THE BLACK VAULT

SCIENCE SPECTRUM

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A NEW TECHNIQUE FOR STUDYING MICROORGANISMS

With differential light scattering you can learn more in minutes about microbial morphology and physiology than others have learned in years. Some examples are given in the accompanying Application Notes.

This technique - new to the study of microorganisms - shares some capabilities of optical and electron microscopy. Sample preparation is simple and rapid; submicron physical details can be studied; a wide variety of samples can be examined in many different environments and states, and physical changes monitored.

The differential light scattering measurements detailed in the accompanying Application Notes were made with our DIFFERENTIAL I photometer. In addition to determining structural parameters, such measurements enable bacterial presence and concentration to be determined easily. The scattering data can be analyzed rapidly, easily and quite accurately using the Atlas of Light Scattering Curves described in the enclosed flyer.

For more information on these or other applications, or on our products, simply fill out the enclosed prepaid card. A copy of our current seminar schedule also is enclosed. If you have any question regarding the study or results reported in the Application Notes, please let us know.

Sincerely yours,

SCIENCE SPECTRUM, INC.

James E. Hawes
Vice President, Marketing

Enclosures

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SEMINARS

Seminars with demonstrations explaining light scattering theory and its many applications are offered by Science Spectrum periodically at various central locations throughout the United States, free of charge. The DIFFERENTIAL I and DIFFERENTIAL II instruments are also exhibited at selected professional meetings. The seminars and exhibits currently scheduled are:

September 14 - 16, 1971	Washington, D.C. - Exhibit
September 17, 1971	Washington, D.C. - Seminar
September 21, 1971	Raleigh, North Carolina - Seminar
September 24, 1971	New York, New York - Seminar
September 27, 1971	Boston, Massachusetts - Seminar
October 5, 1971	Los Angeles, California - Seminar
October 7, 1971	San Francisco, Calif. - Seminar (tentative)
October 15, 1971	Chicago, Illinois - Seminar (tentative)

If you wish to attend one of the seminars listed above, please complete the form below and return it to the Company. A program will be sent to you about two weeks before the scheduled meeting, together with a confirmation of your reservation.

I will attend your seminar to be held on _____

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ATLAS
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Atlas of Light Scattering Curves

Introduction

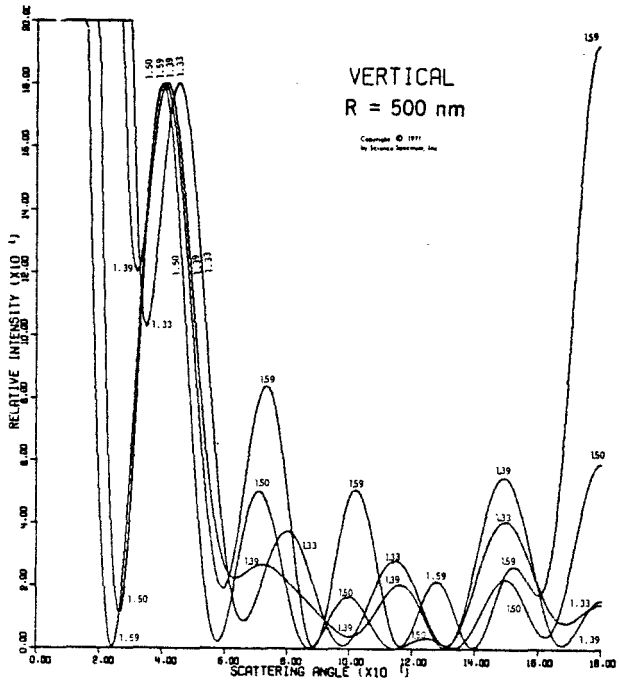
The interpretation of light scattering data has long been an obstacle to the widespread use of this powerful analytical tool. While some scientists devoted their careers to the theoretical understanding of light scattering, their results were not readily adapted for use by workers in other fields. High speed digital computers can now be used economically to generate scattering data for a variety of model particles.

The purpose of the Science Spectrum Light Scattering Atlas is to make this computer-generated information available in a convenient form for a wide range of light scattering applications involving small particles. Computer-generated light scattering patterns are plotted on the same scale as the experimental data measured by the *Differential I* and *II* light scattering photometers. Semitransparent vellum paper has been used for the Atlas so that accurate comparison of theory and experiment is achieved by merely overlaying the two sheets. Tables of normalization constants for absolute scattering power are also provided.

Single Spherical Particles in Air

A wide variety of processes produce homogeneous spherical particles of approximately one micrometer in diameter. For example, photochemical aerosol or "smog" droplets and colloidal particles like those in latex paints are spherical. Such small particles may easily be suspended in air by nebulizing a liquid suspension and their individual scattering patterns are readily measured with the *Differential II* scattering photometer.

This important class of scattering objects is completely described by two parameters: radius and refractive index. The first section of the Atlas displays



A sample page from the Atlas showing the light scattering curves for homogeneous spherical particles of 500 nm radius and four different refractive indices.

the scattering curves of spheres with radius between 0.05 and 1 micrometer in steps of 0.05 micrometer, for refractive indices ranging from 1.33 to 1.59. The refractive index of the surrounding medium is that of air (n ~ 1.0). Curves for both linear polarizations are given. Inspection of the scattering atlas for spherical particles shows that particle size can easily be specified to within 0.1 micrometer diameter. A determination of refractive index to well within ten percent accuracy is achieved for spheres by simply examining the relative intensities at peak amplitudes.

Supplements

Periodically, additional scattering curves are published as supplement sections to the Atlas. Owners of the Science Spectrum Scattering Atlas will receive all supplements issued within two years of the date of

purchase without charge. Subjects selected for early supplements include: the effect of size distribution upon scattering from suspensions of spheres; the effect upon scattering of size and size distribution changes in model bacteria and mitochondria; scattering from conductive particles; scattering from absorbing particles; and scattering from airborne bacteria. Measured scattering curves from known non-spherical particles may also be provided.

Computation of special scattering curve sets for a wide variety of objects will be done at moderate cost using proprietary Science Spectrum computer codes. The scattering from spheres of different diameters or refractive index can be computed on order. For example, curves for small variations in particle diameter at a constant refractive index can be vided for studies of colloidal size distributions. B this means sizing accuracy of $\pm$ 10 nm diameter can be obtained as reported by Phillips *et al* in the *J. Colloid Int. Sci. 34* (1970), p. 159. Scattering curves on absorbing spheres can also be computed as needed. Curves on any spherically symmetric structure with varying complex refractive index can be generated. Specific applications for specialized shell structures include bacteria, bacterial spores, microencapsulation particles, compound aerosol particles with large nuclei, etc. Even more varied shapes can be computed exactly when the particle nearly matches the refractive index of the medium in which it is immersed — as in the case of bacteria in water.

Please send me_____copies* of the Science Spectrum Scattering Atlas at $25.00 each, plus $1.25 sales tax if delivered in California.

I understand that I will receive, without further charge, all supplemental sections to the Atlas published in the next two years. Also, if payment is enclosed with my order, Science Spectrum will pay shipping costs.

If I am not satisfied with the Atlas, I may return it postpaid within 10 days for a full refund.

Name_____

Title _____

Department_____

Organization_____

Street_____

City, State, and Zip_____

My main interest in light scattering is:_____

_____

The supplement I am most interested in is:_____

_____

* Purchasers of a *Differential I* or *Differential II* photometer receive with the instrument two copies of the Atlas and all supplements for two years.

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Physiological Monitoring of Bacteria and Mitochondria

Introduction

Optical methods ranging from microscopy to turbidimetry have long been used to monitor bacterial growth and division. However, the optical microscope is unable to resolve features smaller than a few wavelengths of light in size. Turbidimetric measurements are subject to large errors because the attenuation of light is a function of the product of particle scattering cross section and particle density. Since the particle scattering cross section is not in general the same as the particle's geometrical cross section, significant interpretive problems arise. A given value of transmittance will often correspond to several different products of particle density and particle size. On the other hand, differential light scattering measurements (i.e., recording the pattern of light scattered by such particles as a function of angle relative to the direction of the illuminating beam) are unambiguous, often yielding size and shape information of much higher precision than obtainable with a microscope. Under optimum conditions, cell size determinations of ± 2% accuracy are achievable with differential light scattering measurements.

