also shown that the technique can be used to distinguish between varying states of fibrosis in lung tissues.

Reginald C. Eggleton of the University of Indiana Medical School is using a 100-MHz SLAM to examine muscle tissues. He is able, for example, to image an entire fetal mouse heart while it is functioning in a tissue culture system (Fig. 4). He is thus able to study such things as the effects of cardioactive drugs on internal valves; these valves are not visible in an optical microscope. By focusing attention on only one line of the image on the cathode-ray tube, he is also able to monitor the time course of contraction of various parts of the heart. In particular, he has been able to delineate the pathway of nerve impulses following stimulation of contraction by a pacemaker. He has also examined other muscle tissues, such as the frog sartorius muscle, and has been able to measure elasticity both along the length of the muscle and perpendicular to it. He hopes to be able to relate this

Speaking of Science

Microelectronics: Defense Department Looks to the 1980's

Beginning this fall, the Department of Defense is launching a $150 million effort to develop the microelectronics technology needed for its guidance, surveillance, and communications systems of the mid-1980's and beyond. To be spent over a 6-year period, this outlay will go to a coordinated tri-service program aimed at producing very high speed microelectronic circuits. In addition, a smaller amount will go to a separately managed Advanced Research Projects Agency (ARPA) project with the same general goal that emphasizes high-risk, longer-range research. Because the annual amount under both projects is small compared to that already spent by the semiconductor manufacturers on their own research and product development, the money will be spent in ways calculated to stimulate the industry to advance its timetable for the development of high-speed signal processing and computation circuits by 5 years. (For a look at the impact of military electronics, see News and Comment, p. 1102.)

Much has been made in the last 2 years of the Japanese threat to the long-held U.S. supremacy in microelectronics. The next generation of ultraminiature electronics goes by the moniker of VLSI—for very large scale integrated circuits; Japanese companies are now parlaying both rising sales in the worldwide electronics market and a substantial amount of financial support from their government into an effort to be first in the race to VLSI. But it is not the potential economic threat from Japan that has prodded DOD into action. Instead, it is an increasing divergence between military needs in the next decade and what the U.S. semiconductor industry produces for commercial applications that is said to be the driving force. A second major factor is that what is purported to be a rapidly growing Soviet capability in military microelectronics has eroded a once substantial U.S. lead.

The Soviets have frequently been pictured, at least in the popular press, as being outclassed when it comes to microelectronics. Two years ago, when a defecting pilot landed a MiG-25 (Foxbat) in Japan, newsmagazines gleefully reported that the so-called superaircraft contained only vacuum-tube electronics and no solid-state, let alone microelectronic, circuits. If nothing else, the reputed increases in accuracy of Soviet missiles belie this conception, but the subject is classified and little specific information is publicly available.

In the meantime, the "signal processing intensive" electronics systems of the U.S. military will require the computational power of today's supercomputers to be packed into configurations that are ten times lighter, smaller, and less power-consuming than present systems. Such characteristics will require new microelectronics circuits that are simultaneously faster and more miniaturized than existing devices. One goal is to increase by 100 times the available rates of computation for each microelectronic chip.

The new DOD programs, called VHSI for very high speed integrated circuits, are intended, says Leonard Weisberg of the Pentagon's defense research and engineering office, to develop a few circuit designs with broad functional capabilities that satisfy a large number of the military's needs. At present, military microelectronic circuits are often custom designs. A second goal is to have available military-qualified versions of the new circuits before commercial versions, a reversal of the present pattern. Moreover, adds Richard Reynolds of ARPA, many military systems require a performance far beyond that envisioned for commercial uses even in the distant future. In short, since the military accounts for only about 7 percent of the U.S. semiconductor manufacturers' market, the industry is not rushing to develop on its own the technology the military has to have but cannot purchase in sufficient quantity to make its manufacture profitable.

The tri-service portion of the new microelectronics effort will be partitioned into three areas. The first of these concerns the photolithographic process whereby the intricate patterns that together make up a microelectronic circuit are

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elasticity to the number of cross-links between actin and myosin filaments.

