REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 1988 - Final	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Studying Materials Using Acoustic Waves		5. TYPE OF REPORT & PERIOD COVERED Final Feb.1985-Feb. 1988
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) R.E. Apfel		8. CONTRACT OR GRANT NUMBER(s) N00014-85-K-0215
9. PERFORMING ORGANIZATION NAME AND ADDRESS Dept. of Mechanical Engineering Yale University, P.O. Box 2159 Yale Station 9 Hillhouse Ave., New Haven, CT 06520		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research Physics Division Office Arlington, VA 22217		12. REPORT DATE 1 March 1988
		13. NUMBER OF PAGES 21
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Cavitation, acoustic scattering, interfacial characterization, nonlinear parameter, acoustic levitation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This Final Report summarizes the activity of the contractor in meeting the objectives of the contract. A comprehensive bibliography and list of participants on the contract work are included along with a discussion including: microcavitation, microparticle characterization, interfacial characterization using acoustic levitation, measurements of the acoustic nonlinear parameter for determining the composition of mixtures.

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S/N 0102-LF-014-6601

YALE UNIVERSITY

OFFICE OF NAVAL RESEARCH

1988 - FINAL REPORT

N00014-85-K-0215

STUDYING MATERIALS USING ACOUSTIC WAVES

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1 March 1988

LUX ET VERITAS

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INTRODUCTION

This document is the Final Report for Navy Contract N00014-85-K-0215. Since this also represents the termination of ONR support of our Yale Research (we hope for only a modest period), I have included some background information that reviews the history of our ONR support beginning in 1972.

Reference is made to the Summary Report written on the preceding contract, N00014-76-C-0527, which covered the period of November 1975 - 1 May 1982. The present report discusses our major research focuses, key personnel, and publications since that period of time. A complete personnel history and publications list for ONR supported research is provided in Appendices I and II.

SMALL SCALE SCIENCE

With much attention being paid to BIG SCIENCE (URI's, NSF Centers, Supercomputers, and SSC's), it is a tribute to ONR that it has undergirded a major part of small scale, basic research. Sustained support at modest amounts for large numbers of Principal Investigators must remain the backbone of any federal research program if we are to produce the scientific/technological results and human resources necessary for our country to move ahead in an interdependent world. The buying power of federal money has probably declined, not only because of inflation but also because of the growing Indirect Cost rates that Universities have negotiated with the Federal Government. (Hopefully these trends will reverse.)

Thus, a rough analysis shows that over the 15^+ years of support at a total of about $750K, the dollar value (in purchasing power) of ONR support for our work has gradually declined. Nevertheless, university researchers

(faculty and students) are used to adjusting to these circumstances. Our accomplishments, outlined below, and the comprehensive bibliography attest to the quality and productivity of our work under ONR support.

PRIMARY RESEARCH FOCUSES: 1985-1988

We have emphasized four research topics in the past three years. Each has been described in early Progress Reports, and is summarized briefly below. The last three projects have been supported primarily by the National Institute of Health, with ONR's contribution being directed primarily on the methodology, and less on the medical applications.

1.   Interfacial Characterization of Surfaces in the Presence of Surfactants

Key Questions: How does one model the mechanical characteristics of liquid-fluid interfaces that are contaminated or contain surface-active materials (surfactants)?

Motivation: Surface characterization of liquid-fluid interfaces has applications in the oil industry, chemical industry, and biomedical applications. The state-of-the-art is highly empirical (the "try and see" approach).

Approach: Develop a sensitive experimental method for measuring interfacial mechanics, and relate the experiments to a realistic model of the interface (e.g. something like equations for the constitutive and transport properties of the surface as a function of surface concentration of additives).

