ACOUSTIC MICROSCOPY

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INTRODUCTION

Optical microscopy is a well established investigation technique which furnishes a satisfactory spatial resolution for many applications. However, in some areas, the ability of processing only thin slices of specimen together with the need for tedious selective coloration techniques lead to a time consuming sample preparation. The concept of using acoustic waves for microscopy is relatively new since it appeared in the three latest decades. The acoustic wave velocity in matter is very small (five orders of magnitude lower than that of light waves). The acoustic wavelength falls then in the optical spectrum at frequencies near one gigahertz where the acoustic attenuation doesn't prevent the propagation over some hundreds of wavelengths (see fig. 1).

	Optical microscopy	Acoustic microscopy
Velocity range	1.5 to 3×10^8 m/s	1.5 to 11×10^3 m/s
Frequency range	10^{14} to 10^{15} Hz	10^8 to 10^9 Hz
Range of wavelength	0.4 to 0.8 µm	1 to some hundreds of µm
Relative indexes	no more than 2	greater than 7
Attenuation in water		220 dB/mm/GHz^2

Fig. 1 : Comparison between optical and acoustic microscopy.

The most important point is that optical and acoustical microscopy are complementary techniques. Optical microscopy gives informations about the dielectric properties of the sample and acoustical microscopy gives informations on the mechanical properties of the sample

In this last case the transmitted or the reflected beam amplitude is connected to the acoustic impedance Z.

Where

$$Z = \rho v$$

ρ being the density of the material

v being the velocity of the acoustic wave.

So, we get a new information about the mechanical properties of the sample and the sample preparation may be simpler (without resorting to colorations for optically transparent samples). We can also get, with acoustic microscopy, informations in the bulk of opaque materials.

Transmission acoustic microscope.

Several acoustic microscopes have been studied and tested which rely upon different physical principles : collinear acousto optic interaction(1), radiation pressure detector(2), photopiezoelectric effect(3), optical detection of the dynamic rippling of a surface(4), and scanning acoustic microscope(5). This last system appears to be the more suitable with respect to resolution and ease of use since only standard ultrasonic and electronic methods are implemented. As suggested by professors QUATE, the object is mechanically scanned in the common focal plane of the two lenses (fig.2).

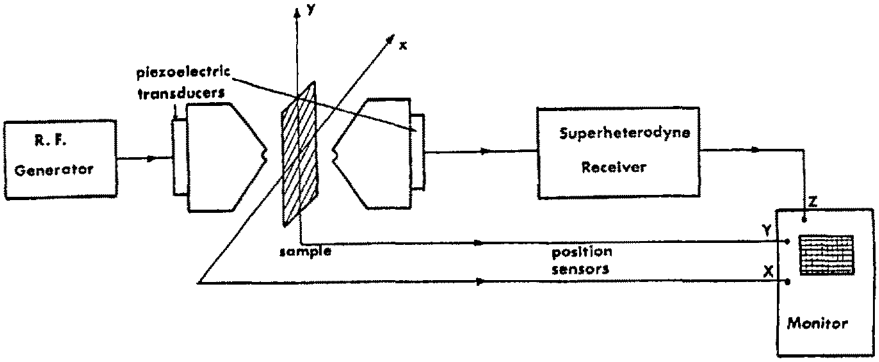

Fig.2 : Synoptic scheme of the transmission acoustic microscope.