Kessler, who is now president of Sonoscan, and Donald E. Yuhas of that company have also looked at heart tissues. They have found that the technique can readily distinguish between healthy tissue and tissue that has suffered an infarct. They have also shown that internal features of fruit fly larvae can be distinguished by acoustic microscopy; these features are not visible in an opitcal microscope.

Acoustic microscopy should also be useful for studying blood cells, Quate says, because variations in their elasticity are quite important. Newly produced erythrocytes are very elastic, but much of this elasticity is lost during the 120 days of their normal existence. It should thus be possible to determine the age of the cells by acoustic microscopy; this could be useful, for example, in studies of certain types of anemia in which erythrocytes are degraded more quickly than normal. The technique should also be useful in studies of sickle cell anemia, in which the cells are much less elastic than normal. Elasticity may also be an important characteristic of malignant cells, which have an enhanced ability to move through narrow, constricted channels during metastasis. Already, Quate says, there is evidence that the velocity of sound is greater in malignant tissues than in healthy ones (Fig. 5).

In one other noteworthy study, Hemantha K. Wickramasinghe of Quate's laboratory has examined Chinese hamster ovary cells with the 1-GHz SAM. He has shown that it is possible to distinguish between cells that have doubled their DNA content and are ready to divide and those that have not. This can be accomplished with conventional radioactive labeling, but the procedure is tedious and time-consuming. There are many potential applications, Quate notes, where it would be useful to detect incipient cell division.

Perhaps the greatest interest in acoustic microscopy now, however, involves its applications in materials science. Those few centers that have acoustic microscopes have been deluged with samples from manufacturers of, for example, semiconductors, circuitboards, in-

delineated. At present, for example, the pattern is transferred onto the semiconductor by means of 4000-angstrom light that is shined through a mask containing the desired pattern onto a photosensitive polymer overlaying the semiconductor chip. Future processes could use vacuum-ultraviolet, x-ray, electron, or ion beams to make patterns with features having dimensions of as small as 0.5 micrometer as compared to about 5 micrometers now.

A second part of the program will be devoted to design and testing of the circuits themselves and of the computer programs that will direct their operation. It is a mammoth job, for example, to arrange from 10,000 to 100,000 devices on a single chip of semiconductor in such a way that they function efficiently. A particular goal will be to minimize the need for customized designs because microelectronics circuits are expensive to design but cheap to manufacture. The microprocessor boom is one result of the same pressure on commercial electronics. (A microprocessor is a general-purpose circuit that can be made to suit any user's needs by writing a specific computer program to control its operation without having to redesign the circuit.) But planned microprocessors will be many times too slow for military systems of the next decade.

The third part of the program entails the actual fabrication of advanced circuits. A principal problem in all microfabrication is increasing the yield—that is, the ratio of the number of circuits produced that have no defects to the total number produced. The VHSI program thus is shooting for a pilot plant facility that can produce quantities of two advanced types of devices: computer memory chips containing 2 million bits of information and logic chips that can execute 1 million instructions per second. The chips must meet military specifications.

Each of the services will spend about $7 million the first year. A possible major stumbling block yet to be overcome is that the funding does not come from new money added to the fiscal year 1979 budget now wending its way through Congress. Rather, the indicated sum is to be reprogrammed by each of the services—that is, the money must be accumulated by cutting or reducing already approved R & D. As one observer noted, "an awful lot of heartburn will be created in making this pot." A new Pentagon wrinkle is that, while the money for the VHSI program is to come from the services, the allocation of the dollars and program direction will be by way of the defense research and engineering office.

The situation at ARPA is more salubrious in this regard. In addition to existing advanced microelectronics research, new components will be added dealing with basic materials and fabrication technology and with circuit design. For the first year, about $3 million will go to the new projects, but the amount is from funds reserved for new programs.