Progress thus far: The characteristics, especially energy dissipation, of capillary waves are strongly influenced by the presence of surfactants or contaminants. We have studied the phenomena by investigating both

theoretically and experimentally the free quadrupole oscillations of a fluid drop in another fluid. A theoretical model has been established in which surface dilatational elasticity, introduced naturally by a hydrodynamic analysis, and surface dilatational and shear viscosities are imposed on the interface. These additional surface properties change the boundary conditions. Strong vorticity is thus generated in the boundary layers when the drop is oscillating, and enhances the energy dissipation of the system. Explicit expressions for the damping constant and the free oscillation frequency for several limiting cases have been derived by the perturbation method and compared to experimental data directly.

An acoustic method for suspending a drop in another liquid and deforming the drop has been combined with an optical detection scheme to measure the frequency and damping constant of the drop. The sample systems that we have studied are hexane drops of different radii in pure water and hexane drops in sodium dodecyl sulfate (SDS) aqueous solutions of different concentrations. The data for the pure system confirm the theoretical predictions. For hexane drops in SDS aqueous solutions, it is found that the most important interfacial property is the surface dilatational elasticity. Furthermore, the measured damping constants are well explained for SDS concentrations lower than 1.75 mM by employing an ideal equation of state for surfactants at the interface. The interfacial tension between hexane and SDS aqueous solutions of different concentrations has also been obtained. Finally, comparing the time history curves for the free oscillation frequency

and damping constant of a contaminated system reveals that the surface dilatational elasticity, which is a function of contaminants, may be the major cause of the time dependent phenomenon.

2. Microcavitation

Key Question: Under what circumstances does high frequency ultrasonic waves produce detectable micron-scale cavitation?

Motivation: In some applications microcavitation is desirable (e.g. cleaning microchips) and in some, undesirable (ultrasonic investigation of the fetus).

Approach: Produce a model which describes the inception and effects of microcavitation, and compare the model with carefully controlled experiments that are sensitive enough to measure single bubble at the micron scale.

Progress Thus Far: Our progress thus far has been both experimental and theoretical. On the experimental front we have sought to measure micron-scale cavitation in three ways: 1) sonoluminescence; 2) passive scattering and listening, and 3) active cavitation detection (ACD). Since results for the first two have been reported in the literature, what follows is a review of our novel ACD technique.

The Active Cavitation Detector (ACD) makes use of high frequency scattered sound waves as an indicator of microbubble activity. We use a lightly focused, narrow band ultrasonic transducer operating in a pulse-echo mode (see Fig. 1). This "detector" transducer, which produces low level acoustic tone bursts, is positioned confocally relative to the "cavitation" transucer. The high frequency (30 MHz center frequency) detector pulse is synchronized with the lower

frequency cavitation pulse in order to insure that the two pulses overlap temporally in the confocal region. If a cavitation event occurs, the bubble will scatter a significant portion of the ACD pulse, which will be detected in the backscattered direction. Prior work suggests that this active scattering system is sensitive enough to detect individual human red blood cells suspended in isotonic saline. A 1 μm diameter gas bubble in water has a scattering cross section which is 425 times larger than that of a typical red blood cell. Needless to say, the ACD is sensitive enough to detect individual microbubbles, which makes it one of the most sensitive cavitation detectors in existence today.
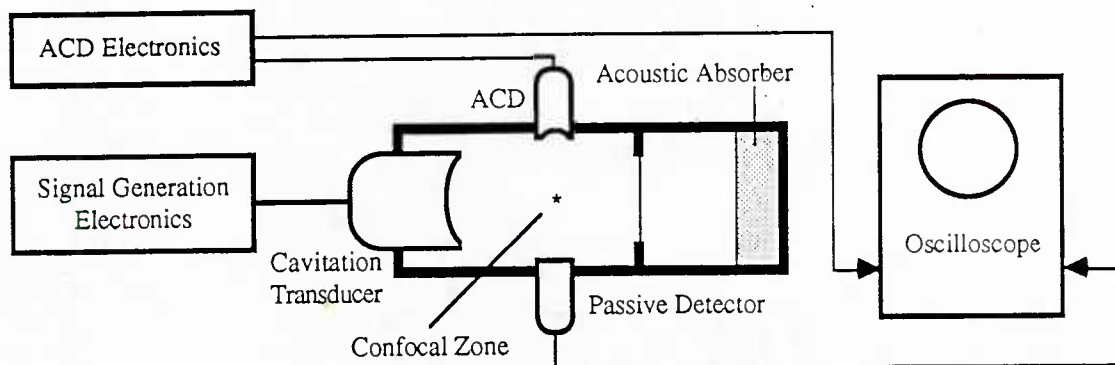

