

CONCISE ENCYCLOPEDIA OF
BIOLOGICAL & BIOMEDICAL
MEASUREMENT SYSTEMS

CONCISE ENCYCLOPEDIA OF
BIOLOGICAL & BIOMEDICAL
MEASUREMENT SYSTEMS

Editor
PETER A PAYNE
UMIST, Manchester, UK

Series Editor-in-Chief
MADAN G SINGH
UMIST, Manchester, UK



PERGAMON PRESS
OXFORD • NEW YORK • SEOUL • TOKYO

UK	Pergamon Press plc, Headington Hill Hall, Oxford OX3 0BW, England
USA	Pergamon Press, Inc, 395 Saw Mill River Road, Elmsford, New York 10523, USA
KOREA	Pergamon Press Korea, KPO Box 315, Seoul 110-603, Korea
JAPAN	Pergamon Press, 8th Floor, Matsuoka Central Building, 1-7-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 160, Japan

Copyright © 1991 Pergamon Press plc

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic tape, mechanical, photocopying, recording or otherwise, without permission in writing from the publishers.

First edition 1991

Library of Congress Cataloging in Publication Data

Concise encyclopedia of biological & biomedical measurement systems / editor, Peter A. Payne. — 1st ed.

p. cm. — (Advances in systems, control, and information engineering)

Includes index.

1. Diagnosis—Encyclopedias 2. Function tests (Medicine)—Encyclopedias. 3. Measurement—Encyclopedias. I. Payne, Peter A. II. Title: Concise encyclopedia of biological and biomedical measurement systems. III. Series: Advances in systems, control & information engineering.

RC71.C65 1991

610'.28'7—dc20

91-19982

British Library Cataloguing in Publication Data

Payne, Peter A.

Concise encyclopedia of biological & biomedical measurement systems.—(Advances in systems, control & information engineering)

I. Title II. Series

574.0287

ISBN 0-08-036188-9

HONORARY EDITORIAL ADVISORY BOARD

Chairman

John F Coales CBE, FRS
Cambridge, UK

Editor-in-Chief

Madan G Singh
UMIST, Manchester, UK

D Aspinall

UMIST, Manchester, UK

K J Åström

Lund Institute of Technology
Sweden

A Bensoussan

INRIA, Le Chesnay, France

P Borne

Institut Industriel du Nord
Villeneuve D'Ascq, France

A W Goldsworthy OBE

Jennings Industries Ltd
Victoria, Australia

J Lesourne

Conservatoire National
des Arts et Métiers,
Paris, France

P A Payne

UMIST, Manchester, UK

A P Sage

George Mason University
Fairfax, VA, USA

Y Sawaragi

Japan Institute for Systems
Research, Kyoto, Japan

G Schmidt

Technische Universität München
Germany

B Tamm

Tallinn Technical University
USSR

M Thoma

Universität Hannover
Germany

R Vichnevetsky

Rutgers University
New Brunswick, NJ, USA

FOREWORD

With the publication of the eight-volume *Systems & Control Encyclopedia* in September 1987, Pergamon Press was very keen to ensure that the scholarship embodied in the Encyclopedia was both kept up to date and was disseminated to as wide an audience as possible. For these purposes, an Honorary Editorial Advisory Board was set up under the chairmanship of Professor John F. Coales FRS, and I was invited to continue as Editor-in-Chief. The new work embarked upon comprised a series of Supplementary Volumes to the Main Encyclopedia and a series of Concise Encyclopedias under the title of the Advances in Systems, Control and Information Engineering Series. This task involved me personally editing the series of Supplementary Volumes with the aim of updating and expanding the original Encyclopedia and arranging for the editing of a series of subject-based Concise Encyclopedias being developed from the Main Encyclopedia. The Honorary Editorial Advisory Board helped to select subject areas which were perceived to be appropriate for the publication of Concise Encyclopedias and to choose the most distinguished experts in those areas to edit them. The Concise Encyclopedias were intended to contain the best of the articles from the Main Encyclopedia, updated or revised as appropriate to reflect the latest developments in their fields, and many totally new articles covering recent advances in the subject and expanding on the scope of the original Encyclopedia.

Professor Peter A. Payne was the Subject Editor responsible for commissioning the articles within the area Measurements in the Biological Area in the *Systems & Control Encyclopedia* and when it came to preparing the *Concise Encyclopedia of Biological & Biomedical Measurement Systems* it seemed natural to ask him to bring his expertise in the field and the knowledge and experience gained during the preparation of the main work to the Concise Encyclopedia.

Many of the top experts in the field of biological and biomedical measurement have contributed to this volume to help create an invaluable reference for medical practitioners and researchers and measurement scientists, covering the boundry between their disciplines. This Encyclopedia will help scientists from both areas who are cooperating in this the most complex area of measurement and instrumentation and to fully understand the specific problems posed by working with living organisms. The volume will undoubtedly be prove of value to all those in the medical and biological community with an interest in measurement or measurement systems.

Madan G Singh
Series Editor-in-Chief

PREFACE

No one can deny the vital role that measurement plays in developing our understanding of biological processes and in furthering our ability to understand and then treat all manner of illnesses and injury to which we are prone. Collaboration and cooperation between medical practitioners and scientists and engineers has long been engaged in and medical scientists have always been quick to take up developments in other areas that seem appropriate to their own specialty. This is also true of the research scientists more concerned with fundamental studies in biology.

The ability to conduct measurements on living organisms and systems has developed at much the same pace as has our technology. Many of the advances reported within this Concise Encyclopedia have arisen directly out of the information engineering explosion of recent years. However, in measurement, and especially in measurements on living organisms, human or otherwise, the following points stand out above all others: the measurand is not entirely stable, there is variability between samples of the same subject and, most of all, the information that we attempt to collect comes in numerous guises. It may be minute electrical signals derived from the muscles of the fetal heart beating inside the mother's womb; it may be small mechanical vibrations detectable on the skin and representing reflections of ultrasound energy sent deep into the body in order to measure, say, the structure of an organ such as the kidney; or it may be minute changes in radio-frequency signals produced by hydrogen ions subjected to magnetic and radio-frequency fields as in magnetic resonance imaging. Whatever form these signals take, if we wish to make use of them in our endeavors to measure the properties and function of a living organism, access to the appropriate transducer, the device that changes the signals from one energy form to another, is required. Following the process of transduction it is necessary to process the signal, now usually in electrical form and more often than not in digital form, and following signal processing it is most often necessary to display the results for use by skilled medical personnel. These stages of transduction, signal processing and display are well known to the measurement scientist and are fundamental to measurement. However, successful application of these in biological and biomedical systems relies very much on detailed knowledge of the organism or the anatomy under study. For this reason, this Concise Encyclopedia considers rather more than just measurement systems. Included also are descriptions of the anatomy and physiology of numerous parts of the human system so that the way in which decisions are made on measurement systems for a given part of the anatomy can be understood more easily.