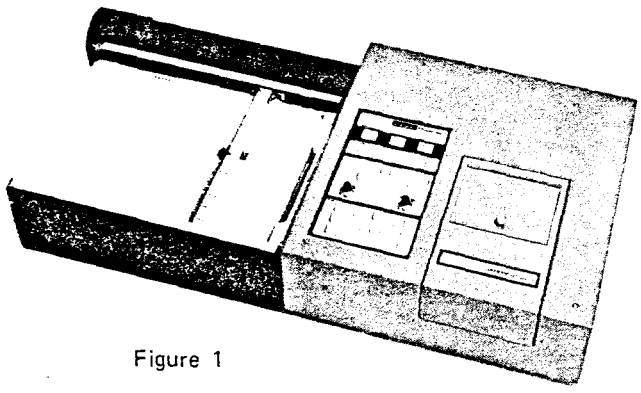

Figure 1

A variety of biologically important processes can be accurately studied by differential light scattering. Physical changes in mitochondria subjected to various enzymes, pH variations, and osmotic stresses can be directly monitored[1-3]. Systematic distortions of bacterial cells by preservatives such as phenol, formalin, or alcohols can be measured precisely[4]. Subtle changes in response to elevated temperatures[5] or pressures are easily determined. Size modification and cellular damage occuring in phage-infected bacteria can be measured. The process of spore germination can be monitored as it proceeds. The response of chloroplasts to various processes including photophosphorylation have been followed by light scattering[6-8]. The susceptibility of bacteria to various antibiotics can be measured within minutes of contact[9]. Changes in mean cell size during synchronous growth can be monitored with an accuracy of ± 20 nm. The effect of different growth media on the size distribution of cells can be seen clearly via light scattering patterns. These measurements can be made without disturbing the growth of the culture. Some details of such studies are discussed below.

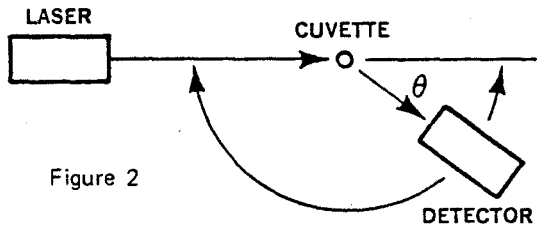

Figure 2

The Differential I Photometer

The *Differential I* light scattering photometer is a highly versatile instrument, uniquely suited to the study of liquid suspensions of bacterial cells. It is shown in Fig. 1 and its operation is represented schematically in Fig. 2. In use, a cuvette containing the suspension is placed in the instrument and illuminated by the intense monochromatic beam of an argon-ion laser. A specially designed scanning detector system records, as a function of the scattering angle θ relative to the beam direction, the intensity of light scattered by the cells. This differential light scattering pattern embodies a wealth of information about the cell ensemble, such as cell size, shape, structure, size distribution[10,11], and even structural details such as cell wall thickness and the refractive indices of the cell wall and cytoplasm[12].

A final example of considerable interest concerns the effects of heat killing on cell size and size distribution. In preparing autologous staphylococcus vaccines, many laboratories use heat as a sterilization procedure. Such a treatment supposedly does not destroy the immunogenic properties of vaccines and would be expected, therefore, to have little or no effect on cell walls. Figure 7 shows the changes in the differential light scattering patterns as a function of heating times for *S. epidermidis* broth suspensions at 60°C. (The curves have been broken at 65° and displaced relative to each other for visual clarity.) A subsequent analysis[5] of this data showed that the un-heat treated cells had an average radius of 432 ± 10nm which decreased to 403 ± 10nm after 30 minutes heating. The average cell wall thickness remained nearly constant at 108 ± 20nm despite the heating, but the breadth of the size distribution increased by 15% after heating.

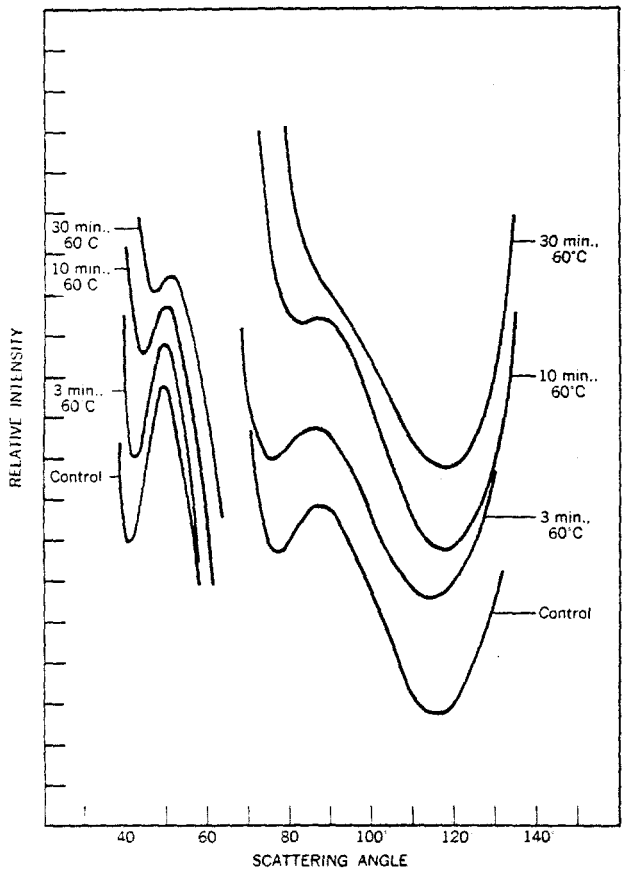

Figure 7

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For further information

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APPLICATION NOTE
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Rapid Assay of Bacteria in Urine

Introduction

The detection of threshold concentrations of bacteria in specimen solutions such as urine presents an important medical challenge. If it were possible to make a *rapid* determination of whether the bacterial count in urine is greater or less than 10^4/ml (0.1 critical level)[1], it would expedite enormously what is now a very time-consuming procedure. A testament to the urgency of this need is the recent work at NASA[2], whose luciferase - ATP assay to detect life on other planets is being considered for detecting bacteria in urine.

A more direct bacterial counting capability, one which is simple, effective and rapid, is available via the technique of laser light scattering using a commercially available table-top instrument, the DIFFERENTIAL I.

DIFFERENTIAL I Photometer

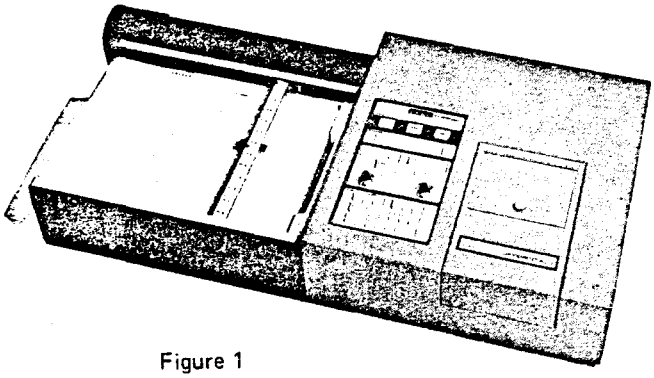


Figure 1

The DIFFERENTIAL I laser light scattering photometer is a highly versatile semi-automatic instrument designed to study liquid suspensions of cells with minimum alteration of their normal environments. The instrument, shown in Fig. 1, records the intensity variation with angle, θ, of the scattered light which results when a cuvette of the solution under study is illuminated by a laser beam. The operation is shown schematically in Fig. 2.

The variation with angle of scattered light intensity is detected by a specially-designed scanning system which records the output on a strip chart or x-y recorder, or on a digital data card punch unit.

When the size and internal structure of the illuminated particles have dimensions approximating the wavelength of the incident light, as do bacteria, the scattered light pattern is particularly sensitive to these particle parameters. The features (amplitude and angular positions of maxima and minima) in the scattering pattern give a precise measure of the size, shape, structure, and size distribution

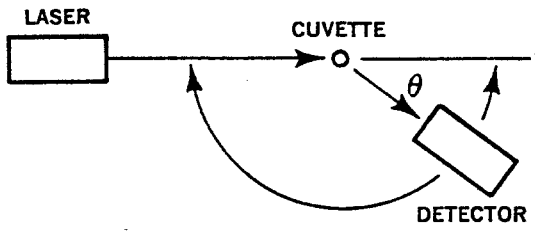


Figure 2

Differential light scattering patterns can be analyzed theoretically using computer software already developed, or simply compared to previously compiled "known" scattering curves in a pattern recognition approach, analogous to fingerprint identification. An *Atlas of Light Scattering Curves*[5] is available which permits even those not previously familiar with differential light scattering to quickly and accurately determine many of the important physical parameters of cells in suspension. In addition, measured changes in the light scattering pattern can be employed to monitor the effects of variation of conditions (heat, nutrient changes, drug treatment, etc.) on bacterial suspensions. A number of these applications have already been carried out using the DIFFERENTIAL I instrument[4,6,7,8].

Urine Specimen Assays

The simple task of determining concentrations of bacteria does not need to utilize these analytical aids however. In studies of bacterial suspensions using the DIFFERENTIAL I the detection of bacterial concentrations of 10^5/ml is routine. Indeed, in applications such as antibiotic susceptibility testing, solutions are prepared at about this concentration for optimal results. At these concentrations and lower, the intensity of the scattered light at any angle relative to the background from the liquid system is approximately proportional to the number and density of cells, especially when the cell size distribution is narrow. Thus, calibration of the light scattering patterns in terms of cell concentration is straightforward.

Figure 3 shows a set of light scattering recordings taken on the DIFFERENTIAL I for pure distilled water and with several bacterial concentrations as indicated. The detectability of these levels can be clearly seen.