Fig. 1   Block Diagram of the Active Cavitation Detector Experiment

In our preliminary experiments we compared the sensitivity of the present embodiment of the ACD with that of a more traditional technique which utilizes a passive acoustic detector which "listens" for lower frequency scattering from cavitating bubbles. Using a 50 μsec long,

750 KHz center frequency cavitation pulse and a 10 µsec long ACD pulse, we initially set the relative synchronization of the two pulses so that they overlapped in the first 10 µsec of the cavitation pulse. While simultaneously monitoring the outputs of the two detectors, we measured the cavitation threshold in relatively clean, degassed water. Next, we increased the delay of the detector pulse relative to the cavitation pulse so that the two pulses overlapped in the second 10 µsec of the cavitation tone burst, and repeated the threshold measurement. By incrementally increasing the delay in this manner, we were able to scan the entire cavitation pulse.

The results of this "proof of principle" experiment are given in Table 1. Note the marked reduction in the thresholds measured by the ACD. This result is significant, for it suggests that the thresholds measured using passive detection may be dependent on the detector sensitivity, and therefore not represent "true" thresholds. This is similar to a conclusion made by others using a sonoluminescence technique. Indeed, the same may be true of the ACD measurements. The only way to determine this will be to vary the sensitivity of the ACD (which we can do) and see if the thresholds change. Also we shall note the variation in measured threshold as we change the temporal overlap position. This serves to illustrate one of the novel features of the ACD: the ability to interrogate the cavitation field during a precisely specified time window. Also since the ACD transducer is finely focussed, it offers spatial resolution as well. Therefore, by introducing appropriate time and position manipulators, it should be possible to determine both where and when cavitation is occurring in a given sample.

Overlap Region	ACD Threshold	Passive Threshold
First 10 μsec	15±2 Bar peak	22±2 Bar peak
Second "	14±3	22±2
Third "	15±2	22±2
Fourth "	15±2	22±2
Fifth "	21±3	22±2

Table 1. Comparison of thresholds measured with the ACD and a passive detector.

Ultimately, we seek to compare the results of active cavitation detection with other techniques (e.g. sonoluminescence) to learn when cavitation is accompanied by free radical formation. Transient bubble formation, by itself, is not necessarily correlated with conditions that produce health risks. Therefore, it is essential that we have the most sensitive bubble detection system possible, so that distinction between bubble occurrence and bubble effects can be made.

3. The Nonlinear Parameter of Materials

Key Questions: How does the acquistion of data on the acoustic nonlinearity parameter of a material add to our understanding of material behavior, and does this additional data offer any special opportunities with regard to determining the composition of mixtures?

Motivation: The "inverse problem" of determining component properties from data on the mixture has applications in material science, the health field, and on other areas of science and engineering. For instance, it would be extraordinarily helpful to be able to determine tissue composition _in vivo_ from non-invasive acoustical measurements.

Approach: Generate a mixture law for the nonlinear parameter of mixtures and apply it to the determination of composition of mixtures (including, for example, tissue phantoms).