It will become evident to anyone scanning the contents of this volume that measurements in biology and biomedicine cover a vast array of different disciplines. Few people can be expert in all of these branches of science and technology and I am fortunate indeed to have the help of the 81 experts in their various fields in covering this vast subject. Without their help and enthusiasm the exercise would of course not have been possible.

This Concise Encyclopedia grew out of a suggestion made to me by the Editor-in-Chief of the *Advances in Systems, Control and Information Engineering* series, Madan Singh, and follows on from an earlier exercise which formed part of the *Systems and Control Encyclopedia*. Madan's support and encouragement in undertaking this project are gratefully acknowledged.

An exercise such as this imposes a colossal administrative and organizational burden on the Editor and I have been much helped by the organizational skills displayed by Audrey M. Richardson and my grateful thanks are extended to her.

Finally, the painstaking work that has been put in by the staff at Pergamon Press must be acknowledged and I wish to extend my thanks to all those staff with whom I have been in contact during this exercise.

Peter A Payne
Editor

GUIDE TO USE OF THE ENCYCLOPEDIA

This Concise Encyclopedia is a comprehensive reference work covering all aspects of biological and biomedical measurement systems. Information is presented in a series of alphabetically arranged articles which deal concisely with individual topics in a self-contained manner. This guide outlines the main features and organization of the Encyclopedia, and is intended to help the reader to locate the maximum amount of information on a given topic.

Accessibility of material is of vital importance in a reference work of this kind and article titles have therefore been selected not only on the basis of article content, but also with the most probable needs of the reader in mind. An alphabetical list of all the articles contained in this Encyclopedia is to be found on pp. xiii.

Articles are linked by an extensive cross-referencing system. Cross-references to other articles in the Encyclopedia are of two types: in-text and end-of-text. Those in the body of the text are designed to refer the reader to articles that present in greater detail material on the specific topic under discussion. They generally take one of the following forms:

...as fully described in the article *Respiratory Systems, Artificial*.

...or on simulation (see *Heart-Valve Prostheses: Performance Measurement*).

The cross-references listed at the end of an article serve to identify broad background reading and to direct the reader to articles that cover different aspects of the same topic.

The nature of an encyclopedia demands a higher degree of uniformity in terminology and notation than many other scientific works. The widespread use of the International System of Units has determined that such units be used in this Encyclopedia. It has been recognized, however, that in some fields Imperial units are more generally used. Where this is the case, Imperial units are given with their SI equivalent quantity and unit following in parentheses. Where possible, the symbols defined in *Quantities, Units, and Symbols* published by the Royal Society of London have been used.

Most of the articles in the Encyclopedia include a bibliography giving sources of further information. Each bibliography consists of general items for further reading and/or references which cover specific aspects of the text. Where appropriate, authors are cited in the text using a name/date system as follows:

...as was recently reported (Smith 1988).

Jones (1984) describes...

The contributors' names and the organizations to which they are affiliated appear at the end of all the articles. All contributors can be found in the alphabetical List of Contributors, along with their full postal address and the titles of the articles of which they are authors or coauthors.

The most important information source for locating a particular topic in the Encyclopedia is the multilevel Subject Index, which has been made as complete and fully self-consistent as possible.

ALPHABETICAL LIST OF ARTICLES

- Absorption Spectroscopy
- Acoustic Microscopy
- Amplifiers and Isolation
- Applied Potential Imaging
- Auditory Perception
- Auditory Prostheses
- Auditory System: Physiological Measurements
- Biotelemetry
- Blood Flow: Invasive and Noninvasive Measurement
- Blood Gas Analysis
- Blood: Mechanical Properties
- Blood Pressure: Invasive and Noninvasive Measurement
- Bone: Biomechanics
- Bone: Measurements of Properties
- Cells
- Circulatory System
- Computerized Axial Tomography
- Connective Tissue: Biomechanics
- Coulter Counters
- Data Acquisition and Analysis, Computer-Aided
- Dentistry: Equipment and Devices
- Dentistry: Research
- Dentistry: Stomatognathic Physiology Research
- Dentistry: Visual and Tactile Measurement Methods
- Digestive System: Myoelectric Activity
- Electrical Safety
- Electrocardiograms: Computer Analysis
- Electrodes
- Electroencephalography
- Electron Microprobe Analysis
- Emission Spectroscopy
- Fetal Monitoring
- Gait Analysis: Measurements
- Heart: Electrical Characteristics
- Heart-Valve Prostheses: Performance Measurement
- His Bundle Electrocardiography
- Insects: Measurements
- Jaws and Teeth
- Joints
- Joints: Biomechanics
- Kidney Prostheses, Portable
- Kidneys, Artificial
- Kidneys, Human
- Lung Function
- Lung Modelling
- Lung Modelling: Measurements
- Lungs, Artificial
- Magnetic Resonance Imaging
- Magnetic Resonance Spectroscopy
- Mechanical Aids for Locomotion
- Microbiology Measurements
- Muscle
- Muscle-Bone Connections
- Musculoskeletal System
- Nervous System
- Neuromuscular Control
- Olfactory Measurements and Modelling
- Optical Microscopy
- Physiological Temperature Measurement
- Posture and Balance: Measurements
- Radiography in Dentistry
- Radioisotopic Imaging
- Respiratory System
- Respiratory Systems, Artificial
- Scanning Electron Microscopy
- Sensory Systems: Transduction Mechanisms
- Skin: Biomechanics
- Skin: *In Vitro* Imaging
- Skin: *In Vivo* Imaging
- Skin: Measurement of Function
- Soft Tissue: Biomechanics
- Soft Tissue: Dielectric Properties
- Speech: Measurements
- Stereological Analysis of Heterogeneous Solids
- Tactile Measurements and Modelling
- Taste Measurement and Modelling
- Telemetry in the Musculoskeletal System
- Telemetry, Underwater
- Thermal Imaging
- Transmission Electron Microscopy
- Ultrasonic Imaging
- Urinary System: Functional Measurements
- Visual System: Psychophysics
- Visual System: Structure and Function
- X-Ray Imaging