In specimen solutions such as urine, appreciable background light may be scattered from various materials other than the bacteria, materials such as tissue cells, granules, cell debris, leukocytes, erythrocytes and various crystals. To gauge the magnitude of this background scattering, bacteria in known concentrations were added to *unprocessed* urine and the samples examined in the DIFFERENTIAL I. Some of the typical scattering patterns are shown in Fig. 4. It can

Figure 3
S.aureus in water

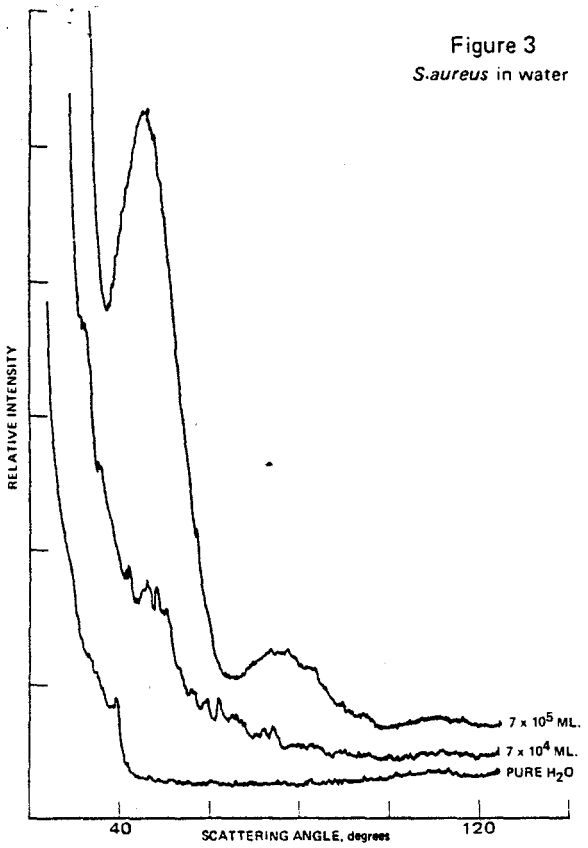


Figure 4
Bacteria added to urine

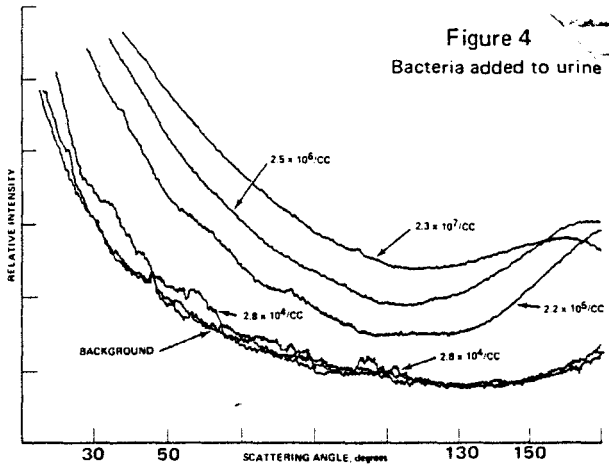
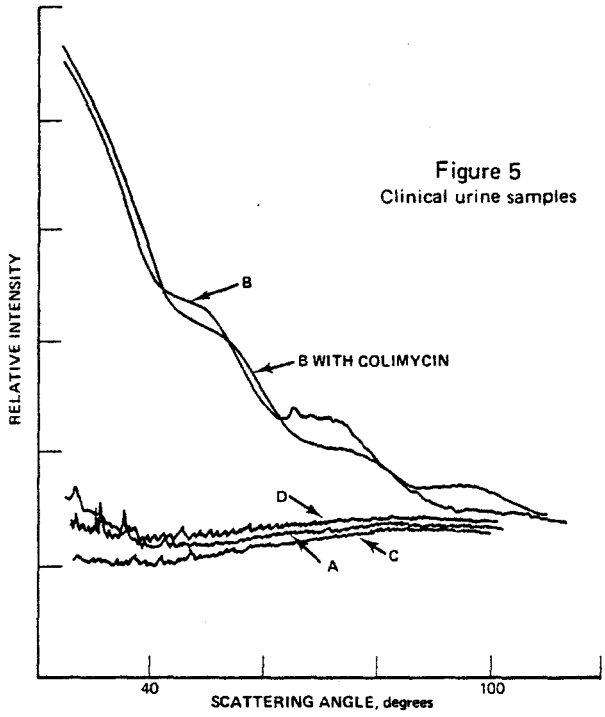


Figure 5
Clinical urine samples



able, as expected; even at a lower concentration of 2.8 x 10^4/ml the pattern is emerging from the background signal.

These results for untreated urine are very encouraging. Simple techniques such as warming, mild acid treatment, and sedimentation all of which are compatible with rapid processing, and should not affect the bacteria, can remove most of the background-producing material, thereby reducing the background scattering levels so that bacterial concentrations appreciably lower than 10^5/ml can be measured.

Qualitative Studies

Figures 5A through 5D show light scattering patterns, taken with the DIFFERENTIAL I, based on four patient urine samples[9]. The protocol for all four was:

- 1 loopfull of urine was placed in trypti case broth and incubated for six hours;
- 0.5 ml of the incubated solution was placed in 13.5 ml of distilled water in a cuvette;
- the cuvette was placed in the DIFFERENTIAL I and scanned.

Samples A, C, and D showed no growth when incubated overnight on a nutrient plate. Sample B showed the presence of growth after incubation overnight on a plate, corroborating the clear indication of bacterial cells in the light scattering pattern (specimen curve). The effect of treatment with an antibiotic (colimycin) was also determined. As shown in Fig. 5B, simply by adding it to sample B and then rescanning its resulting light scattering pattern[8], susceptibility was clearly indicated by the dramatic change in the pattern. This illustrates the rapid antibiotic susceptibility test capability of the DIFFERENTIAL I. When coupled with the simple yet sensitive capability of the instrument to measure bacterial concentrations in urine, the importance of differential light scattering as a clinical tool is evident.

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SCIENCE SPECTRUM
APPLICATION NOTE

Standardization of Bacterial Culture Media and Suspensions Using the Differential I

Introduction

In the process of growing bacteria, it is often necessary to maintain a strict uniformity of the cultural conditions from one day to the next. The success of a long and often costly experiment is directly dependent both on the quality and on the uniformity of the growth media. In clinical laboratories, the lack of uniformity of culture media can lead to grave consequences. For example, in antibiotic susceptibility testing, errors not only in preparation of the culture medium[1], but also variation between batches of media supplied by the manufacturer can produce erroneous results. In commercial laboratories (drug houses, chemical manufacturers, etc.) a major obstacle to obtaining optimum biological or biochemical yields arises from lack of a precise means to measure and standardize growth media.

The formulation printed on bottles of dehydrated culture media, unfortunately, only approximates many of the actual components. The chemical composition of components such as peptones, tryptic hydrolysates and meat extracts are known to vary greatly from one medium to another. In addition, analyses or descriptions of amino acid sequences, lengths of peptide chains, vitamin contents, and contents of all other growth and inhibitory substances which may be present in complex media are, of course, not normally provided nor available.

In rehydrating and sterilizing a culture medium, the chances of altering the medium to an unknown degree are high. Temperature and duration of autoclaving, temperature of medium when plates are poured, ambient temperature and humidity, thickness of the medium, and age of the plates affect subsequent growth.

The difficulties in standardizing cultural conditions are quite apparent. However, Science Spectrum now provides an instrument to monitor with high reproducibility and precision, variability in culture media and cultural conditions in general. The *DIFFERENTIAL I* measures the physical and physiological state of growing bacteria, providing a means to quantify variability of the growth conditions.

By comparing data obtained with bacteria grown on different media, one can immediately determine similarities or differences in the growth patterns. In addition, this simple comparison technique can easily be extended to measuring the effects of toxins, antibiotics[2], temperature[3], chemicals[4], irradiation, and moisture on growing bacteria.

An *Atlas of Light Scattering Curves*[5] can be used to determine quickly and accurately particle parameters such as size, refractive index, and size distribution for various species of bacteria and similar particles.

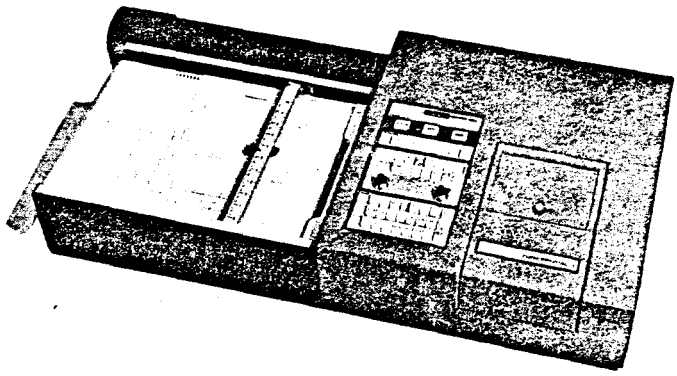


Figure 1

Differential I

The *DIFFERENTIAL I* laser light scattering photometer is a highly versatile semi-automatic instrument designed to study liquid suspensions of both viable and nonviable cells. The instrument, shown in Fig. 1, records the directional pattern of scattered light intensity from a bacterial suspension illuminated by a laser beam. The operation is shown schematically in Fig. 2.