Progress Thus Far: The measurement procedure and apparatus for measuring the nonlinear parameter have been refined to allow accurate and repeatable measurements of the acoustic nonlinear parameter, B/A, for liquid and gel-like substances. As outlined in our earlier reports, an acoustic interferometric technique is employed to measure the small changes in sound speed which occur as pressure is changed in the substance to be measured. Our system is distinct from that of other researchers in that we make rapid adiabatic pressure changes as small as one atmosphere. In addition, we use pulsed ultrasound to avoid standing wave effects, making it suitable for both lossy and not-so-lossy materials, and our system requires no temperature ramping. Because our data collection procedure is computer-controlled and each measurement takes only a few seconds, it is possible for us to make thousands of independent measurements of B/A and perform statistics on the ensemble to infer the true B/A value (see accompanying Figure 2). The resulting mean values of the nonlinearity parameter, made on a wide range of substances, are as accurate or more accurate than those in existing published data. This high level of accuracy is necessary in order to allow us to study quantitatively our tissue composition methodology, compare it with other such models, and to improve it with a view toward its use in in vivo imaging.

Scattergram of B/A of H20 at 20°C
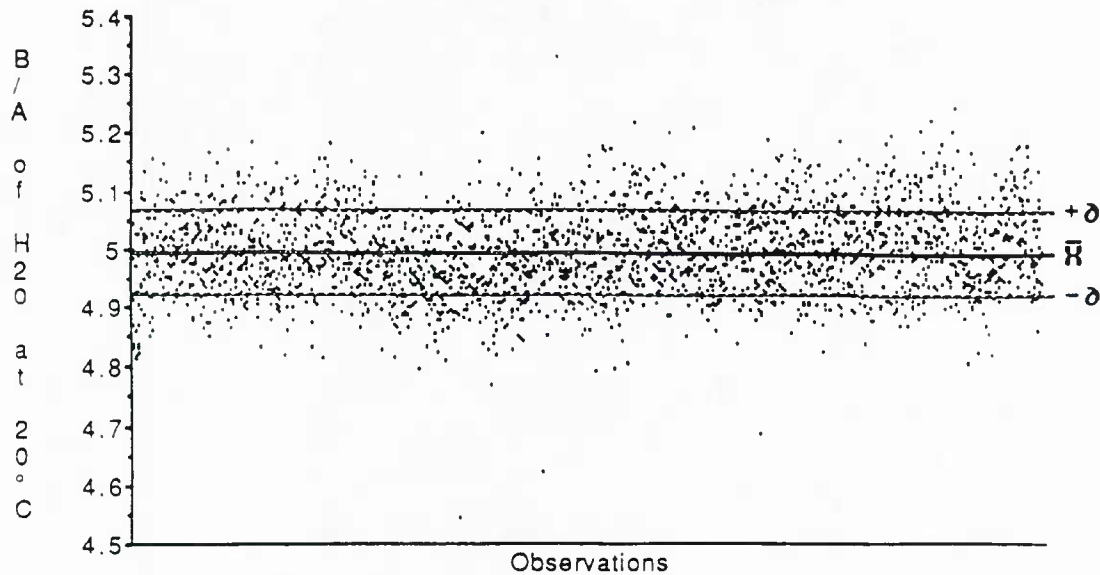
B/A of H20 at 20°C

Mean:	Std. Dev.:	Std. Error:	Variance:	Coef. Var.:	Count:
4.996	.073	.001	.005	1.467	2565

Minimum:	Maximum:	Range:	Sum:	Sum Squared:	# Missing:
4.544	5.332	.788	12814.756	64036.366	0

