Ultrasonic Absorption Microscope*

FLOYD DUNN AND WILLIAM J. FRY
Biophysical Research Laboratory, University of Illinois, Urbana, Illinois
(January 27, 1959)

This paper constitutes a progress report on the development of an ultrasonic instrument for detecting microstructure. The principle of operation of the device (designated by the term "ultrasonic microscope") is as follows: The specimen is imbedded in a liquid, which closely matches it in acoustic impedance, and is irradiated with short pulses of high-frequency sound. Some of the ultrasonic energy is absorbed in the specimen and the remainder leaves it and excites a small thermoelectric probe placed immediately adjacent to the region being investigated. As the specimen, which is between the sound source and the probe, is moved in a direction *normal* to the direction of sound propagation, a varying acoustic signal will be detected by the probe. This is a consequence of the fact that the amount of acoustic energy absorbed by the specimen in the portion immediately adjacent to the probe is determined by the structure of that part of the specimen.

Preliminary experimental results obtained with such a "microscope" operating at a frequency of 12 mc/sec are presented.

A VARIETY of methods are available for presenting information obtained from acoustic field distributions or signals as visual images. A number of these methods were reviewed by Rozenberg several years ago (1955).[1]

The method of interest in this paper, which has been under study at this laboratory[2,3] and which to our knowledge has not been proposed elsewhere, is, in principle, capable of producing high resolution. The goal is an acoustic device ("ultrasonic microscope" or "micrograph") which will lend itself to investigations of biological systems and will be capable of resolving the microstructure of these systems.

The development of such an instrument is important when considered as follows. As new tools for detecting microstructure have become available, they have been applied to the examination of biological structure and much new information has resulted in each case. The light microscope (absorption type) exhibits certain structural features of cellular and subcellular organization by means of the contrasting pattern of light and shade resulting from the absorption of electromagnetic energy to different extents by various parts of the biological structure of interest. The use of various selective staining procedures permits other aspects of structure to be detected which are not directly observable in unstained material. With the advent of phase contrast microscopy it became possible, without an increase in resolving power, to identify structural features not observable with the light microscope employing the absorption principle.[4] With electron microscopy, new details of cellular structure could be detected because of the higher available resolving power. Selective impregnation methods (retention of material at certain sites in the structure), employing the salts of heavy metals for producing contrast in electron transmission, are used in the preparation of tissue for electron microscope study.[4]

Study of the transmission characteristics of mechanical energy, in the form of pulsed sound waves through tissue or suspensions of biological material, can be expected to yield information concerning the structure of biological systems which is not obtainable from either light or electron microscopy work. This follows from the fact that the interaction of the sound waves with the tissue structure will be of a different nature from that of light or electrons. Reported experimental results[5,6] indicate that the protein constituents of tissue are largely responsible for its absorption of acoustic energy in the ultrasonic frequency range. This reported work also shows that *some* different types of protein molecules, at equal concentrations, absorb sound at different rates. Therefore, a suitably designed acoustic device could yield information on both spatial distributions and identification of types of protein in tissue.

The principle of operation of the ultrasonic microscope is illustrated in Fig. 1. High-frequency sound waves are generated in a "coupling" medium by a piezoelectric crystal vibrating in a thickness mode (an X-cut quartz plate, with an area of approximately $\frac{1}{4}$ sq in. in contact with the coupling medium, having a thickness of 0.01 in. to resonate at approximately 12 mc/sec is in use in the present first model of the device). The coupling liquid which fills the irradiation chamber, serves to conduct the sound to and from the specimen under observation which is interposed between the crystal and a small thermoelectric probe (in the present apparatus, an iron-constantan junction having a maximum dimension at the junction of 0.0005 in.). The piezoelectric crystal is excited electrically by voltage pulses with a rectangular temporal envelope (0.1 sec duration at present). The small probe detects the acoustic energy level of the sound which passes through the portion of the specimen in its immediate neighborhood. The variation in this transmitted energy level, as a function of the position of the probe relative to the specimen, constitutes an acoustic image of the ultrasonically detected structure.