The pattern (i.e. the intensity of scattered light as a continuous function of angle relative to the incident beam

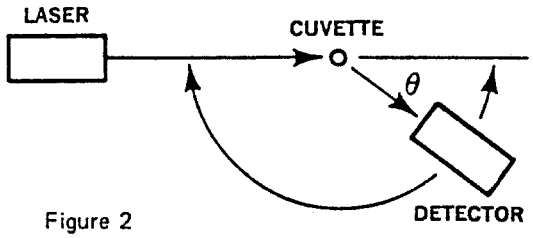


Figure 2

direction) is recorded by an automatic scanning detector system, the output of which is designed to drive either a chart recorder or a digital data card punch unit.

If the average radius of the bacteria approximates the wavelength of the laser light, the interaction between the cells and the light is strong. Hence, variation in intensity of the scattered light is particularly sensitive to the size and structure of the bacteria. Conversely, the features of the light scattering pattern generated by and measured with a *DIFFERENTIAL I* can be used to deduce average size, size distribution[6] and even general structure of the particles.

Measuring Relative Cell Concentrations

To determine cell numbers by differential light scattering, a standard set of light scattering measurements for different known concentrations of bacteria is used to establish control patterns. In making subsequent light scattering measurements, a quick comparison to the control curves allows a very accurate determination of bacterial concentrations. Differences in cell concentration of less than five percent are easily recognized by this method.

Figure 3 shows a typical standard set of curves which were used as control patterns. In this case, *Staphylococcus aureus* (Seattle) was the test organism. The cells were spread evenly on Heart Infusion Agar (HIA) and after incubation at 37° C for 10 hours, an aqueous suspension of cells was prepared. The initial cell concentration was 2.0×10^7 cells per ml; by dilution concentrations of 1.5×10^7, 1.0×10^7, and 5.0×10^6 bacteria per ml were also prepared. Note from the figure that large differences in overall scattering were produced with cells differing by only 25 percent in concentration. The position (scattering angle) of the maxima, i.e. the primary peak (arrow) provides a measure of the average size of the bacteria. Note that dilution has not altered the angular position of this peak, nor has it significantly changed the overall *appearance* of the curves. If the bacteria were of smaller average size, the position of the peaks would have been shifted to larger angles (to the right) or to smaller angles were the average size larger. But the overall intensity level of the curves is essentially only a function of cell concentrations.

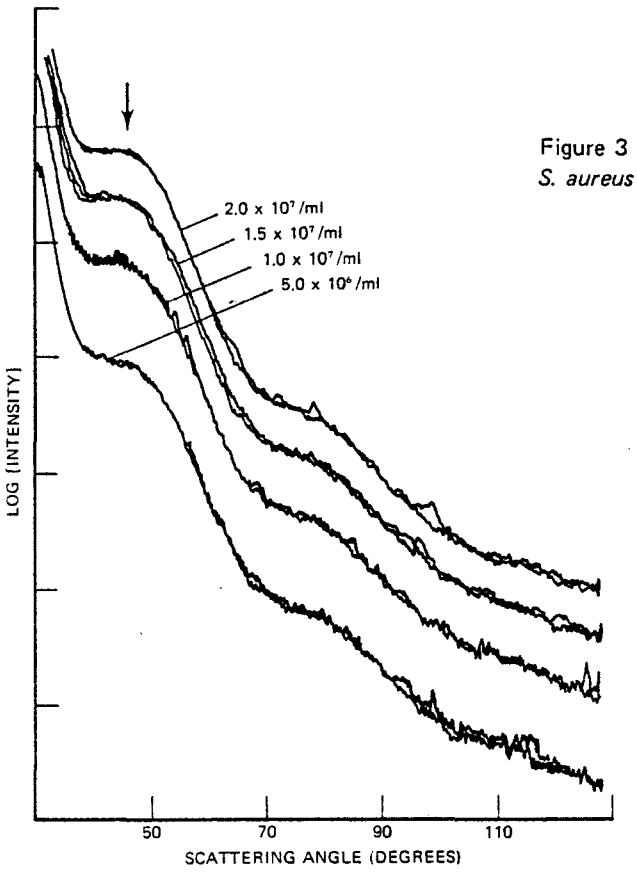


Figure 3
S. aureus

Monitoring of Solid Media for Growth Potential

Difco Tryptic Soy Agar (TSA), Nutrient Agar (NA), and Heart Infusion Agar (HIA) were evaluated using *S. aureus* (Seattle) and the *DIFFERENTIAL I.* Cells removed from each plate after incubation for 8 hours at 37°C were resuspended in water to an optical density (OD) of 0.38 (λ = 650 nm). For the differential light scattering measurements, the cells were then diluted 1/40, corresponding to cell counts of approximately 8×10^6 bacteria per ml. In addition, a set of four Tryptic Soy Agar plates, freshly pre-

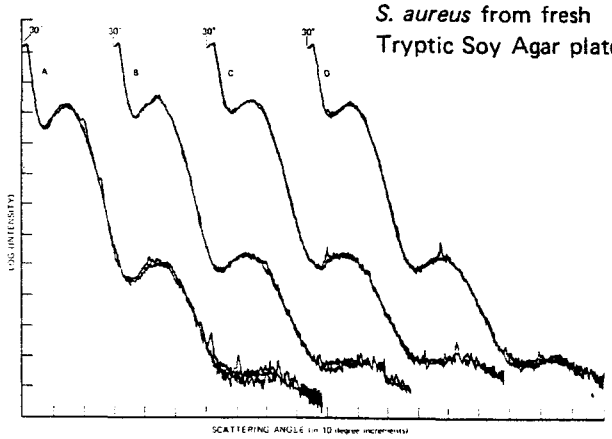
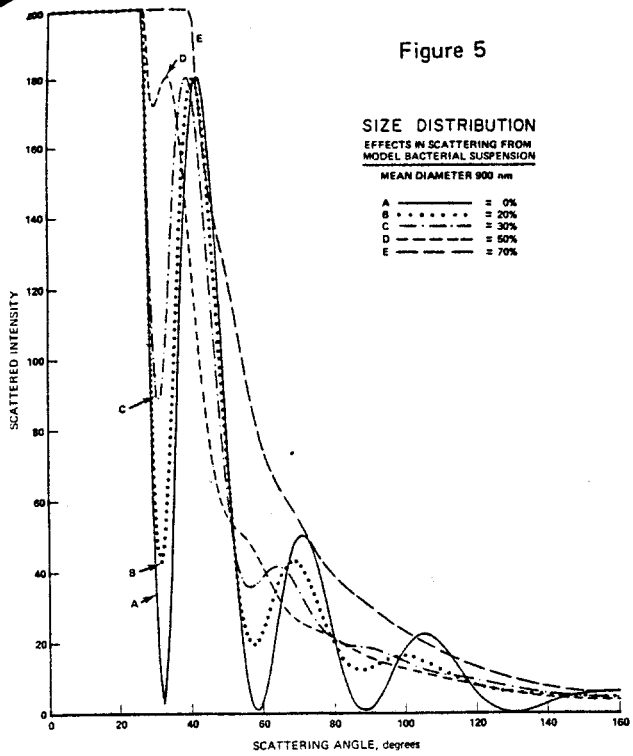


Figure 4
S. aureus from fresh Tryptic Soy Agar plates

Figure 5



pared, served as a control for variability within a single medium batch. The four control curves are shown in Fig. 4. Note that while the relative heights of the peaks differ slightly, the four curves are almost identical in shape. (The differences in peak height suggest that the cell concentration of each sample differs by a small percentage, an error resulting from lack of sensitivity of the spectrophotometer used to prepare the suspensions.)

Figure 6 Effects of media on growth of *S. aureus*

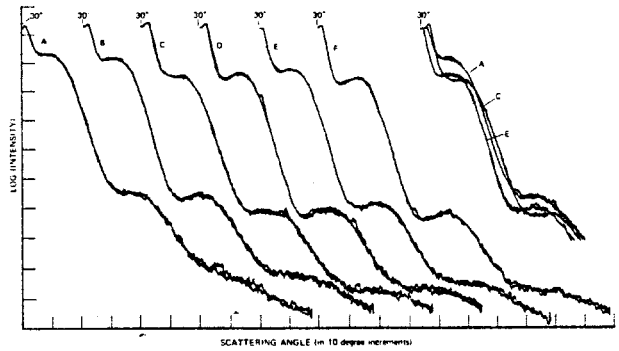


Figure 5 shows a series of computer-plotted curves for cells of varying size distribution. The loss in peak definition is clearly increasing with widening of the size distribution. The Science Spectrum *Atlas of Light Scattering Curves*[5] can be referred to for excellent approximations of size and size distribution.