CONTENTS

Honorary Editorial Advisory Board	vi
Foreword	vii
Preface	ix
Guide to Use of the Encyclopedia	xi
Alphabetical List of Articles	xiii
Articles	1
List of Contributors	453
Subject Index	461

A

Absorption Spectroscopy

Absorption spectroscopy is a widely used technique of analysis for biological measurements, including clinical chemistry. In principle, the sample is arranged so as to absorb radiation from a suitable source, and the amount of energy absorbed is related to the concentration of particular substrates in the sample, or the wavelength of absorption is used to elucidate structural information. Most regions of the electromagnetic spectrum may be used, each yielding different information. Often the instrumentation required is essentially simple, but a major limitation in many biological systems may be spurious losses of energy by processes such as scattering. There is thus a need to prepare most samples before analysis and this limits *in vivo* applications. A general outline of the relevant theoretical considerations will first be given, followed by an indication of appropriate instrumentation and some absorption techniques.

1. General Considerations

1.1 Electromagnetic Radiation and Matter

Electromagnetic radiation exhibits both wave and particle properties. The wave is described either in terms of wavelength λ , the distance of one complete cycle, or in terms of frequency ν , which are related by the speed of light c , i.e.,

$$\lambda = c/\nu \quad (1)$$

The reciprocal of the wavelength is called the wave number. The wavelengths of radiation used for absorption spectroscopy vary from picometers (for γ rays) to nanometers for ultraviolet and visible light and centimeters for microwave radiation. The frequency of a given radiation is the same in every medium but the velocity and wavelength may change. As the name indicates there are electric and magnetic components of the wave; these oscillate in planes perpendicular to each other and to the direction of propagation of the radiation. The square of the amplitude of the wave is a measure of intensity.

The energy of electromagnetic radiation is quantized into photons. The energy of a photon is given by Planck's equation:

$$E = h\nu = hc/\lambda \quad (2)$$

where h is Planck's constant (6.62×10^{-34} J s). Photons may interact with matter (atoms, ions or molecules) so that the energy is transferred to the matter in a discontinuous process (i.e., only complete quanta of radiation

are absorbed). This arises because the energy levels in matter are also quantized. Various "selection rules" permit "allowed" transitions (Hollas 1987).

Thus, when a beam of radiation, of intensity I_0 , passes through an absorbing medium of path length b , a component of that medium at a concentration c may absorb some of that radiation such that the transmitted or emergent radiation may have a reduced power I . It can be shown (Strong 1952) that the following relationship applies:

$$-\log(I/I_0) \propto bc \quad (3)$$

The above logarithmic term is usually known as the absorbance A , and in molecular absorption in solution the proportionality constant ϵ is termed the molar absorptivity (where c is in mol l^{-1} and b in cm), i.e.,

$$A = \epsilon bc \quad (4)$$

This relationship is commonly referred to as Beer's law.

1.2 Principles of Instrumentation

Instruments for measuring absorption will have five basic components: an appropriate source of radiation; a monochromator or other device which allows investigation of a closely defined wavelength region; a container or position for the sample; a detector which will convert radiant energy to a measurable signal (usually electrical); and an amplification and readout system.

A wide variety of sources of radiation are employed to cover the whole of the spectral range of analytical interest. It is necessary that the source have sufficient energy at the wavelengths of interest for ready detection of small changes in intensity, and be sufficiently stable for I and I_0 to be measured without error-inducing fluctuations. For the visible region a tungsten filament lamp fulfills these requirements, provided its electrical supply is closely controlled. Its useful wavelength region is approximately 350–2500 nm. The deuterium arc lamp allows coverage of the region between 180 nm and 375 nm provided quartz windows are used. About 40 V dc are required to maintain the arc between a heated, oxide-coated filament and a metal electrode which produces the arc in the low-pressure deuterium filler gas. The Globar and Nernst glower are the most widely used infrared sources. The former is a silicon carbide rod heated to approximately 1200 °C which gives a stable output in the region 1–40 μm . The Nernst glower is a hollow rod of refractory oxide heated electrically to about 1500 °C. Its spectral range is 0.4–20 μm .

Lasers have been used as sources for Raman spectroscopy and for some infrared techniques (Hollas 1987). Since the radiation obtained from a laser is much

more intense than that obtained from a mercury arc source, much weaker Raman scattering may be detected. Tunable lasers (e.g., diode lasers) have been used to obtain infrared absorption spectra.

Most of the above sources of radiation emit continuous radiation and narrow bandwidths have to be selected from the radiation to allow resolution of different absorption bands and increase the sensitivity and accuracy of measurements. Filters of either the absorption or interference type (Willard *et al.* 1988) may be used. The former, as the name implies, operate by absorbing certain parts of the spectrum and offer bandwidths of perhaps 30–200 nm. The latter operate on optical interference, consisting of a transparent dielectric sandwiched between two semitransparent metallic films. The bandwidths obtained are more typically 10 nm. As the cost of monochromators has fallen these have largely replaced filters, as much narrower wavelength bands can readily be isolated. Such monochromators are also increasingly likely to use gratings rather than prisms as the dispersing medium. Gratings give a constant angular dispersion over the entire wavelength region covered. Thus, with a constant-width exit slit, a constant bandwidth is obtained. With reflection gratings there is no loss in intensity due to absorption by the optical material. By “blazing” the grating (Willard *et al.* 1988) optimally, approximately 75% of the energy can be concentrated in the first-order spectrum. High efficiency and minimal stray light can be obtained by holographically ruling the grating. Another approach to reducing stray radiation is to combine two dispersing elements into a double monochromator.