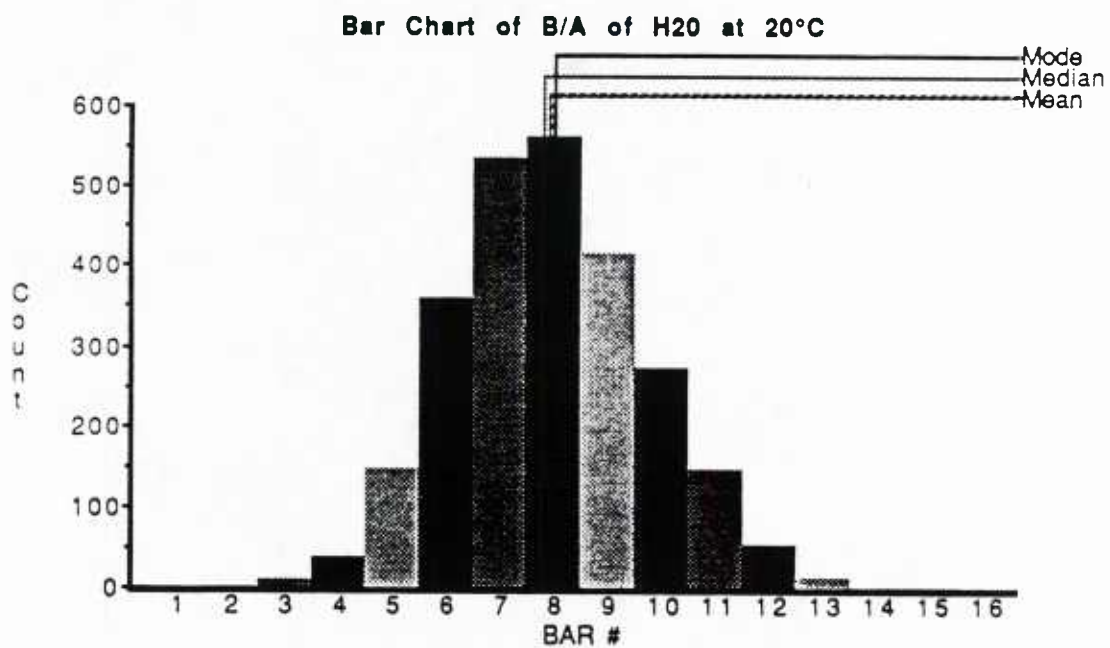| Median:	Geo. Mean:	Har. Mean:	Kurtosis:	Skewness:	
4.992	4.995	4.995	.9	.05	

Bar Chart of B/A of H20 at 20°C

Figure 2: Data and Statistical Measures for B/A Experiments

In the past year the apparatus has been fully automated, the temperature stability and monitoring have been improved, and the electronics redesigned to yield more consistent measurements (see apparatus Figure 2). Because we will soon be analyzing protein and lipid suspensions of precisely controlled molecular structure, our measurement cell has been modified to accept small quantities (30 ml) of solution. Earlier in the year we measured the density, sound velocity, and B/A of water, methanol, dextrose solutions, various gelatins, and mixtures of these materials. To help us investigate the role of molecular structure in our methodology, however, we must make a more systematic study. We anticipate making measurements of density, sound velocity, B/A, acoustic absorption, and MRI proton relaxation time on biochemically well-characterized materials (amino acids, proteins, lipids, and their mixtures), varying quantities such as pH, temperature, and surfactant concentration for each. Our goal is to analyze and compare the various mixture rules which predict, on the basis of measurements made on the bulk materials, the volume fractions of water, protein, and fat contained in the mixture. Once the theory has been refined, it should be possible to characterize a tissue by measuring its bulk properties and inferring its water, protein, and fat content. Eventually, it may be possible to perform this technique _in vivo_ by imaging parameters such as the acoustic absorption, sound velocity, and B/A, and using our improved methodology to infer the components. We have already collected _in vitro_ data on "phantom" mixtures, i.e. tissue-equivalent mixtures for which the water-protein-fat ratios are known, in order to see under what conditions the existing models correctly predict the components.

For example, below is a summary of some recent tests that compare our experimental results with predictions from the Apfel model.

Table 2

Mixture	Measured or Inferred Parameters				Known Percentages	Predictions (Apfel Methodology)
	ρ	c	β	B/A		
50% gel4* 50% chicken fat	943.2	1470.1	4.91	8.85	53.5% water 3.5% protein 43% fat	56% water 0.8% protein 43.1% fat
75% gel4 25% chicken fat	990.0	1505.4	4.46	7.43	74.5% water 4% protein 21.5% fat	75.3% water 5.6% protein 19% fat

* gel4 = 4% gelatin (by volume) in water

In the coming year we will complete our analysis of the existing mixture models by testing their ability to predict the components of many different kinds of mixtures. We hope to be able to extend our theory to allow us to resolve differences between similar tissues (e.g. cancerous and non-cancerous tissue). We will then begin making measurements on tissues of this type, as well as on pure materials whose material properties (density, sound velocity, B/A) are not well-characterized. Finally, by performing a detailed study of mixture properties with concentration and temperature, we hope to gain insights into the molecular structure of the various mixtures. (This work is largely supported by NIH, now. Early work and non-biological applications supported by ONR.)