The scattering curves shown in Fig. 6 were obtained when *S. aureus* was grown on various solid media for 8 hr at 37° C. The plates contained (A) TSA, stored at 4° C for 16 days, (B) TSA, freshly prepared, (C) NA, stored for 6 wks at 4° C, (D) HIA, 6 wks at 4° C, and (F) HIA, sealed 2 mos at 4° C. At first glance the differences in scattering appear small; however, a composite of Curves A, C, and E, shown to the right, clearly reveals some significant quantitative and qualitative differences in three of the cultures. Such differences cannot be measured with other conventional particle sizing or monitoring instruments. Only by differential scattering measurements could it be ascertained that, for example, the cell concentration is highest for

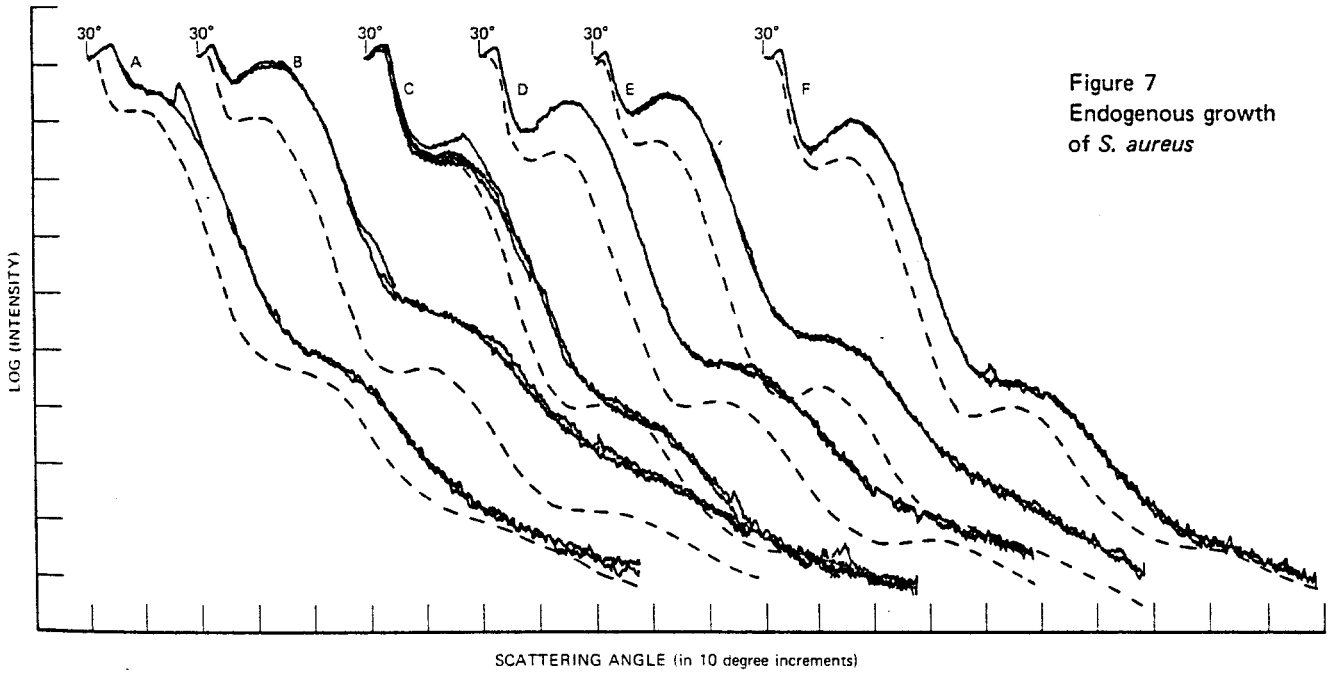


Figure 7
Endogenous growth of *S. aureus*

Curve A and lowest for Curve C. (The OD's for all three suspensions were the same. The so-called OD as determined with conventional spectrophotometers depends critically upon the average size of the particles and is not a monotonic function of this average size.) Furthermore, cells producing Curve C are smaller than the others, as evidenced by the shift of the scattering peak to higher angles. Lastly, the cells producing Curves A and E are similar in size, but differ in that the size distribution among the latter cells (E) is narrower.

Of equal significance is the ability of the *DIFFERENTIAL I* to measure physiological differences in the cells taken from these six cultures. The method is described in the next section.

Endotrophic Metabolism, a measure of cell quality

The cultures tested directly from agar media have characteristics which differ not only with respect to size or size distribution, but also with respect to nutritional makeup. The *DIFFERENTIAL I* can also measure these nutritional or physiological differences simply by measuring changes in light scattering among cells reincubated for several hours in liquids having no nutritional value, such as water or certain buffer solutions.

Figure 7 shows the scattering curves of the same cell suspensions as Fig. 6 taken after changes due to endotrophic metabolism had occured. The data of Fig. 6 are included in Fig. 7 as broken lines for the purpose of comparison. The solid, unretouched curves show how the scattering signatures changed for each of the six cultures when they were allowed to stand in the cuvettes containing water for an additional 10 hours at 25° C. The changes observed consisted of changed in cell numbers, average cell size and size distribution. Endotrophic growth was apparently best among cells previously grown on fresh TSA (Curve B) since the scattering curve was shifted upwards significantly. On the other hand, the residual growth of *Staphylococcus aureus* taken from Nutrient Agar (Curve C) was barely detectable. Note also how cell shrinkage, readily deduced from shifts of the primary peak to larger angles (A, B, D, E, F) can be observed to various degrees for each of the six preparations. Lastly, one can deduce that cell size distribution had narrowed significantly in some cases (e.g., curves B, D, E, and F) as is evident from the significant sharpening of the primary peaks.

In a set of control determinations (not shown) inocula on four plates made from a single batch of TSA were found to change uniformly when held in water for 8 hours.

Summary

Differential light scattering measurements provide a sensitive means to measure numbers, size, size distributions, refractive index, and refractive index distribution of bacterial cultures. With the *DIFFERENTIAL I* laser light scattering photometer the most critical applications involving standardizing of culture media are possible and practical. In addition to its ability to monitor quantity and quality of bacterial growth, the instrument provides a sophisticated means to study physical and physiological changes in growing and resting cells.

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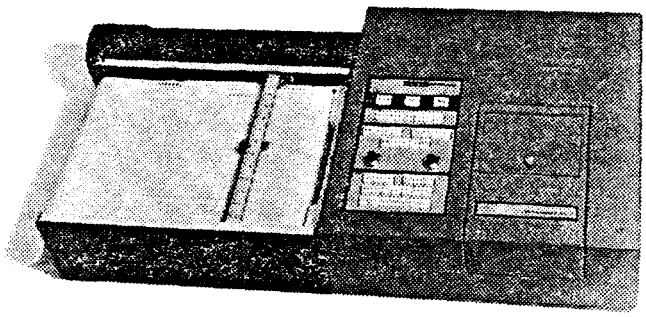
For further information

Call or write the Director of Technical Liaison, Science Spectrum, Inc., P.O. Box 3003
1216 State Street, Santa Barbara, California 93105; telephone (805) 963-8605.

SCIENCE SPECTRUM

ASM NEWSLETTER

New Laser Instruments for Microbiology Demonstrated



The *Differential I* instrument
for studying microbial suspensions and molecular solutions.

Periodic Demonstrations Scheduled

Periodic demonstrations of each instrument will be given by Company personnel at its exhibit booths 812-813.

Every two hours, beginning at 9:30 a.m. various applications of the *DIFFERENTIAL I* will be presented. These applications include -

- bacterial growth and morphology
- the effects of various alcohols on bacterial suspensions
- antibiotic susceptibilities
- accurate measurement of bacterial concentrations in suspension

Many other applications will be discussed. Also, Application Notes will be available for inspection and discussion with Company personnel.

The *DIFFERENTIAL III* instrument for the automatic determination of antibiotic susceptibilities will be demonstrated each morning at 10:30 A.M. Afternoon demonstrations will start at 12:30, 2:30, and 4:30 P.M. During each demonstration, susceptibilities of viable bacterial isolates to several antibiotics will be automatically computed by the *DIFFERENTIAL III.* Samples of the data card printed with these computed susceptibilities will be distributed, together with brochures describing the instrument and test.

Differential Light Scattering Papers

Exciting results and potentials of differential light scattering investigations were discussed in papers presented during the Annual Meeting. W. Khan *et al* described "Rapid Detection of Bacteria and Antibiotic Sensitivity in Body Fluids by Differential Light Scattering", paper M45 presented in Session 24 Monday afternoon. M. W. Wolfe and D. Amsterdam mentioned some preliminary differential light scattering measurements related to their study of the "Interactions of Bacteria of Medical Importance with Plant Agglutinins", paper M107 of Session 69 Tuesday afternoon.