Many samples of biological interest are solutions and thus can be placed in cells, which should be optically transparent at the wavelength of interest and inert to the sample. While long path lengths increase absorbance, the problem of scattering also increases. Glass and certain plastics (which may be disposable) provide suitable materials for use in the visible region, quartz can be used in the ultraviolet region, and materials such as sodium chloride, potassium bromide or calcium fluoride in the infrared region. The availability of fiberoptic devices may mean that in future more samples may be examined remotely (e.g., *in vitro*, without insertion).

Most modern detectors convert the energy of the photons to electrical energy which is known as the signal. Noise will also be generated in the detector either by random changes within the detector, by electrical responses to other signals in the vicinity of the detector or from spurious radiation reaching the detector. The first two of these problems are intrinsic to detector design and the important requirements for a detector are high signal-to-noise ratios, short response time, stability and a readily amplified signal. Detectors for the ultraviolet and visible region are based on the photoelectric effect. Photovoltaic cells may be used for the visible region. They consist of an electrode on which a thin layer of semiconducting material such as

selenium is deposited; on the surface of this is a transparent metallic film which serves as a second collector electrode. The interface between the selenium and the metal acts as a barrier to the passage of electrons, until irradiation produces electrons in the semiconducting layer of sufficient energy to flow into the metal film. If the two electrodes are connected via a suitable meter the current flow may be monitored. Such a device is expensive but lacks sensitivity and stability. For the measurement of low radiant power, photomultiplier tubes are preferred. Light enters the sealed tube via a suitable window where it strikes a cathode coated with photoemissive material. When the photons strike the cathode, electrons are emitted which are then accelerated down a chain of electrodes (dynodes) at successively more positive potentials. Each electron impacting on a dynode causes a number of secondary electrons to be ejected which are focused on the next dynode. Thus significant currents can be produced from very small numbers of photons. By suitable choice of the photoemissive material, responses in the range 150–900 nm can be readily obtained. Additionally, photomultipliers offer fast response times but care must be taken that they are not damaged by exposure to strong light. Diode arrays, linear arrangements of light-sensing electronic components, have become increasingly popular because of the speed with which wavelength scans may be made. A microcomputer-based signal analyzer both controls the detection system and processes the data. Both 256 or 512 channel diode arrays are commonly used. The 512 channel diode array may scan between 200 nm and 840 nm in a few microseconds. Resetting the diode array takes only 40 μ s and, therefore, multiple scans may be made in very short periods of time.

The design of detectors for the infrared region is more demanding as it may be difficult to obtain the necessary rapid response. Thermocouples, resistance thermometers (bolometers) and gas thermometers (pneumatic or Golay cells) may be used on the basis that the photons when absorbed are converted to thermal energy and temperature changes or gas expansions are monitored. Pyroelectric detectors use ferroelectric materials operating below their Curie points which produce signals when the intensity of the incident radiation changes.

The amplifier takes an input from the detector and electronically increases this to a useful output. The ratio of the output to input signals is known as the gain of the amplifier. Usually, voltages are amplified and when the detector generates an electric current this is usually converted to a voltage drop by a resistor. A review of amplifier operation is outside the scope of this article but is covered by DeMarre and Michaels (1983) and Willard *et al.* (1988). Advantage is frequently offered in spectroscopic instrumentation by modulating the analytical signal, for example, by the superimposition of a sine wave. The encoded analytical signal can then be distinguished from spurious, noise signals and

selectively amplified by a tuned ac amplifier. As the output from most detectors is proportional to the transmission of the sample and the concentration of analyte is proportional to absorbance, a logarithmic amplifier may be employed to effect this conversion. If, however, a photomultiplier is operated at constant current, rather than at constant voltage, the output is very nearly logarithmically related to illumination. Such operation can simply be achieved by means of a feedback amplifier.

Historically, after amplification the signal may be read out on an analog meter or potentiometric recorder. An X-Y recorder coupled to the signal and wavelength, retrospectively, facilitates spectral scans in molecular spectroscopy. Increasingly, even simple instruments may be under microprocessor control to increase flexibility with a choice of forms of readout such as digital meters, visual display units or printers. Advantage is often taken of the possibilities of direct output to a microcomputer for data manipulation and correlation with other measurements on the same substrate.

Two basic instrument designs are often encountered. Single-beam instruments are simpler and less expensive in that only one beam of light is required, as the blank and sample are measured sequentially to obtain absorbance. Stable, high-quality components are, however, required to obtain high precision, and spectral scanning is extremely difficult. Double-beam instruments are more complex in that the light beam is split so that half is directed through the sample and the other half through the blank. The two beams are then recombined, but remain encoded, so that the detector can compare both beams at frequencies typically in the range 10–50 Hz. This compensates for fluctuations in source intensity, detector response and amplifier gain. This is particularly valuable when scanning spectra because of the wide variation of source intensity and detector response with wavelength.

2. Absorption of Radiation

2.1 Ultraviolet and Visible Molecular Absorption Measurements

The ultraviolet and visible spectra of molecules arise from transitions between electronic energy levels of the molecule. The large energies associated with these transitions ($\sim 500 \text{ kJ mol}^{-1}$) cause associated vibrational and rotational changes. As resolution of these associated changes is not possible, the absorption appears as broad bands several nanometers wide. Observations of molecular spectra are usually made in solution. Groups of atoms within molecules which give rise to absorption are known as chromophores. In organic compounds, of particular interest in biology, most applications are based on transitions from non-bonding (n), or overlapping paired electrons in π covalent bonds, to the π^* excited or antibonding orbital.

Both transitions require the presence of an unsaturated functional group to provide the π orbitals. The $\pi \rightarrow \pi^*$ transitions produce the strongest absorption and these are usually shifted to longer wavelengths as the polarity of solvent is increased. Typical organic chromophores of interest in biological molecules include: alkene, carbonyl, carboxyl, -azo, nitro and nitrate groups. Aromatic rings also absorb strongly. For analytical purposes the most important type of absorption by inorganic species is charge-transfer absorption. This occurs when one component of a complex has electron-donor characteristics and the other electron-acceptor properties, the excited state being the result of an internal pseudoredox process. Examples include the I_2^- complex and the thiocyanate complexes of Fe(III). Less intense, but characteristic, colors may be observed from transition metal ions in solution. In this case the electronic transitions are within the d orbitals and the energy levels are considerably affected by complexing groups according to the ligand field theory. Many analytes of interest do not exhibit strong absorption in accessible regions of the spectrum, but may be converted to absorbing species by derivatization or reactions, in the case of organic molecules, or complexation, with color-forming reagents, in the case of inorganic ions.