4. <u>Microparticle Characterization Using Acoustic Scattering</u>

Key Question: Can one characterize the mechanical properties of

distributions of microparticles suspended in a liquid, distinguishing

among subpopulations, etc.?

Motivation: A wide range of equipment is available to size and

characterize particles, drops, and bubbles, but none of these focuses

on mechanical properties of individual particles that are part of a

large group of particles. Applications abound in biomedicine, the

chemical industry, and in several other areas in the public and

private sector.

Approach: Develop an experimental procedure that can provide size,

density, and compressibility information on each particle in a

suspension passing through the focal zone of a pair of 30 MHz

acoustic transducers, and compare the information gained from the

scattered signals with theory that has been appropriately modified so

as to allow the inversion of the scattering data into material

properties.

Progress Thus Far: The topic of this work is an acoustic scattering

technique for determining the compressibility and density of

individual particles. The particles, which have diameters on the

order of 3-10 μm, are modeled as fluid spheres. Ultrasonic tone

bursts of 2 μsec duration and 30 MHz center frequency scatter from

individual particles as they traverse the focal region of two

confocally positioned transducers. One transducer acts as a receiver

while the other both transmits and receives acoustic signals. The

resulting scattered bursts are detected at 90° and at 180°

(backscattered). Using either the long wavelength (Rayleigh) or the weak scatterer (Born) approximations, it is possible to determine the compressibility and density of the particle provided we possess a priori knowledge of the particle size and the host properties. The detected scattered signals are digitized and stored in computer memory. With this information we can compute the mean compressibility and density averaged over a population of particles (typically 1000 particles) or display histograms of scattered amplitude statistics.

An experiment was first run to assess the feasibility of using polystyrene polymer microspheres to calibrate the instrument. A second study was performed on the buffy coat harvested from whole human blood. Finally, chinese hamster ovary cells which were subject to hyperthermia treatment were studied in order to see if the instrument could detect heat induced membrane blebbing (blistering).

In these experiments, we demonstrated the utility of the instrument for isolating subgroups in a mixed population. This suggests that the acoustic scattering apparatus would be well suited to applications involving selective operations, such as particle sorting or counting. Because the demodulated acoustic signals are available in real time, it is possible to use acoustic information to provide a sorting criterion.

A distinct disadvantage of our acoustic apparatus is that there are two independent bits of information available from our experiments, but there are three unknowns (volume, density, and compressibility) in the theory (assuming that the wavelength is sufficiently long and the scatterer is sufficiently weak so that

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shape is not a factor). This fact leaves us in the dilemma of not being able to determine physical properties without a priori information.

Our most recent work is devoted primarily to incorporating a particle volume sensing device into the scoustic scattering system in order to overcome the above-mentioned problem. We have built and tested our own Coulter-type sensing orifice. From the raw data obtained with our new apparatus we should be able to calculate density, and compressibility for each cell passing through the system. (This data was taken on Oct. 22, 1987, and has not been evaluated in detail for quantitative information.)