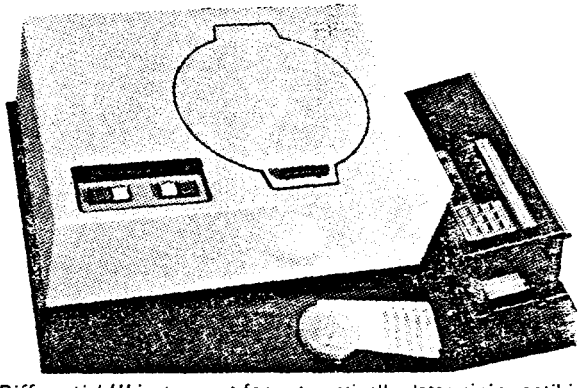
A survey of the potential of differential light scattering was discussed in a seminar, Session 25, held Monday afternoon and titled "Instrumental Approaches to the Rapid Detection and Characterization of Bacteria". Convened by David Amster-

With the advent of new laser light scattering instrum tation, on display by Science Spectrum at booths 812-813, is no longer necessary to wait for days to determine the resu of an experiment involving microorganisms. Bacteria, spo and other microorganisms now may be examined with lc powered lasers, permitting their structural characteristics a their responses to various environments and processes to quantified accurately and rapidly.

One of the basic laser instruments for such studies is t *DIFFERENTIAL I*™ light scattering photometer, shown the left. Applications of this new instrument, and this n approach to microbiology, demonstrated by the Company its exhibit include quantifying the effects on microorganis of pesticides and germicides, antisera, and various chemica Antibiotic susceptibility determinations also will be demc strated.

Another basic laser instrument being demonstrated Science Spectrum is the *DIFFERENTIAL II*™ photome for studying *single* microorganisms. Using this unique inst ment, the structure of single bacteria and spores can be det mined while the individual particles are still viable and ir natural environment.

Another instrument, one very important to the clini laboratory, is the *DIFFERENTIAL III*™. Shown below, t instrument enables antibiotic susceptibilities to be determir automatically and rapidly. It will be demonstrated periodica by Company personnel for the duration of the exhibit usi viable organisms and several antibiotics. Susceptibilities will computed by the instrument within two minutes of sample troduction. Samples of the data card printed with the compu susceptibilities will be distributed together with brochures scribing the instrument and test.



The *Differential III* instrument for automatically determining antibic susceptibilities.

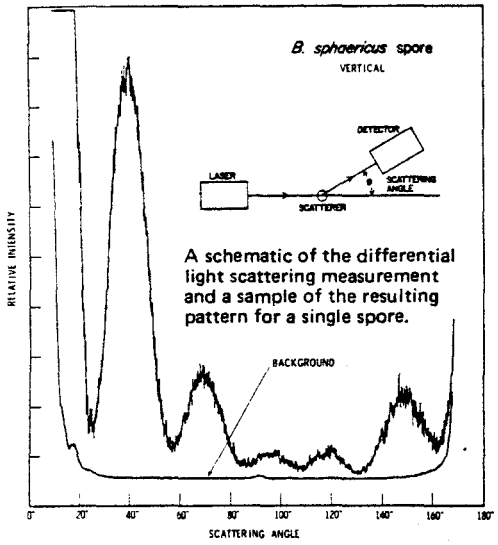
The DIFFERENTIAL III™ Instrument

The new *DIFFERENTIAL III* instrument, shown abc rapidly and automatically determines antibiotic susceptibili of microbial specimens in an entirely different and nc manner. It will be demonstrated periodically throughout exhibit, using viable cells and several antibiotics and deterr

Differential Light Scattering Papers cont.

dam, Kingsbrook Jewish Medical Center, Brooklyn, the speakers were Donald A. Glaser, University of California, Berkeley; Henry Lubatti, University of Washington, Seattle; Norman G. Anderson, Oak Ridge National Laboratory; Philip J. Wyatt, Science Spectrum, Inc., Santa Barbara; and Henry D. Isenberg, Long Island Jewish Medical Center. An abstract of Dr. Wyatt's presentation "Applications of Differential Light Scattering in the Clinical Microbiology Laboratory" follows:

Dr. Wyatt described a powerful new approach to many of the problems of clinical microbiology. Noting that the size of bacteria and the wavelength of visible light are about the same, Dr. Wyatt pointed out that as a consequence a variety of unusual effects are observed whenever laser light is scattered from bacteria. By carefully measuring and interpreting the manner by which such microorganisms scatter laser light, numerous microbiological phenomena may be rapidly and accurately characterized. These light scattering techniques have thus opened the way for extensive new instrumentation that promises dramatic changes in near future for the clinical laboratory. Dr. Wyatt described instrumentation and techniques currently available, including a new and revolutionary automated system *(DIFFERENTIAL III)* that determines the antibiotic susceptibilities of exponential phase isolates within 12 minutes. He also described other instrumentation to be developed within the next decade using light scattering techniques that could permit rapid identification and susceptibility testing of clinical specimens without the requirement for initial isolation and incubation. All the techniques described appear to be equally applicable to aerobes and anaerobes, as well as the more fastidious mycobacteria. In this latter regard, a highlight of his talk was the preliminary report that Dr. Claude Reich of Johns Hopkins (Leonard Wood Memorial) had measured *an antibiotic effect on a species of mycobacteria in less than 15 minutes using the differential light scattering technique.*



A schematic of the differential light scattering measurement and a sample of the resulting pattern for a single spore.

The DIFFERENTIAL III™ Instrument cont.

ing susceptibilities in a few minutes. During each demonstration, first sample suspensions of the bacterial isolate are prepared and exposed to different antibiotics. Then in the instrument, each sample is illuminated in succession with a laser beam of low power. The illuminated bacteria scatter the incident radiation, producing characteristic light scattering patterns which respond in a manner corresponding to the response of the cells to the antibiotic. The patterns produced by suspensions incorporating antibiotics are compared in succession to the pattern produced by a control suspension without antibiotics. The degree of susceptibility indicated by pattern changes then is automatically computed and printed on a data card.

A *DIFFERENTIAL III* placement and evaluation program will begin shortly after the ASM meeting in several laboratories throughout the nation. The results of this evaluation program will be made available periodically to interested clinical laboratories.

Differential Light Scattering, Briefly

When particles are illuminated by light, they will in general scatter this light in all directions. The intensity of the scattered light as a function of the *direction* has been termed the *differential scattered light intensity.* This is illustrated schematically in the figure on this page. The trace adjacent the schematic shows such a differential light scattering pattern (measured in a plane with respect to the direction of the incident light) for a single *Bacillus sphaericus* spore. Comparison of this pattern with theory permits the unique determination of the spore's diameter and refractive index of the spore's cortex and coat. For the example shown, the radius of the spore was found to be 483 ± 5nm, the coat thickness 80 ± 10nm, the refractive index of the cortex 1.56 ± 0.02 and of the coat 1.48 ± 0.03.

Your Invitation

To see the instruments, and to discuss the application of particular interest to you, please drop by booths 812-813 at your convenience. Company scientists will be there, and literature and data folios will be available for your inspection. Also, you may fill out a request form to receive - free of charge - any Company publications you desire.

Should you care for more information, or copies of our publications, please call of write the Vice President, Marketing, Science Spectrum, Inc., 1216 State Street, Post Office Box 3003, Santa Barbara, California 93105, telephone (805) 963-8605.

PUBLICATIONS AVAILABLE FROM SCIENCE SPECTRUM

Reprints

Differential Light Scattering: A Physical Method for Identifying Living Bacterial Cells, P. J. Wyatt. Applied Optics **7**, 1879 (1968).

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Dielectric Structure of Spores from Differential Light Scattering, P. J. Wyatt, Spores V, (1972) in press.

Bibliographies

DIFFERENTIAL I bibliography - selected reference material including particle suspensions and molecular studies.

DIFFERENTIAL II bibliography - selected reference material including single particle measurements.

Application Notes:

Physiological Monitoring of Bacteria and Mitochondria

Rapid Assay of Bacteria in Urine (currently being updated)

Standardization of Bacterial Culture Media and Suspensions Using the DIFFERENTIAL I

Size Measurements of Single Microparticles

Characterization of Airborne Particulates Using the DIFFERENTIAL II

The Structure of Individual Microorganisms

Measuring Antibiotic Susceptibilities and MIC's by Differential Light Scattering

Brochures describing -

DIFFERENTIAL I instrument for studying microparticle suspensions and molecular solutions

DIFFERENTIAL II instrument for studying individual microparticles

DIFFERENTIAL III instrument for automated antibiotic susceptibility testing

were used to identify the cells which were preparing for division and colloidal saccharated iron oxide was used to identify the active phagocytic cells. In livers of mice whose reticulo-endothelial system was stimulated by estradiol, it was established that the cells preparing for division and those which had recently divided were actively phagocytic. In livers of mice whose reticulo-endothelial system had been "blockaded" with saccharated iron oxide, it was established that the cells which had phagocytized colloid were able to divide in the process of recovery from "blockade." No evidence was found for a stem cell which proliferates and differentiates to provide the active phagocytic population.