In this spectral region, tungsten filament lamps and deuterium arc lamps are suitable sources, and grating monochromators with photomultiplier detectors are used increasingly (see Sect. 1.2). Samples are placed in the light beam contained in glass or plastic cells (for the visible region) or quartz cells (for the ultraviolet). If it is desired to follow the rate of chemical reaction, the cell is thermostatted. The use of fiber-optic light guides does enable the sensing of absorbance *in situ* but this is only practical in the visible and near ultraviolet regions. Scanning instrumentation is of the double-beam type (see Sect. 1.2).

The application of ultraviolet and visible spectrophotometry to qualitative analysis is more limited than that of infrared absorption (see Sect. 2.2) because the absorption bands are broad and lack detail. Spectra may, however, reveal evidence of chemical identity, particularly the presence of certain functional groups (e.g., carbonyl, aromatic, nitro or conjugated diene). An important application is the detection of highly absorbing impurities in nonabsorbing media. It should be noted that the solvent, particularly if it is polar, may affect the spectrum. Tables of ultraviolet and visible absorption data for organic compounds are available in several publications.

More use is made of absorption spectra in this wavelength region for quantitative analysis. This is because of the wide range of absorbing species, sensitivity down to the micromolar level, good precision and convenience of use. In wide areas of clinical and biological monitoring, such measurements are highly automated although the principles are the same as those for manual methods. The analyte is mixed with reagents

Table 1
Examples of typical continuous flow procedures for absorption measurements in clinical analysis

Determinand	Procedure	Wavelength of measurement (nm)
Alkaline phosphatase	incubate with <i>p</i> -nitrophenylphosphate substrate, dialyze into recipient stream of aminomethylpropanol buffer	410
Uric acid	dilute with normal saline, dialyze into recipient stream of sodium tungstate and hydroxylamine hydrochloride; phosphotungstic acid added for color development	660
Inorganic phosphate	dilute with sulfuric acid, dialyze into sulfuric acid, add ammonium molybdate, tin(II) chloride and hydrazine	660
Glucose	dilute with saline, dialyze into sodium carbonate, add copper neocuproine; heat to 90 °C	460
Calcium	mix with hydrochloric acid and 8-hydroxyquinoline, dialyze into hydrochloric acid, 8-hydroxyquinoline and cresolphthalein complexone	570
Blood urea nitrogen (BUN)	dilute with water, dialyze into diacetyl monoxime with thiosemicarbazide; treat with acid iron(III) chloride, heat to 90 °C	520

and buffered to produce a color; after a fixed time the absorbance is measured and the concentration of analyte determined from standards, which have been through a similar procedure, and a calibration curve of absorbance vs concentration based on Beer's law. Automating these procedures not only allows analysis at rates in excess of 50 tests per minute but considerably improves precision.

Automatic instruments for clinical analysis usually employ the following functions: aspiration of the sample from its container; dispensation of an aliquot of sample; dilution; deproteinization; reagent addition; heating in delay coils; delivery to the sample cell; detection and recording of absorbance; data processing (correction for blanks, nonlinear calibration, calculation of means, precision and correlation of all tests with sample identifier); printout of results with unusual results highlighted. Two major types of automatic analyzer are those which deal with the sample in a discrete cell and those based on continuous flow. Usually they will be of the multichannel type to allow several determinands to be measured simultaneously. Continuous flow analyzers may be based on peristaltic or proportioning pumps. Often, flowing streams are segmented by air bubbles to prevent successive samples mixing and thus reduce wash time. Automated clinical analysis is a rapidly growing field and the reader is referred to specialist texts for a full appreciation of the field and the chemistry involved (e.g., Thomas 1979). Some examples of typical continuous flow procedures are given in Table 1.

2.2 Infrared Absorption Measurements

In order to absorb infrared radiation a molecule must undergo a net change in dipole moment as a consequence of its vibrational or rotational motion. The

absorption peaks are much sharper than in the ultraviolet or visible regions and consequently easier to identify. Each molecule has a fingerprint spectrum unique to that molecule. Catalogs of infrared spectra are available for many compounds and the technique is widely used for qualitative analysis, for example, to identify carbohydrate structure, steroids, biopolymers and coenzymes.

The energy required for rotational changes is very small and, except for gases, molecular vibrations are more analytically useful. The frequency of the bond between groups of atoms is characteristic and not markedly affected by molecular environment, hence information on structure or functional groups is readily obtainable.

Commonly, the Globar or Nernst glower (Sect. 1.2) is used as a source, with possibly a prism or probably a grating scanning double-beam monochromator, or the spectral analysis may be by autocorrelation techniques (Fourier transform methods). The sample for examination may be either a gas, solution or solid. Gas cells may be a few centimeters to several meters in length, reflecting internal surfaces enabling long path lengths to be achieved in compact cells. The choice of suitable solvents for liquid samples may be troublesome as many solvents absorb strongly. Carbon disulfide may be used for the region $1330\text{--}625\text{ cm}^{-1}$ and carbon tetrachloride in the $4000\text{--}1330\text{ cm}^{-1}$ region. Short (0.1–1 mm) path-length cells with sodium chloride windows are used. Often a smear of a pure liquid between sodium chloride plates may act as a suitable sample. Finely ground solids can either be mixed with a heavy hydrocarbon oil (Nujol) to form a mull which is viewed as a thin film, or milligram or smaller amounts of solids may be intimately mixed with potassium bromide and pressed under vacuum to form a transparent disk.

Qualitative applications are more common, and have been discussed above, but the intensity of absorption peaks may be used for quantitative analysis. An example of obvious biological importance is the measurement of carbon dioxide by a gas sampling technique.