Two mechanisms are involved in the detection of the ultrasound by the thermoelectric probe.[7-9] First, an increase in the temperature of the wire results from the conversion of acoustic energy into heat by the viscous forces acting between the wire and the fluid medium. Second, acoustic energy is converted into heat by

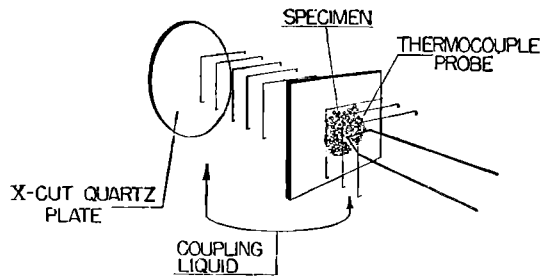

FIG. 1. Schematic representation of ultrasonic microscope showing transducer plate which is excited to produce pulses of ultrasound in the coupling liquid, the specimen under examination which is movable in the coupling liquid, and the thermocouple probe whose junction is placed immediately adjacent to the specimen.

the absorption of sound in the body of the coupling medium which surrounds the probe and specimen. The thermoelectric emf of the probe acts as the input to a dc amplifier, the output of the latter driving the vertical deflection plates of a cathode-ray oscilloscope. Thus, when the sound source is driven by a suitable pulse, the cathode-ray beam is transiently deflected from its equilibrium position and the magnitude of this deflection is a measure of the relative amount of acoustic energy detected by the probe. As the specimen is moved through the pulsed acoustic field, the changing deflection of the cathode-ray beam is observed and recorded. The data are then plotted and a "picture" of the disturbance to the sound field distribution, caused by the presence of the specimen, is obtained.

An example of the type of data obtained in this fashion is shown in Fig. 2. Here, a nylon filament 0.003 in. in diameter is moved past the probe starting from a position in the field where its presence does not appreciably influence the level of acoustic energy detected by the probe. The movement is in a plane parallel to the crystal face and perpendicular to the filament axis. During this motion, the quartz plate is excited to radiate pulses of ultrasound at a frequency of 12 mc/sec. The abscissa of the figure indicates the position of the specimen in a plane parallel to the crystal face. The ordinate indicates the relative acoustic intensity detected by the probe and the scale unit is 0.1-in. deflection on the oscilloscope screen. The minimum in the curve corresponds to the position of closest approach of the nylon filament as the specimen is moved past the probe. The presence of the nylon filament at the position of closest approach causes a reduction of 30% in the acoustic intensity below the undisturbed level. At half of this reduction, the curve is 0.007 in. wide while it is equal to the filament diameter (0.003 in.) at 0.8 of the total reduction.

Not all of the observed reduction is produced, in this case, by absorption of sound in the nylon. There is a mismatch in impedance between the coupling liquid and the filament of at least 10%, so that some of the incident energy is scattered. In addition, some of the sound energy is converted to heat at the interface between the nylon filament and the coupling liquid as a result of the viscous forces acting there.

Measurements similar to those shown in Fig. 2, but obtained with filaments of 0.001 in. diam in the field, demonstrate that at a frequency of 12 mc/sec a structure with a diameter of 25 μ can be resolved.

An approximate analysis (based on formulas derived to describe the behavior of a thermoelectric probe in a pulsed acoustic field)[7] of the operation of the ultrasonic microscope indicates that a structure with a "radius"† of 0.4 μ and having an acoustic

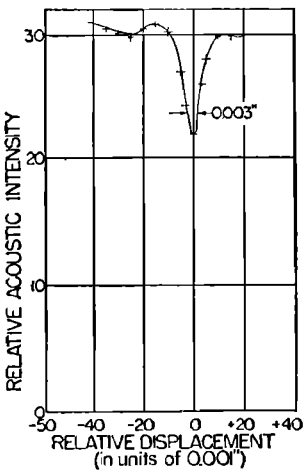

FIG. 2. The detection of the presence in the coupling liquid of a 0.003-in. nylon filament by the ultrasonic microscope operating at a frequency of 12 mc/sec.

intensity absorption coefficient per unit path length differing from the average value of the specimen by 5%‡ should be detectable if the following acoustic and other parameters are employed: frequency—1000 mc/sec, ultrasonic intensity—1000 w/cm², acoustic pulse duration—1.0 μsec, and thermocouple lead diameter —0.1 μ. It would be desirable to use a pulse repetition rate of the order of 1000 pps.

It should be noted that a considerable amount of knowledge of tissue structure could be obtained from the examination of tissue by an "ultrasonic microscope" of considerably less resolving power than that just indicated. This follows from the fact that many structural components of biological materials, with different ultrasonic absorption coefficients, may not be detected at all by the light microscope. There is no *a priori* reason why materials with greatly different ultrasonic absorption coefficients should have either detectably different absorption coefficients or indices of refraction for light.