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Origin of the Galvanic Skin Response.* (27579)

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(Introduced by C. P. Richter)
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If a metal plate electrode is placed on the skin surface of the human palm, or on the foot or toe pads of the cat, and the body of the experimental subject grounded by means of another metal plate electrode at some point on the body at a distance from the first electrode, a change in electrical potential will occur between the 2 electrodes in response to a stimulus transmitted to the skin by the sympathetic nervous system. There is a simultaneous decrease in the skin's resistance to the passage of an electric current. When the potential change is elicited by any painful, startling, or threatening event in the environment of the experimental subject, it is referred to as the galvanic skin *reflex*. When the stimulus is applied peripherally as when a peripheral sudomotor nerve, or the sympathetic trunk to the hind extremity is stimulated, the potential change is called the galvanic skin *response*. The wave forms of the galvanic skin reflex and the galvanic skin response, when recorded from metal plate electrodes (hereafter referred to as "macroelectrodes") covering a portion of the skin's surface, are identical. Their latent periods differ as would be expected from the difference in the path length and conduction time of the nerve fibers carrying the stimulus to the skin(1).

Various theories of origin of the galvanic skin reflex and the galvanic skin response have been advanced at one time or another since their discovery by Fere(2) and Tarchanoff(3), respectively, over 70 years ago. Veraguth(4), by implanting the grounded, or indifferent, electrode beneath the skin surface, demonstrated that the potential change originated within the skin, rather than from muscle and other underlying tissues. The controversy primarily centers around the question of whether this phenomenon is caused by sweating, the widely accepted view. These theories and the experimental evidence supporting them have been reviewed by Wang (1).

Richter(5) pointed out that the skin po-

* This project was supported in part by grant from U. S. Public Health Service.
† Fellow of Maryland Heart Assn.
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tential tracings obtained from the palm of the human subject contain 2 components: a fast component, negative in direction, and a slow component, positive in direction. He recorded the slow positive component from the skin of a patient with congenital absence of the sweat glands. The fast negative component was absent in this patient. He concluded that the fast negative component was related to sweating, but that the slow positive component was not, and had its origin in capillary or epithelial cells of the skin.

Lloyd(6), studying the galvanic skin response of the skin of the cat's paw pad, recently discovered that Richter's observation (5) of 2 components was valid in that species also. However, Lloyd interpreted his data to indicate that the slow component was a sweat gland "secretory potential" that was related to reabsorption, and to the amount of moisture in the sweat gland ducts.

The present study was undertaken to determine which of these conflicting interpretations is valid. Cats were employed as the experimental subjects. The use of micropipette electrodes permitted direct measurement of the electrical potential from the lumen of individual sweat glands and from the epidermal and dermal tissues surrounding them. Simultaneously, the galvanic skin response was recorded from a macroelectrode covering the surface of a paw pad of the same extremity.

Methods. In our experiments, the galvanic skin response was studied by measuring the electrical potential arising in the skin of the paw pad of anesthetized mongrel cats in response to electrical stimulation of the lumbar sympathetic trunk supplying the homolateral hind extremity. A nerve preparation similar to that of Dale and Feldberg(7) was used. The apparatus and technics employed are similar to those used in the microelectrode impalement of single nerve fibers. Glass capillary tubes were drawn out into micropipettes with a pipette puller similar to the one described by Alexander and Nastuk(8). The electrodes were filled with methanol by boiling under vacuum, and subsequently with 3-Normal potassium chloride by diffusion replacement of the methanol. Electrical contact with the electrolyte solution within the pipette was provided for by inclusion of a silver-silver chloride electrode as an integral part of the electrode holder.

Indifferent, or ground return, electrodes of several kinds, placed in a variety of locations were used with uniform results. Electrode materials included platinum, steel, stainless steel, and zinc. Locations included the abdominal cavity of the laparotomized animal, skin of the thigh, muscle of the thigh, the tongue, and the external ear. The recorded responses were not affected by the nature or location of the indifferent electrode, unless the latter was placed near the stimulating electrode within the abdominal cavity. In the latter case, as would be expected, considerable stimulus artifact was introduced into the tracings.

The signal from the recording micropipette electrode was fed into a coupling amplifier employing a single-ended CK-5889 electrometer tube. The measured electrometer tube grid current was 10^{-14} amperes. The signal was further amplified by Offner transistorized voltage and power amplifiers.

The electrical resistance of the micropipette electrodes used in these experiments ranged from 20 to 60 megohms. This resistance was continuously monitored by means of the pulse injection technic, and did not significantly change during the experiments reported here. The usual precautions in selecting micropipette electrodes with low tip potentials were observed. Negative feedback was used to cancel out input capacitance.

The microelectrode implantations were made under direct vision with the aid of a dissecting microscope and a Zeiss micromanipulator. To obtain the galvanic skin response in the same animal, a small stainless steel cup electrode, 1.5 cm in diameter, covered another toe pad of the same paw. A layer of electrolyte-containing paste was interposed between the toe pad and the cup electrode. The latter electrode was connected to an independent direct-current differential amplifier.

Because of its ready availability and convenience, the electrolyte paste used in most of these experiments was that made by the Sanborn Co. to be used between the skin and an electrocardiograph electrode. To exclude

polarization phenomena at the skin and indifferent electrodes, the results obtained when using the Sanborn paste and a stainless steel macroelectrode were compared with results obtained when using a zinc macroelectrode covered with a paste composed of kaolin and a saturated aqueous solution of zinc sulphate. The zinc-zinc sulphate macroelectrode was used in Richter's experiments(5,10), and is a combination in which polarization over the voltage range in these experiments is negligible. Under the conditions of the experiments reported here, the results obtained with the 2 electrode and paste combinations were identical.

The output signals from both the amplifier connected to the microelectrode, and the amplifier connected to the macroelectrode covering the toe pad, were fed into a 2-channel Offner recording galvanometer by means of which the 2 signals could be recorded simultaneously.

A Grass stimulator supplied the stimulating pulses to a platinum boot electrode placed at laparotomy around the lumbar sympathetic trunk supplying the hind extremity of the animal. Squarewave pulses, varying from threshold, usually 2 to 3 volts, to 7 to 10 volts were employed. Pulse duration was 1 to 5 microseconds.

Results. Single pulse stimulation. In tracings recorded simultaneously from the macroelectrode covering a toe pad and from a microelectrode with the duct of a single sweat gland of the adjacent toe pad, the initial fast negative (downward) component of the two curves ran an almost identical course. The exponential decay of the fast component in returning toward the base line proceeded more rapidly in the case of the recording made from the macroelectrode (galvanic skin response) and became positive with respect to the resting potential, whereas the tracing from the lumen of a single sweat gland did not (Fig. 1). The electrical response from over a thousand individual sweat glands in 20 cats always returned to the base line after a decay period of from 4 to 6 seconds following the initial negative deflection. In no instance did the tracing from a microelectrode in the lumen of a single sweat gland (Fig. 1A) show this slow positive component, but the simultaneously recorded galvanic skin response (Fig. 1B) frequently did. All of the microelectrode responses obtained from individual sweat glands were identical in waveform to that shown in Fig. 1A. The latent period varied from 0.6 to 0.8 second, the mean and median values being 0.7 second. The magnitude of the peak negative response varied with stimulus intensity, the more electronegative responses being obtained with the higher voltage stimuli. The positive component of the galvanic skin response (Fig. 1B) was not always present in the tracings obtained from the macroelectrode. It appeared most consistently in response to higher voltage stimuli. Its presence or absence appeared to be related to the intensity of the stimulus, discussed below.

Repetitive squarewave stimulation. In the experiments performed with repetitive stimulation, a stimulating frequency of 10 per second was employed. In response to repetitive stimulation (Fig. 2), the microelectrode within a sweat gland duct lumen held a uniform negativity for the first half minute and thereafter began to decay toward the base line, and finally rose to lumen positivity by the end of the third minute of repetitive stimulation, whereas the galvanic skin response recorded from the macroelectrode covering a toe pad became positive early in the first minute of stimulation after an initial negative deflection.

Effect of stimulus voltage on fast and slow components of galvanic skin response. The voltage threshold necessary to elicit the slow positive component was higher than that required to elicit the fast negative component. In one experiment, the latter was obtained from the macroelectrode with a squarewave pulse of 2 to 3 volts in a fresh preparation. Increasing the stimulus to 4 volts, the fast negative and the slow positive components were obtained in the same preparation. The voltage threshold for the slow positive component was more variable than that required to elicit the fast negative response and became lower with the application of frequent stimuli. If the preparation was rested, this effect was reversed. The same threshold effect was