A related but complementary technique uses visible light to excite molecular vibrations—the Raman effect. If a photon is scattered inelastically by a molecule it may gain energy and be scattered at a frequency higher than the original, or lose energy and be scattered at a lower frequency. The frequency displacements are characteristic of particular molecular vibrations. Raman lines are only produced if the vibration changes the polarizability of the molecule.

Fourier transform infrared is a technique that has several advantages over ordinary dispersive infrared techniques. The instruments used are commonly based on the Michelson interferometer. Light from the source is split into two beams by a half-silvered 45° mirror so that the resulting beams are at right angles to each other. When the sample is placed in one of the beams the resulting interferogram will carry spectral information on the sample. The interferogram can be transformed to an infrared spectrum using the fast Fourier algorithm and an integral microcomputer. Since filtering or dispersion is not required, there are no slits and so there is no subsequent loss of energy. A helium-neon laser may be used as a reference and, therefore, there is near absolute frequency accuracy (better than 0.01 cm^{-1} over the range $4800\text{--}400\text{ cm}^{-1}$). Other major advantages of Fourier transform infrared include the achievement of the same signal-to-noise ratio in a fraction of the time. Therefore, a number of scans may be made in the time it takes a dispersive instrument to perform one scan. This results in an improvement in resolution.

2.3 Atomic Absorption Spectroscopy

Ground state atoms will absorb radiation of the correct frequency to excite them to an excited electronic state, and the absorbance is again related to the concentration of atoms in a similar relationship to the Beer–Lambert law for solutions. The peculiar requirements for atomic absorption spectroscopy (AAS) are thus an atomizer, to produce atoms from samples, and a light source which provides radiation of the correct frequency. Conventionally a monochromator of medium resolution is used of the Czerny–Turner configuration with a photomultiplier (see Sect. 1.2) as detector. Modulation of the light source (see Sect. 1.2) is an important feature as this enables discrimination of atomic absorption signals from emission signals from the atomizer. Several excellent accounts are available of AAS and its applications, including a self-teaching textbook (Ebdon 1982).

A premixed air/acetylene flame is an excellent atomizer for AAS. The solution sample is sucked by capillary into a corrosion-proof, concentric, pneumatic nebulizer where a fine aerosol of the sample is formed

using the air flow. The finest droplets ($<10\text{ }\mu\text{m}$, i.e., 10–20%) pass through a spray chamber into the burner, the rest drains away. In the flame the aerosol is desolvated and atomized. Air/acetylene flames are usually burned on a 100 mm slot burner viewed end-on to maximize the path length of the light in the flame. Some elements form refractory oxides or other compounds which are difficult to atomize in the air/acetylene flame (temperature 2500 K) and then the hotter and more reducing fuel-rich nitrous oxide/acetylene flame may be used. Typical flame detection limits are in the ng ml^{-1} to $\mu\text{g ml}^{-1}$ range. The inefficiency of sample transport into the flame may be seen as a disadvantage. Certain elements form gaseous covalent hydrides when their compounds are mixed with reagents such as sodium borohydride. The hydrides are then swept with an inert gas into a flame or furnace for atomization. This so-called hydride generation technique is particularly useful for arsenic, bismuth, antimony, selenium, tin and tellurium. Mercury can actually be chemically reduced to an atomic vapor without a flame. Electrothermal atomizers, usually small 20–30 mm graphite tubes, heated electrically to 2000–3000 °C within 2 s, in an inert atmosphere, are becoming increasingly popular. Samples of 1–50 μl are pipetted directly into the tubes, avoiding the dilution associated with flames. Thus, detection limits in the pg ml^{-1} range are obtained on very small samples. This has great advantage in biological and clinical monitoring where sample size may be limited. When flame atomizers are practicable, however, they should be used as they are convenient and less prone to interference.

Since the absorption line profiles of atoms are much narrower than the spectral bandpass of the monochromators used, negligible absorption is observed using continuum sources and conventional monochromators. If, however, a line source is used, the source profile is similar to the absorption profile and high selectivity, as well as excellent sensitivity, is assured. A hollow cathode lamp is usually used as the source although rf excited electrodeless discharge lamps may be employed. The profiles of the lines ensure that the possibility of overlap of atomic lines arising from ground state absorption by other than analyte atoms is virtually negligible. Molecular species in the flame or furnace give rise to broad-band spectra and these may overlap the source line. Fortunately such interferences can be eliminated by background correction. This is usually accomplished by alternating the line source with a continuum source. While both the analyte and molecular interferents absorb the line source, only the latter will appreciably absorb the detectable continuum radiation. Subtraction of the two signals gives the true atomic absorption signal. Background correction is usually essential when using biological samples in electrothermal atomizers.

There are two other types of background correction system used for electrothermal atomization. The

Zeeman effect occurs when an atomic vapor is subjected to a magnetic field of several teslas. The magnetic field splits the spectral lines into several components of slightly different wavelength. The π component is situated at the "normal" wavelength of the line but the σ^+ and σ^- components lie an equal distance on either side. If the field is strong enough, these components will lie outside the atomic observation profile and the background can be corrected by difference.

The Smith-Hieftje method of background correction is based on the self-reversal obtained when hollow cathode lamps are operated at high lamp current. The lamp is first run at low current and the light it emits is absorbed by both the analyte and the background. The lamp is then pulsed at much higher lamp current and the light it emits is absorbed predominantly by the background. Again, the corrected signal is obtained by subtraction. These latter types of background correction are most useful for overcoming the effects of structured background interferences, such as that of phosphate on arsenic or selenium determinations.

Atomic absorption spectroscopy is normally the first-choice method for trace metal determinations in biological samples. Samples are usually brought into aqueous solution by ashing and/or acid digestion for flame analysis. It is possible to use solid samples in graphite furnaces and whole, or diluted, serum is frequently analyzed by electrothermal atomization for a range of toxicological clinical tests. Both flame and furnace instruments can be automated by the provision of autosamplers and microprocessor control. The frequency of such operations is a reflection of the high sample loadings often found with this invaluable technique.