The visualisation monitor is driven in synchronism with the object displacements and intensity modulated by an electrical signal proportionnal to the object acoustic transparency function. The fast motion of the sample is operated at about 100 Hz frequency and the slow motion at a 1Hz frequency. To observe the image of a sample we must use a remanent cathode ray-tube or we must photograph the screen of a classical cathode ray-tube : A 0.5 µm resolution has been reported at an operating frequency of three gigahertz(6). The good performances obtained follow from the characteristics of the acoustic lenses used. The grinding of spherical sapphire-water interfaces leaves us with lenses of 7.3 relative refractive index. For such an unusual value, when thinking in optical terms, the geometrical aberrations which vary as $\frac{1}{n^2}$ (if n stands for the lens index), are very small. For an angular aperture of 120 degrees the least confusion circle is then 0.25 µm in radius. It follows that, up to gigahertz frequencies, the resolution of the confocal system remains diffraction limited. It can be shown that such lenses follows the classical relationships of paraxial optics despite the lens aperture is greater than 120 degrees. This feature arise from the fact that the dephasing on the lens input face is divided by its refractive index The most important difficulty to implement an acoustic microscope technological one. Indeed we must hollow a spherical lens of 120 µm radius to image samples at a 600 MHz operating frequency. For such a value of the lens radius the attenuation during the propagation of the acoustic wave in the liquid coupling medium is not very prohibitive, say 15 dB. But at a 2 GHz operating frequency the lens radius of curvature must be 10 µm. The technological limit attained actually is a 50 µm radius of curvature. The losses in the system increase and the image contrast becomes poorer. Actually the total losses of a transmission acoustic microscope, operating at a 600 MHz, are 80 dB and we can get a good image contrast using a heterodyne receiver which has a very high sensitivity. Some very good images have been obtained at a 700 MHz frequency in the Molecular Acoustic laboratory of Strasbourg(7) about biological samples. A very specific biological sample preparation has been worked out in this laboratory which allows to bypass the classical paraffin which sustains the thin biological specimen, and to replace this by a more homogeneous polystyren film. A test grid has been imaged with a 250 magnifying power (fig.3) and a 630 magnifying power (fig.4). The bars are 50 µm large and we can see on these photographs some very small details with a good contrast. We may observe that the contrast in these photographs is reversed. The fig.5 shows the acoustic image of a piece of thyroïd tissue with a 160 magnifying power and the fig.6 shows the details of the former image (mark b) with a 400 magnifying power. The next photograph fig.7 shows the acoustic image of breast tissue with a 160 magnifying power. All these photographs show the very high contrast characterizing conjunctiva tissue, which tissue is hardly seen with an optical microscope.

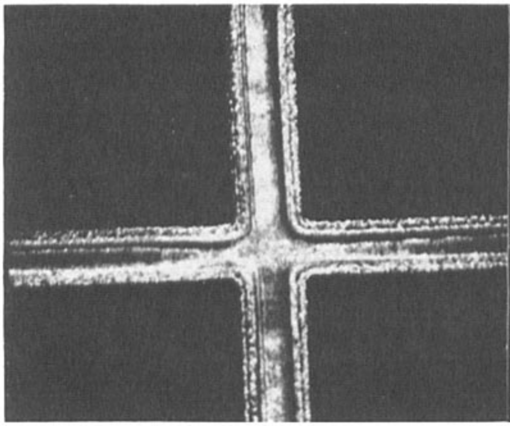

Fig.3 : acoustic image of a test grid.(x 250)

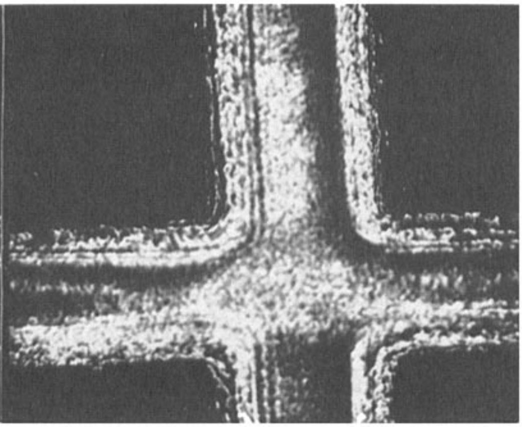

Fig.4 : acoustic image of the same test grid.(x 630).

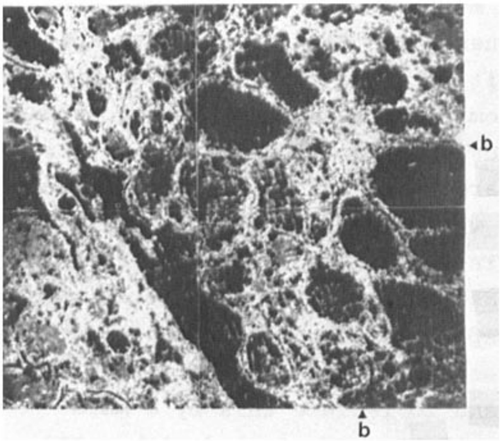

Fig. 5 : acoustic image of thyroïd tissue (x 160).