In the course of experimentation with the device described in this note, it was observed that the intensity absorption coefficient per unit path length in highly absorbing viscous liquids could be readily determined. This can be accomplished by moving the probe along the axis of the sound beam and observing its output (transiently deflected spot on oscilloscope screen) as a function of position. In this way, the decrease in acoustic intensity as a function of the distance from the sound source is obtained. Although time has not yet permitted a thorough study of this application of the "microscope," it appears that this method of determining ultrasonic absorption coefficients in highly absorbing liquids does not suffer from the major disadvantages which plague the interferometric pulse reflection and optical methods at high ultrasonic frequencies, *viz.*, extremely critical alignments, undetermined deviations from plane wave configurations, pulse transit time limitations, and possibly others.

The authors wish to acknowledge the work of Professor F. J. Fry in designing the first model of this device.

* Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[1] L. D. Rozenberg, Soviet Phys.-Acoust. (translation) 1, 105–116 (1957).
[2] W. J. Fry, J. Acoust. Soc. Am. 30, 387–393 (1958).
[3] F. Dunn and W. J. Fry, J. Acoust. Soc. Am. 31, 120 (1959).
[4] See, for example, the chapters on light and electron microscopy in *Physical Techniques in Biological Research*, edited by G. Oster and A. W. Pollister (Academic Press, Inc., New York, 1955 and 1956), Vols. 1 and 2.
[5] E. L. Carstensen and H. P. Schwan, "Ultrasound in biology and medicine," Am. Inst. Biol. Sci., Washington, D. C., 1–14, 1957.
[6] H. P. Schwan and E. L. Carstensen, WADC Tech. Rept. 56–389, Wright Air Development Center, 1956.
[7] W. J. Fry and R. B. Fry, J. Acoust. Soc. Am. 26, 294–310 (1954).
[8] Reference 7, pp. 311–317.
[9] F. Dunn and W. J. Fry, Inst. Radio Engrs. Trans. on Ultrasonic Engineering PGUE-5, 59–65 (1957).

† The "radius" of the structure is defined to be that distance from the "center" at which the deviation of the absorption coefficient from the average value is down to 0.7 of the maximum deviation.

‡ If the absorption coefficient of the structure differs from the average by a greater percentage, then a smaller structure can be detected.

On the Intensity of Ultrasonic Beams Diffracted by a Wire Grating

GOPALA MENON SREEKANTATH AND C. A. VERGHESE
University College, Trivandrum, Kerala State, India
(Received November 13, 1958)

A theoretical investigation of diffraction of plane waves by a transmission grating shows that the expressions for the intensity of a given order of diffracted beam are not the same for light and sound waves. The latter is $\cos^2\theta$ times the former, where θ is the angle of diffraction. This result has considerable effect on the relative distribution of intensity among the various orders in the two cases. The foregoing theoretical result is verified experimentally in the case of high-frequency sound waves in a liquid diffracted by a wire grating immersed in the liquid.

THE accepted theory of diffraction of plane waves by a transmission grating of N vertical slits each of width b at distances $b+c$ apart yields the expression

$$I_m=\frac{N^2a^2\sin^2\{m\pi(b/(b+c))\}}{\{m\pi(b/(b+c))\}^2} \tag{1}$$

for the energy of the mth-order beam diffracted in the horizontal plane at angle θ_m. In deriving this expression the actual direction of vibration at points on the plane wave front is not taken into consideration. Hence, the same expression is assumed to give the energy of the diffracted beam whether the plane waves are of light or sound.

In developing the theory, any single slit of the grating is looked upon as made up of a number of elementary slits of width δb. When a plane wave front is incident normally on the slit, the vibration transmitted radially in any horizontal direction from each of the elementary slits is assumed to have the same amplitude δa, whatever the direction of vibration. Then the resultant amplitude of the vibrations proceeding from a single slit in a given direction θ_m and arriving at the focus of a lens is given by

$$\frac{\Sigma\,\delta a\sin\{\pi b\sin\theta_m/\lambda\}}{\pi b\sin\theta_m/\lambda}=\frac{a\sin\{\pi b\sin\theta_m/\lambda\}}{\pi b\sin\theta_m/\lambda}, \tag{2}$$

where a is the resultant amplitude in a direction normal to the slit. From this it follows that the energy of the mth-order beam diffracted by the grating at an angle θ_m is given by expression (1). However, there seems to be no justification for the general assumption that the amplitude of vibration δa from the elementary slit is independent of the direction of vibration. For instance, in the case of unpolarized light the direction of vibration is ever changing though always in the plane of the wave front. At a particular instant it makes an angle α with the horizontal; and the resolved parts of the amplitude—horizontal and vertical—transverse to the direction θ_m in the horizontal plane are

$$\delta a\cos\alpha\cos\theta_m \quad \text{and} \quad \delta a\sin\alpha,$$