3. Optical Activity

Ordinary radiation can be represented as a bundle of electromagnetic waves in which the amplitude of vibrations is equally distributed among all planes centered along the path of the beam. The vector in any one plane can be resolved into two perpendicular components, and if these two components are combined and one of the two resultant planes removed, the beam is said to be plane polarized. Optical activity is a measure of the ability of certain substances to rotate this plane polarization. Probably the most familiar examples are the sugars and quartz. The rotation is said to be dextro (+) if it is clockwise to an observer looking toward the light source and levo (−) if counterclockwise. The extent of rotation depends on the number of molecules in the light path, the wavelength and the temperature. The specific rotation $[\alpha]'$ is defined as

$$[\alpha]' = \alpha/dc \quad (5)$$

where α is the angle of rotation of a solution of

concentration c (g cm^{-3}) in a cell d (dm) long at a temperature t . The 589.3 nm sodium line is frequently used as the measurement wavelength.

The polarimeter is generally used to make measurements of optical activity. This consists of a source of monochromatic radiation (e.g., sodium lamp and filter), a polarizer (e.g., a calcite prism) and a sample cell (e.g., a 10–20 cm long glass tube). A Nicol analyzer in a rotating eyepiece completes the apparatus, the angle of rotation to the crossed position being measured. Polarimeters are usually equipped with a half-shadow device to permit the determination to be made by matching two halves of a field at a radiation intensity greater than the minimum. Photoelectric polarimeters with servomechanisms and recorders for automating the measurements are available.

The most extensive applications are in the sugar industry, as sucrose, β -D-glucose, β -lactose and β -maltose are dextrorotary in water, whereas β -D-fructose is levorotary. Structural information, for example, on steroids, carbohydrates, amino acids and penicillins, may be obtained from polarimetry or from the related techniques of optical rotary dispersion and circular dichroism. These are more powerful tools for structural analysis and are based on the resolution of plane-polarized radiation into two beams circularly polarized in opposite directions. The variation of the refractive index and absorptivity with wavelength of these two components is measured. The structural information yielded has been used in the study of amino acids, polypeptides and proteins, and of complex natural products such as steroids, terpenes and antibiotics.

4. Magnetic Resonance

In the presence of a strong magnetic field the energies of the nuclei of certain elements are split into two or more quantized levels; electrons may behave in a similar way. Energy levels between the magnetic quantum levels of the atomic nucleus correspond to the radio frequency portion (0.1–100 MHz) of the electromagnetic spectrum. For electrons the energy differences are larger, corresponding to the microwave region (10 000–80 000 MHz). The study of nuclei in this way is termed nuclear magnetic resonance (NMR) and is a very powerful tool for structure determination (see *Magnetic Resonance Spectroscopy*). Electron spin resonance (ESR) also provides structural information but of a less general nature than NMR. Most molecules fail to exhibit an ESR spectrum as they contain an even number of electrons and these are spin paired, thus the magnetic effects are cancelled. Therefore, ESR is most widely applicable to the study of free radicals, molecules with triplet state electrons and transition metal ions. This is a small and closely defined field, as far as biological applications are concerned, and ESR will not be further considered, although it should be noted that

the principles of ESR are broadly analogous to those of NMR.

4.1 Nuclear Magnetic Resonance Spectroscopy

A nuclear charge spinning around an axis possesses angular momentum given in terms of a spin number I assigned half-integral values $0, \frac{1}{2}, 1, \frac{3}{2}, \dots, \frac{I}{2}$ depending on the particular nucleus. Nuclei which have either an odd number of protons or neutrons, but not both odd, exhibit half-integral spin numbers (e.g., ^1H , ^{13}C , ^{19}F and ^{31}P) and, hence, generate a magnetic field as they spin. There is an associated nuclear magnetic moment μ along the axis of spin. Magnetic nuclei interact with an external magnetic field by assuming $2I + 1$ possible orientations with corresponding energy levels. The difference between these levels ΔE , in an applied field of H_0 , is given by

$$\Delta E = \mu H_0 / I \quad (6)$$

A typical applied field is about 1.4 T; for protons in such a field ΔE is about 23.9 J mol^{-1} , which corresponds to a frequency of approximately 60 MHz.

The essential components of any high-resolution NMR spectrometer are a magnet, sample tube, sweep generator, transmitter coil and receiver coil. The magnet may be either a permanent magnet or electromagnet, yielding a strong, stable, homogeneous magnetic field. The sample tube is rotated at several hundred rpm to average out small magnetic field inhomogeneities. Dilute solutions (2–10%) are normally used and for proton resonance, solvents ideally should not contain hydrogen (e.g., carbon tetrachloride, deuterated chloroform or deuterated benzene are used). Repetitive scans are often made to improve the signal-to-noise ratio by a computer average of transients.

The behavior of an isolated nucleus yields no chemical information but the variation of the resonance line with chemical structure, known as the chemical shift, yields considerable structural information. Thus, proton magnetic resonance has been very widely used in the determination of the structure of biochemicals and pharmaceuticals (Campbell and Dobson 1979) and in the study of important behavior such as ion binding (Forsen and Lindman 1981).

Fourier transform NMR is a technique that has several advantages over the conventional continuous wave NMR spectrometry technique. It is a technique in which an rf pulse lasting 10^{-5} s is used to excite the atoms. The signals are obtained after such an excitation. Since all frequencies are measured simultaneously data acquisition is extremely fast. The recording of hundreds of transients may take only a couple of minutes. Since time averaging is used, greatly enhanced signal-to-noise ratios may be obtained and the resulting enhanced sensitivity means that insensitive nuclei such as ^{15}N may be studied more readily.

Recently, *in vivo* NMR has developed very rapidly (Radda 1986). It is a technique that has branches in biology, biochemistry and medicine. ^{31}P is the main nucleus used. Compounds containing ^{31}P such as ATP, ADP, inorganic phosphate and phosphocreatine may be analyzed *in vivo*. The technique may therefore be used to measure metabolite turnover, increase the understanding of some muscle diseases and provide information on hormonal status and has helped in the elucidation of causes of disease. In addition to these, it may also be used to evaluate organ transplants, recovery from heart attacks and the efficacy of drugs used to treat brain tumors.