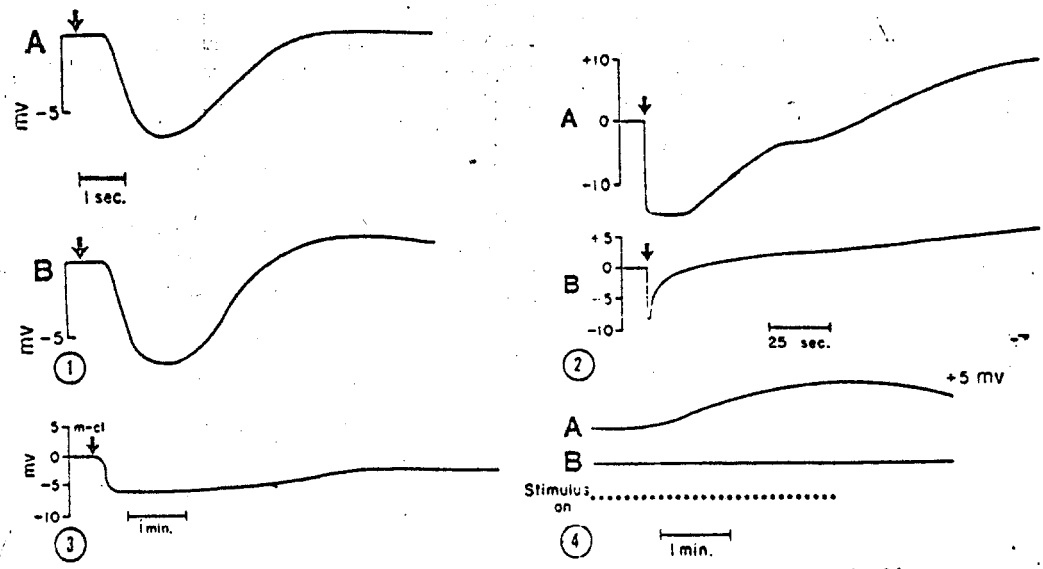

FIG. 1. (A) Microelectrode response from a single sweat gland. (B) Galvanic skin response from a macroelectrode covering toe pad of same extremity, recorded simultaneously with (A). A single monophasic squarewave pulse stimulus was applied at the arrow. Negative deflection is downward in this and succeeding figures.

FIG. 2. (A) Microelectrode response from lumen of a single sweat gland. (B) Galvanic skin response from a macroelectrode covering toe pad of same extremity. Repetitive stimulation, 10 per sec., begins at arrow and continues to end of tracing. (A) and (B) recorded simultaneously.

FIG. 3. Microelectrode response from lumen of a single sweat gland to intra-arterial inj. of methacholine chloride.

FIG. 4. (A) Microelectrode response from epidermal cells of skin of cat's paw pad obtained during the life of the animal. A similar tracing could be obtained for nearly one hr post-mortem, long after sweating had ceased. (B) Microelectrode response from dermis of skin of living animal. Repetitive stimulation of 10 per sec. was used in obtaining both tracings.

noted in the experiments in which repetitive stimulation was used. Using low voltage stimuli in a fresh, or rested, preparation, the tracing from the macroelectrode became negative and remained so for several minutes, as reported by Richter and Wheelan(10). At a slightly higher stimulus voltage, often a differential of as little as 1 volt, the positive component appeared (*cf.* Fig. 2A, and Lloyd (6)). Stimulus pulse duration over 1 to 25 microseconds had no appreciable effect on the responses obtained.

Pharmacologic stimulation. The microelectrode tracing from a sweat gland of a cat's toe pad in response to the intra-arterial injection of methacholine chloride (*via* the abdominal aorta) is shown in Fig. 3, which is included to show the similarity of the response obtained by repetitive stimulation to that produced by a parasympathomimetic drug (compare Fig. 2 and 3), and to indicate that electrical responses from the lumen of the individual sweat glands and from the skin surface are not artifacts related to the electric shock stimulus.

Localization of the slow component of galvanic skin response. The superficial cornified layer of the epidermis was dissected away so that the papillary layer of the epithelium was exposed. A microelectrode was touched to the surface of the exposed epithelium, but not in proximity to a sweat gland duct orifice, and a single pulse was applied. No negative deflection was obtained with a stimulus which readily elicited the fast negative component when the microelectrode was subsequently inserted into the lumen of a sweat gland in the same prepared area. With the microelectrode in contact with the epidermal layer of the skin but not near a sweat gland, repetitive stimulation identical to that used in Fig. 2 was applied. There was observed a slow posi-

tive component of the galvanic skin response from the macroelectrode. If the animal was killed, visible sweating soon ceased, and the fast component of the galvanic skin response could no longer be detected. However, the slow component could be obtained from the epithelium with the microelectrode for nearly an hour post-mortem (Fig. 4A), long after the sweat glands had ceased to function.

To exclude the possibility that the slow positive component originates from the skin structures below the epidermis, a strip of epidermis was dissected away leaving the dermis exposed. Sweat glands in this area poured forth sweat when stimulated repetitively, but the positive component was not recorded when the microelectrode was in contact with the exposed dermis (Fig. 4 B).

Discussion. It is unlikely that curves such as Fig. 2A would be seen under physiological conditions. If a flat electrode is placed on the skin surface of the palm, and another on the back of the hand of a human subject (the same obtains for the paw of the cat), spontaneously arising negative waves will be recorded which are identical to the microelectrode responses from the lumen of a single sweat gland of the cat. These responses occur in random fashion in the unstimulated subject and by their identity with the microelectrode responses (Fig. 1A), are presumably the electrical potentials generated by the individual sweat glands or groups of sweat glands covered by the skin electrode. In a resting patient with extreme hyperhydrosis of the palms of the hands from whom such tracings were taken, the sweat gland potential changes recorded from a skin electrode with an area of 6 sq cm occurred as often as 100 per minute. These responses often were superimposed on each other because of their frequency, but in no case was fusion observed such as occurs with strong repetitive stimulation of the lumbar sympathetic trunk or a peripheral sudomotor nerve(6,10). On tracings obtained from the resting, unstimulated cat or human subject, only the fast negative component was seen. It appears that under the enormous stress upon the sweat glands when repetitive stimulation is used (Fig. 2A), the sweat glands become fatigued and are unable to maintain their lumen negativity. That the site of this fatigue is the sweat glands and not the sudomotor nerve, is supported by the experiments in which pharmacologic stimulation was used (Fig. 4) (See Thaysen and Schwartz(9)).

We can account for the data presented in Fig. 1 and 2 if, as Richter(5) contended, the positive component is not related to the sweat glands, but originates in other skin structures. Our data substantiate Richter's view and are consistent with the following mechanism. With single shock stimulus, the fast negative component originates in the sweat glands. With repetitive stimulation, the fast components fuse to become a sustained negative potential as has been shown by Richter and Wheelan(10) and by Lloyd(6). This would also appear to be the case with a pharmacologic stimulus of long duration of action as methacholine chloride (Fig. 3). In response to an enormous stress such as repetitive stimulation or with intra-arterial methacholine chloride, the sweat gland begins to fatigue (after approximately 600 shocks in Fig. 2) and is unable to maintain its lumen negativity. The skin surface has simultaneously become positive as the epidermal cells have become positive (Fig. 4A). When the sweat gland is no longer able to maintain its lumen negativity (after approximately 1400 shocks in Fig. 2A), the microelectrode within the sweat gland duct lumen records the positive potential of the surrounding epidermal cells.

We agree with Lloyd(6) that the slow positive component is obtained in response to a single pulse stimulus only after a period of rest following repetitive stimulation. The slow positive component in response to repetitive stimulation of a given intensity reaches a maximum or ceiling for that stimulus strength and frequency. Since the positive component runs an exceedingly long time course(6), subsequent stimulation, either single shock or repetitive, would not elicit the slow positive component of the galvanic skin response until a sufficient period of time had elapsed for the decay of the slow positive component. During this period of rest, reabsorption of water may be taking place in the sweat gland ducts as Lloyd(6) has suggested.

However, this hypothesis does not appear to be supported by Lloyd's data, since, as we have shown here, the slow positive component is unrelated to sweat gland activity. If one must assign the term "secretory potential" to either of the 2 components of the galvanic skin response, it should be applied only to the initial fast negative component since the slow positive component originates in the cells of the epidermis. The physiological significance of these potentials recorded from within the lumen of single sweat gland ducts will be reported later.

In addition to the Emf generated by sweat glands and epidermal cells described above, another component of the galvanic skin response is the simultaneous fall in skin resistance. Although resistance measurements were not made during these experiments, this report would not be complete without relating skin resistance to the present work.

According to Lloyd(6), the slow phase of the galvanic skin response and the impedance (alternating current resistance) change in response to repetitive stimulation "show an identical course and are, therefore, considered as signs of the same fundamental process at work." Considering the data presented above, it appears that the skin resistance changes recorded by Lloyd originate in the epidermal cells and not in the sweat glands. Further evidence to support this view is given by Edelberg(11) who employed a microsurgical technic to isolate physically and electrically a slab of epidermis from surrounding sweat ducts. By measurement of resistance on the surface of such a preparation with microelectrodes, he demonstrated that the epidermis contributes significantly to the resistance changes of the skin in the galvanic skin response. Therefore, interpretations relating to the physiology of sweating which have been based on the slow phase of the galvanic skin response(6) and on skin impedance changes (12,13,14) should be reevaluated in the light of our report.

Summary. The galvanic skin response from a macroelectrode covering the toe pad of the cat was compared with simultaneously recorded potentials arising from individual sweat glands and cells of the surrounding epidermis and dermis of the toe pad skin of the same animal. By direct measurements employing microelectrode technics, the fast negative component of the galvanic skin response was shown to originate in the sweat glands, and to be related to sweat gland activity. The slow positive component of the galvanic skin response was shown to originate in the cells of the epidermal layer of the skin and is therefore unrelated to sweating.

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