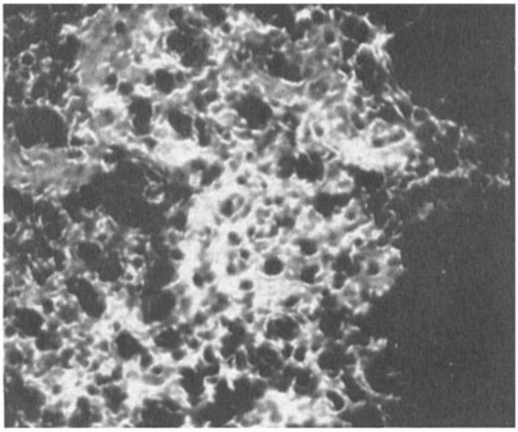

Fig. 6 : details of thyroïd tissue (x 400) in mark b)

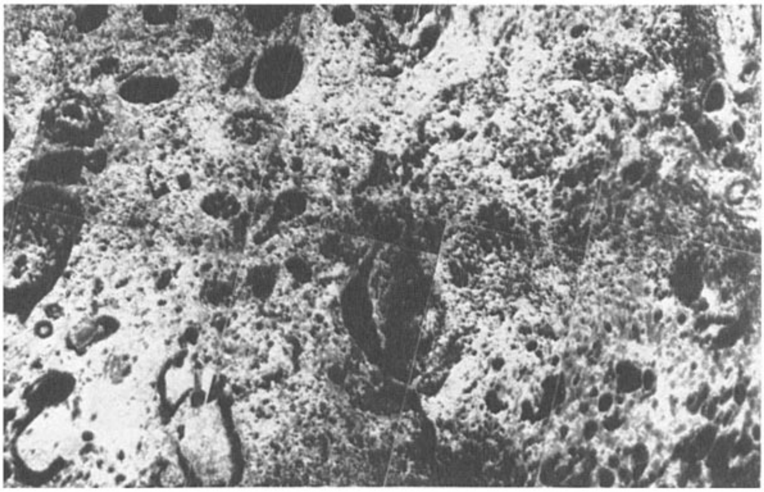

Fig.7 : acoustic image of breast tissue (x 160)

Reflection acoustic microscope

But, a transmission acoustic microscope cannot image thick specimens because the two confocal lenses distance is , at a 600 MHz frequency, 150 µm. So for thick specimens it is also suitable to use reflection acoustic microscopy in some areas like geology, metallurgy and generally non destructive testing where the thickness of the sample may be equal to several millimeters. The reflection scheme allows the visualization of internal structures lying under the specimen surface using acoustic waves. This would require careful lapping and polishing techniques if optical microscopy was to be used. In the reflection acoustic microscope there is a single transducer-delay line and a single lens grinded in this delay line. A great simplification results then in the mechanical design of the apparatus. However, the occurence of several parasitic reflected electrical signals requires a more sophisticated electronic system. One signal comes from the reflection F of the electrical energy (Fig. 8) at the piezoelectric transducer since the electrical match between the generator and the transducer is never perfect. The other signal comes from the reflection of the acoustic energy R on the highly acoustically mismatched spherical sapphire water boundary. The pulse mode of operation allows us to separate in time the usefull information E from the parasitic ones Fand R. Adelayed gate is used to prevent the receiver from being saturated by the parasitic signals(fig.9).

A boxcar integrator is used to get a continuous bright image.

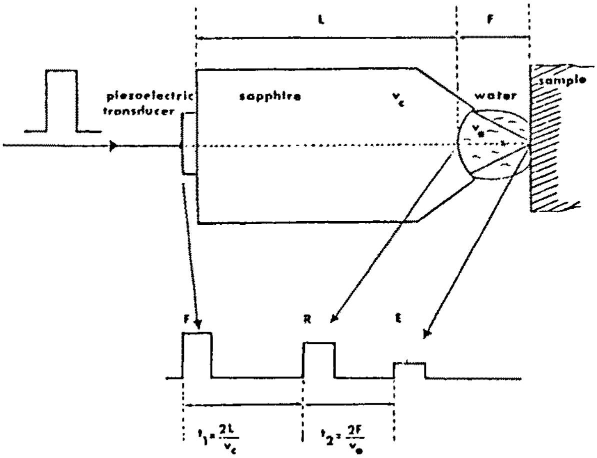

Fig. 8 : Received electrical signals

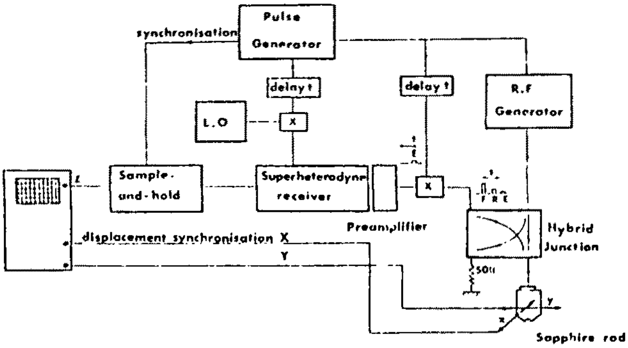

Fig. 9 : synoptic scheme of the amplifier.