See also: Amplifiers and Isolation; Emission Spectroscopy; Magnetic Resonance Imaging; Magnetic Resonance Spectroscopy

Bibliography

- Campbell I D, Dobson C M 1979 The application of high resolution nuclear resonance to biological systems. In: Glick D (ed.) *Methods of Biochemical Analysis*, Vol. 25. Interscience, New York
- DeMarre D A, Michaels D 1983 *Bioelectronic Measurements*. Prentice-Hall, Englewood Cliffs, NJ
- Ebdon L 1982 *An Introduction to Atomic Absorption Spectroscopy*. Heyden-Wiley, London
- Forsen S, Lindman B 1981 Ion binding in biological systems as studied by NMR spectroscopy. In: Glick D (ed.) *Methods of Biochemical Analysis*, Vol. 27. Interscience, New York
- Hollas J M 1987 *Modern Spectroscopy*. Wiley, Chichester, UK
- Radda G K 1986 The use of NMR spectroscopy for the understanding of disease. *Science* 233, 640–5
- Strong F C 1952 Theoretical basis of the Bouguer–Beer law of radiation absorption. *Anal. Chem.* 24, 338–42
- Thomas H E 1979 *Handbook of Automated Electronic Clinical Analysis*. Reston Publishing, Reston, VA
- Willard H H, Merritt L C, Dean J A, Settle F A 1988 *Instrumental Methods of Analysis*, 7th edn. Wadsworth, Belmont, CA

L. Ebdon and A. S. Fisher
[Polytechnic South West, Plymouth, UK]

Acoustic Microscopy

The mechanically scanned acoustic microscope (SAM) was first demonstrated by Lemons and Quate (1974) of Stanford University. After research and development, the instrument is now becoming a commercial reality. It is therefore appropriate to review the progress made since 1974 and comment on possible future advances.

Experience has shown that whenever a microscope based on a new class of radiation is developed, our understanding of the microscopic structure of nature has improved. The introduction of acoustic radiation to microscopy can be expected to have a similar impact.

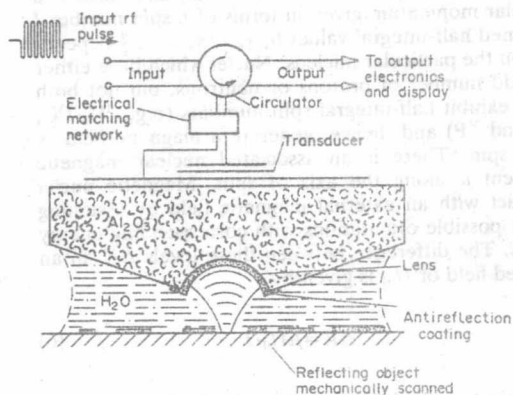
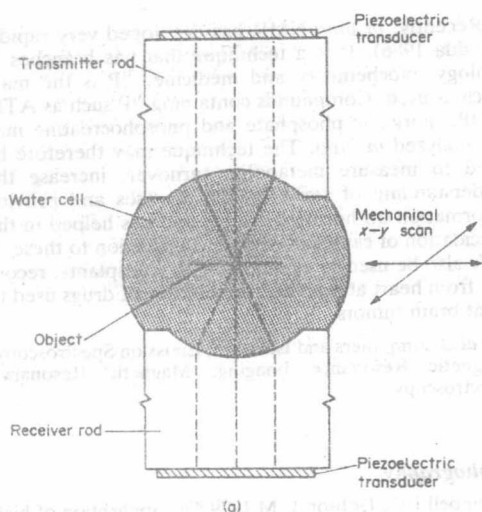


Figure 1 (a) Transmission scanning acoustic microscope configuration, and (b) basic lens geometry for reflection acoustic microscopy (radius of the lens $r_0 = 40 \mu\text{m}$, focal length $f = 1.13r_0$, radius of lens aperture $R = 0.7r_0$)

Contrast in acoustic micrographs derives from changes in mechanical properties (e.g., density, elasticity and viscosity) within the sample; for this reason, the images obtained with an acoustic microscope are fundamentally different to those obtained with other techniques such as light, x rays or electron microscopy.

The basic idea of an acoustic microscope is not new; it was first put forward by the Russian scientist S. Ya. Sokolov who realized that acoustic waves in water at gigahertz frequencies had wavelengths comparable with that of visible light. In the years before World War II, he described his investigations on an "acoustic microscope" which was based on the use of a high-resolution electron probe to scan the acoustic field. Although he mentioned the possibility of operating at acoustic frequencies of a few gigahertz, the technology to generate acoustic waves efficiently at such high frequencies did not exist at the time and his work culminated with the demonstration of a system working at frequencies around 1 MHz (Sokolov 1949). Interest in acoustic microscopy was revived during the latter part of the 1960s following the development of techniques for depositing efficient thin film acoustic transducers. In the 1980s, several novel acoustic microscopes have been demonstrated. This article, however, will be exclusively concerned with a review of the mechanically scanned acoustic microscope as invented by Quate and Lemons (Quate *et al.* 1979).

Section 1 describes the basic operating principles of the SAM both in reflection and transmission. Many of the imaging modes available in optical microscopy have their acoustic counterparts; these will be discussed in

Sect. 2. Techniques for measuring the elastic properties of a sample on a microscopic scale will be discussed in Sect. 3; such measurements are invaluable for materials characterization. Section 4 presents some examples of high-resolution acoustic imaging as applied to biology and materials science. Acoustic waves are capable of penetrating substantial distances into most solids; a unique application is, therefore, interior imaging of objects opaque to optical radiation. Methods for improving the resolving power of the SAM are presented in Sect. 5. Finally, Sect. 6 is a brief discussion of possible future advances.

1. Principles of Operation

The schematic of a transmission SAM is shown in Fig. 1a. Two identical lens assemblies are rigidly mounted and separated by a coupling fluid. On the back surface of each sapphire lens crystal is a piezoelectric transducer; at frequencies below 150 MHz, this would typically be a slab of lithium niobate bonded via a thin indium film, while at higher frequencies a zinc oxide film is sputtered directly onto a gold electrode deposited on the sapphire surface. A radio frequency (rf) input signal is applied to the transmitting lens transducer which, in turn, excites longitudinal acoustic waves in the sapphire rod. These propagate (without significant loss) to the far side of the rod, where they encounter the lens—a simple spherical depression ground into the sapphire. Because of the small ratio between the acoustic velocity in the coupling fluid (in the case of water 1500 m s^{-1}) and that in sapphire