The present relationship between the DOD and the semiconductor industry seems a curious turnabout. Once upon a time, government and industry were locked in a synergistic embrace that, if it did not make microelectronics possible, greatly speeded its development. Not only research funding, but infusions of capital for purchasing the equipment needed to fabricate microelectronics circuits and the providing of a market for the circuits produced were essential to the rapid growth of the industry (*Science*, 18 March 1977, p. 1107).

Now some in the semiconductor industry are taking exception to elements of the Pentagon's thinking, although the specifics of the VHSI project have yet to be made public. The claim is that, by concentrating on its own unique requirements, the Pentagon is acting like the proverbial tail that tries to wag the dog. Overriding all concerns, however, is that over the Japanese VLSI challenge.

While the VHSI project is not overtly intended to help the U.S. semiconductor industry meet this test, it has the potential to do just that because the technology overlap between VHSI and VLSI is nearly 100 percent. Moreover, the government will be helping those companies selected to work on the project to develop some advanced fabrication equipment and DOD will be the first major customer of the new circuits. It could be a repeat of a familiar story.

Such an outcome would please the industry no end; the fear is that, if the program focuses on specific, even exotic, circuits rather than on advancing the health of the industry by developing new technology, the VHSI project will end by helping no one very much.—ARTHUR L. ROBINSON tegrated chips, ceramics, and metals. Preliminary results indicate that the technique will be very useful for nondestructive analysis of such materials. Particularly valuable is the capability of the technique to detect voids and other defects hidden below the surface of a specimen.

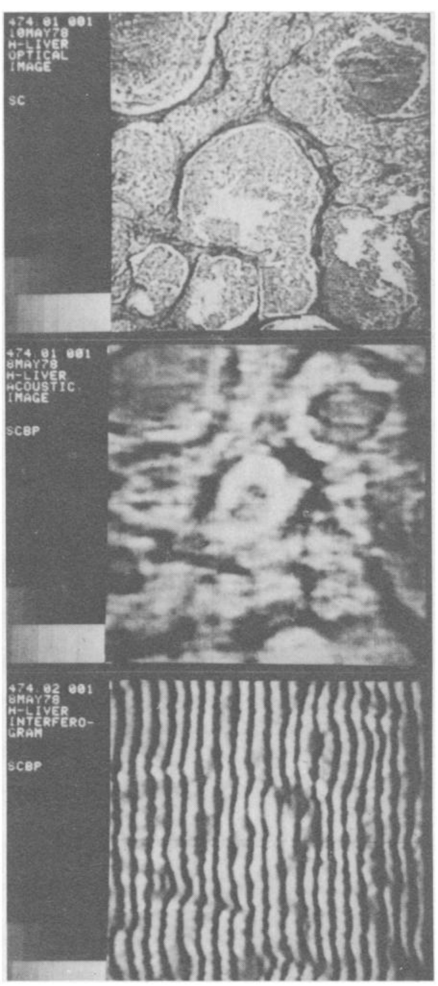
Chen S. Tsai of Carnegie-Mellon University has shown that the depth of focus, and thus the ability to detect subsurface defects, can be increased by what is known as a nonconfocal mode of operation. This is accomplished by operating his 150-MHz SAM in the transmission mode and positioning the sample between the two lenses at a point other than the common focus. In one particularly dramatic illustration of the capabilities of this mode of operation, Tsai bonded a thin copper grid between two thin sheets of brass. An image of this specimen obtained with an optical microscope shows only the polished brass surface, but an acoustic image clearly shows the copper mesh (Fig. 6). The dark area near the center indicates an area where bonding is not good.

Tsai has shown that defects in bonding can be identified in a nondestructive manner in a wide variety of materials. Acoustic microscopy should prove particularly useful in quality control of microelectronic components, he argues, because their electrical, thermal, and mechanical characteristics are greatly influenced by elastic faults in the interfacial regions. In just one example of a potential application, Tsai examined production line die-bonded transistor headers, each of which consists of a silicon wafer bonded to a copper plate with Au-Sn solder. The copper plate is a heat sink, and therefore the bonding must be uniform to prevent hot spots. Tsai has found that voids in the solder and other bonding defects can be easily observed with acoustic microscopy. Quality control for production of these transistors is currently achieved with x-radiography, but that technique is applied only with great difficulty, it does not provide magnification, and small defects are often missed. The only other alternative is destructive testing. Tsai has also demonstrated the utility of acoustic microscopy for quality control in production of multilayer chip capacitors, where it is necessary to determine whether metal and ceramic components are making good contact.