MeanDiameter = 4.9
SD(acoustic) = 9.3%
SD(coulter) = 12.0%

MeanDiameter = 5.9 μm
SD(acoustic) = 7.3%
SD(coulter) = 8.0%

MeanDiameter = 7.4 μm
SD(acoustic) = 6.4%
SD(coulter) = 8.1%

MeanDiameter = 9.7 μm
SD(acoustic) = 3.0%
SD(coulter) = 2.1%

MeanDiameter = 10.5 μm
SD(acoustic) = 7.4%
SD(coulter) = 8.7%

MeanDiameter = 14.6 μm
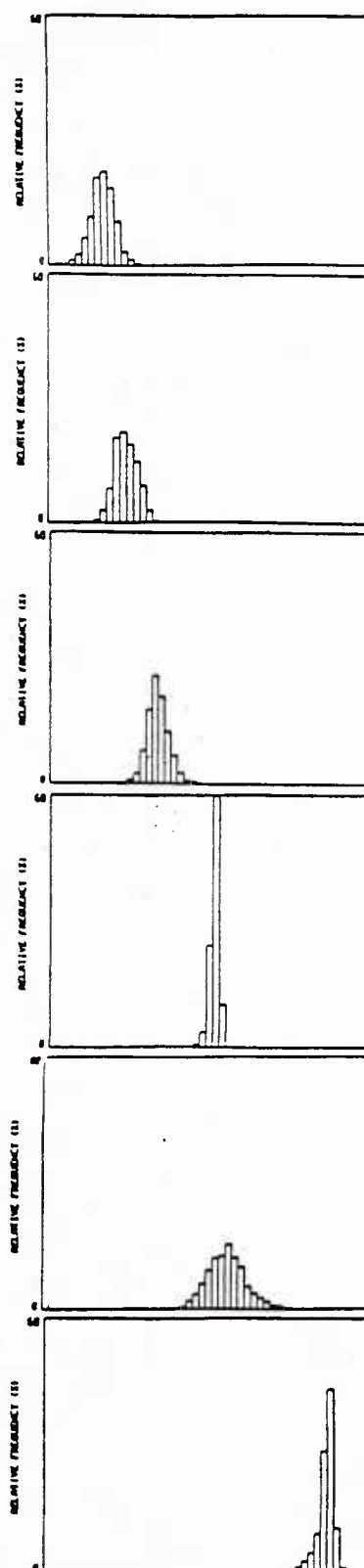SD(acoustic) = 3.4%
SD(coulter) = 2.7%

Fig. 3 Histograms of the 90° scattered signals for polystyrene microspheres in water.
The horizontal axis corresponds to the cube root of the scattered signal amplitude,
which for polystyrene is proportional to size.

PERSONNEL WHO HAVE WORKED ON OUR ONR CONTRACTS:
N00014-67-A0097-0023; N00014-76C-0527; N00014-85-K-0215

NAME	TITLE	TIME ON PROJECT
Robert Apfel	Principal Investigator	11/76 - present
K. Baxter	Undergraduate student and part-time summer employee	During term and summer, 1977
M. Cheng	Summer Research Student	11 weeks, 1985
B.-T. Chu	Professor	1979 - present
Howard Clifton	Summer Research Program Participant	Summer 1978
Wesley Cobb	Graduate student	1978-1982
Jonathan Denlinger	Summer Research Program Participant	Summer 1983
R. Eligator	Summer Research Program	June 1982
E. Everbach	Ph.D. student	1985-1987
Henry Fischer	Summer Research Student Participant	Summer 1983
James P. Harbison	Summer Research Program Participant	Summer 1972
Chaur-Jian Hsu	Graduate student	1977-1982
H.-L. Lu	Ph.D. student	1984-1987
P. Marston	Research Fellow	1976-1978
R. McGowan	Graduate student	1983-1985
E. Neppiras	Consultant	1 week each Fall
R. Roy	Ph.D. student, Postdoc.	1984 - present
C. Smith	Ph.D. student	1984-1985
M.P. Smith, Jr.	Summer Research Program Participant	Summer 1972
E. Trinh	Graduate student, Postdoc.	1972-1978
Victoria Wagner	Summer Research Program Participant	Summer 1977

M.A. Weiser	Graduate student	1976-1981
Roberta Young	Summer Research Program Participant	Summer 1976
Zhu Zhe Ming	Visiting Fellow	1981-1983

Often support has also come from other sources (e.g. Yale); these individuals have in one way or other supported the objectives of the contract.

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