Our reflection system works at a 130 MHz frequency with a spatial resolution of 10 µm inside water. This resolution has been verified using test grids with bars of 12 µm width (fig.10). The total loss in the system amounts to 50 dB and the signal to noise ratio is nearly equal to 60 dB for a typical + 20 dBm electrical power emission. So a very high quality image may be obtained.

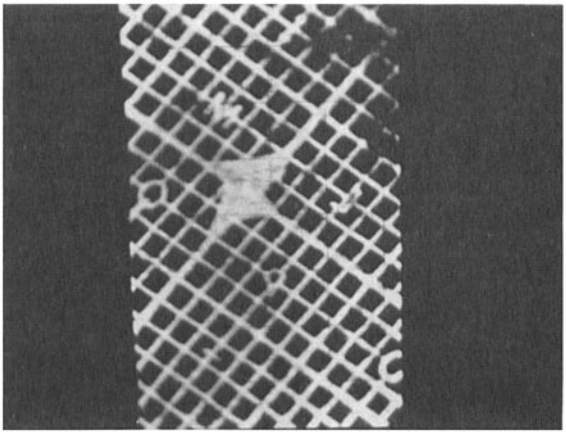

Fig. 10 : acoustical image of a test grid.
at a 130 MHz frequency.

The practical interest of the reflection acoustic microscope for seing structures lying under the surface of an opaque material or of a thick sample has led us to the numerical calculation of the acoustic field distribution behind the surface of a plane boundary. The results of this computation allow us to determine the lens radius of curvature R together with its distance to the sample surface(8) :

$$R = \frac{n-1}{n} (e + n'Z)$$

Where n is relative index of the lens with respect to water.
n' the relative index of the sample with respect to water.
Z the exploration depth under the sample surface.

The choice of the radius of curvature and angular aperture of the lens determines the minimal distance propagated in the water coupling medium inside which the attenuation is very high. The sample, when homogeneous, has a much lower attenuation. It is the case when we search for some defects inside a sample. So the radius of curvature must be kept minimum in order to preserve the high contrast. Thin slices, 2 mm in thickness, have been analyzed and images have been taken at different depths, using a 1 mm lens radius and a 1 20° angular aperture. A typical optical image of a fossil is given in fig. 11 and the acoustical image of the sample surface is shown in fig. 12a together with that of a plane lying at nearly 150 µm depth (fig. 12b).

Fig. 11 : Optical image

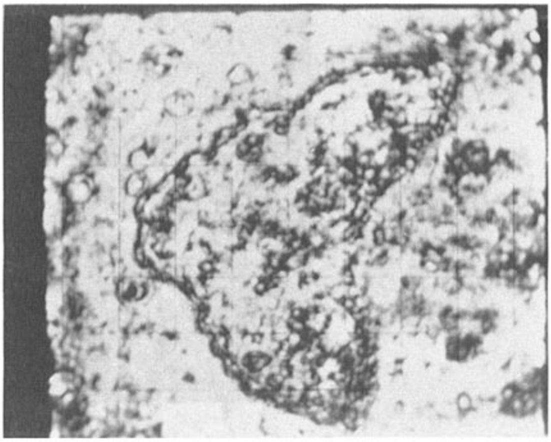

Fig. 12a : acoustical image of the sample surface.

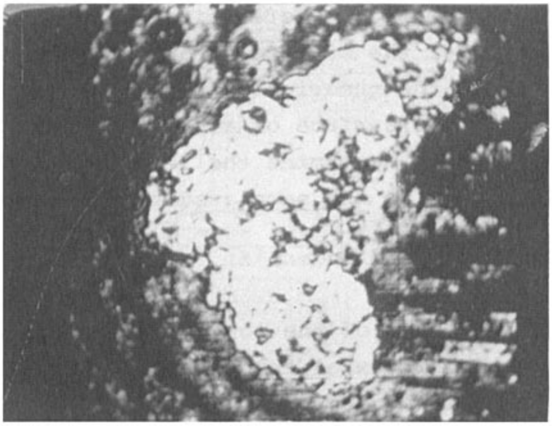

Fig. 12b : Acoustical image at 150 µm depth

To conclude, the rated resolution compares actually well with that given by the best optical microscopes and in some instances structures have been imaged acoustically which don't appear on optical micrographs. It is the case for structures lying in the bulk of opaque materials. Some images have been obtained at Standford University at a 3 GHz frequency which show some defects in integrated circuits. Some applications of acoustic microscopy to non destructive testing area are also now under study. However a particular study and a matching of the basic apparatus must be devoted to each new problem. For example, the application to the localization of very small diameter defects is now underway in our laboratory.

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