Weglein and Robert G. Wilson of the Hughes Research Laboratories have used a 375-MHz SAM to examine different kinds of integrated circuits. They have found that different layers of the integrated circuit can be imaged by varying the distance of the specimen from the lens; subsurface layers can thus be checked for cracks, voids, and other defects. In addition, they have shown that characteristic acoustic signatures can be obtained by moving the specimen perpendicular to the focal plane without scanning. In this manner, it is possible both to identify unknown materials and to determine the thickness of known materials, thereby assessing the reproducibility of the fabrication process.

The interference mode of the SLAM is also useful for identifying such defects, say Kessler and Yuhas. Examination of ceramic capacitor chips, for example, in the interference mode clearly shows small voids, areas of delamination, and other defects (Fig. 3). In collaboration with David S. Kupperman of Argonne National Laboratory, they have also used the technique to identify voids and other defects in ceramic structural materials, such as turbine blades; the technique showed defects that could not be seen at all or could be seen only with great difficulty by other means.

The number of potential applications of acoustic microscopy thus seems almost limitless. Even so, investigators are working on improvements. Quate is investigating the possibilities of replacing the input acoustic lens with an optical lens, operating in the transmission mode, and illuminating the specimen with a pulsed laser. In this case, the instrument would operate as a photoacoustic microscope (*Science*, 25 March 1977, p. 1317), and could be used, for instance, to identify the chemical composition at specific sites within a sample, Also being investigated is a way to increase resolution by "illuminating" the specimen with sound at one frequency and imaging with sound waves emitted by the specimen at harmonic frequencies. This may also reveal new types of information about the material being studied. Even without these newer variations, however, acoustic microscopy seems destined for a bright future.—THOMAS H. MAUGH II



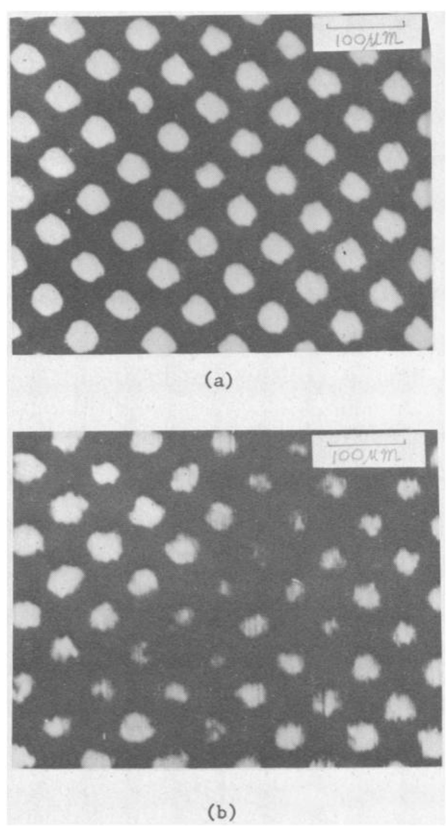


Fig. 5 (left). An optical micrograph (top), acoustic micrograph (center), and acoustic interferogram (bottom) of a metastatic adenocarcinoma in human liver. Dark areas in the acoustic micrograph indicate collagen in the septae and necrotic centers of tumor cells. Fringes in the interferogram bend to the right as they traverse the septae and the necrotic tissue, indicating that the speed of sound is greater in them than in the surrounding tissues. [Source: Robert C. Waag, University of Rochester Medical Center] Fig. 6 (right). (a) Acoustic image of a 400-mesh copper grid. (b) Acoustic image of the same grid bonded between two thin brass sheets. [Source: Chen S. Tsai, Carnegie-Mellon University]