Advances in Optical and Electron Microscopy

Volume 7

Advances in

OPTICAL *and* ELECTRON MICROSCOPY

Volume 7

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ACADEMIC PRESS · 1978
LONDON, NEW YORK AND SAN FRANCISCO
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road,
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number: 65–25134
ISBN: 0–12–029907–0

PRINTED IN GREAT BRITAIN BY
THE WHITEFRIARS PRESS LTD.
LONDON AND TONBRIDGE

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Preface

In the Preface to the first volume in this series (1966) we excused its appearance on the grounds of a need for informative reviews "in a subject like microscopy which is not only advancing rapidly but impinges on so many branches of science and technology". That we had identified a real need in our chosen field has been confirmed by the reception accorded to successive volumes by readers and reviewers. Volume 7 continues the aim of wide-ranging coverage without, we hope, sacrificing depth for breadth.

Two of the articles on optical microscopy deal with problems arising from the study of moving objects. Berg offers a solution to the frustrating problem of keeping a motile organism, moving randomly in three dimensions, both in view and in focus. The construction of a microscope capable of doing this automatically would have seemed impossible at one time, but modern technology has provided several solutions. Castenholz applies a principle well known in radiology but little used in microscopy to the recording and quantitative evaluation of certain types of movement of microscopic objects. Pincus discusses optical diffraction analysis in some detail. Similar techniques have been used to analyse electron micrographs but the method has been less widely used in optical microscopy. Pincus' examples are mainly geological but there may be many suitable problems in biology and materials science.

In electron microscopy the emphasis on this occasion is on physical principles of image formation and interpretation rather than on aspects of instrumentation. Hawkes treats at length the subject of coherence and partial coherence. Although there is a considerable literature on the related problem in light optics, this is the first thorough treatment for electron optics. Misell deals with the phase problem in similar detail. Here the literature has been quite extensive, on the experimental as well as the theoretical side, partly because of the related (but more difficult) situation in X-ray structure research. Misell brings it all together, including the prospects for imaging single atoms. Finally Vainshtein describes recent progress at the next level of analysis, the determination of the three dimensional structure of macro-molecules.

As previously, we believe it will be of interest to list the topics on which articles for future volumes are being commissioned. The editors will be grateful for suggestions for, and especially offers of, articles on other subjects of current importance in microscopy.

Use of lasers in microscopy
Microscopy with polarized light
Reflection interference microscopy
Reflectance microscopy
New methods of investigating chromosome structure
High resolution electron microscopy
Cryomicroscopy
Image intensifiers for electron microscopy
Optimisation of conditions for very high resolution
Phase contrast electron microscopy
Characteristics of electron guns
Radiation damage and image resolution

V. E. COSSLETT
R. BARER

August, 1978

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B. K. VAINSHTEIN

The Tracking Microscope

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I. Motivation

Many bacteria swim. Cells of diameter 10^{-4} cm may move steadily at speeds of the order of 30×10^{-4} cm/sec, then abruptly reverse or choose new directions at random. If able to respond to sensory inputs, they bias the probability of the occurrence of these all-or-none events and migrate to regions that are hot or cold, light or dark, or of favourable chemical content.‡ How is one to study such behaviour? At magnifications high enough for close observation, the cells move out of focus in a fraction of a second; when confined to a thin chamber, their motion is perturbed by its walls. These difficulties have been overcome by the development of a microscope that automatically follows the motion of individual cells in three dimensions (Berg, 1971). The design and operation of this instrument are reviewed here.

II. Rationale

If a chamber containing a suspension of bacteria is moved so that the position of a given organism remains fixed, the displacement of the chamber will provide a measure of the motion of that organism relative to the medium

‡ For recent reviews on bacterial behaviour, see Adler (1975, 1976), Berg (1975a,b,c), and Koshland (1976).

in which it is suspended. Since the propulsion of a micro-organism is governed by viscous rather than inertial forces (Ludwig, 1930; Taylor, 1952; Purcell, 1976), the displacement of the chamber will not affect the motile behaviour of the bacterium in any way (Berg, 1971). One needs a microscope with a detector that monitors changes in the position of the image of the bacterium and an electro-mechanical transducer that drives the chamber so that the image moves back toward the centre of the detector. If the bacterium swims 30 diameters/sec and its image is about the size of the detector, the signals generated by the detector will change appreciably in 1/30 sec. The transducer must be able to move in the right direction in times shorter than this. It can do so only if its resonant frequencies exceed 30 Hz; the mass and moments of inertia of the moving parts of the transducer must be small and their suspension stiff. If the transducer is driven at frequencies higher than its resonant frequencies, the system will oscillate; the gains of the servo-loops must be attenuated at high frequencies. The filter of choice is an RC circuit, since its phase shift is at most $\pi/2$.

III. Design

A system embodying these principles is shown schematically in Fig. 1 (Berg, 1971). The chamber containing the bacteria is mounted between a long-working distance phase-contrast condenser and a bright contrast phase objective.‡ The beam emerging at the top of the trinocular is split so that three images of the organism being tracked fall on six fibre-optics fibres, each leading to its own photomultiplier. The diameters of the fibres are all the same, about the width of the image of the bacterium. One image is focused on the ends of the set x_1, x_2, y_1, y_2, the second in front of z_1, the third behind z_2. When the bacterium moves in the $+X$ direction, the image moves so that more light falls on x_1 than x_2; when it moves in the $+Y$ direction, more light falls on y_1 than y_2; when it moves in the $+Z$ direction, more light falls on z_1 than z_2 (because the image focuses more sharply on z_1 and less sharply on z_2). Signals proportional to the errors in displacement are obtained from the differences in the photomultiplier outputs $x_1 - x_2$, $y_1 - y_2$, and $z_1 - z_2$. They are amplified, filtered (RC = 8 sec) and used in current-injection circuits to drive the coils in the electromagnetic transducer. Each coil is mounted in the annular gap of a cylindrical magnet. Its equilibrium position is determined by the balance between the electro-

‡ A dark-contrast phase objective also can be used, provided that the error signals are inverted. Success depends on the absolute difference between the intensity of the image and that of the background, not on the image contrast. Images obtained with dark-field microscopes look brighter, but they may not be suitable.

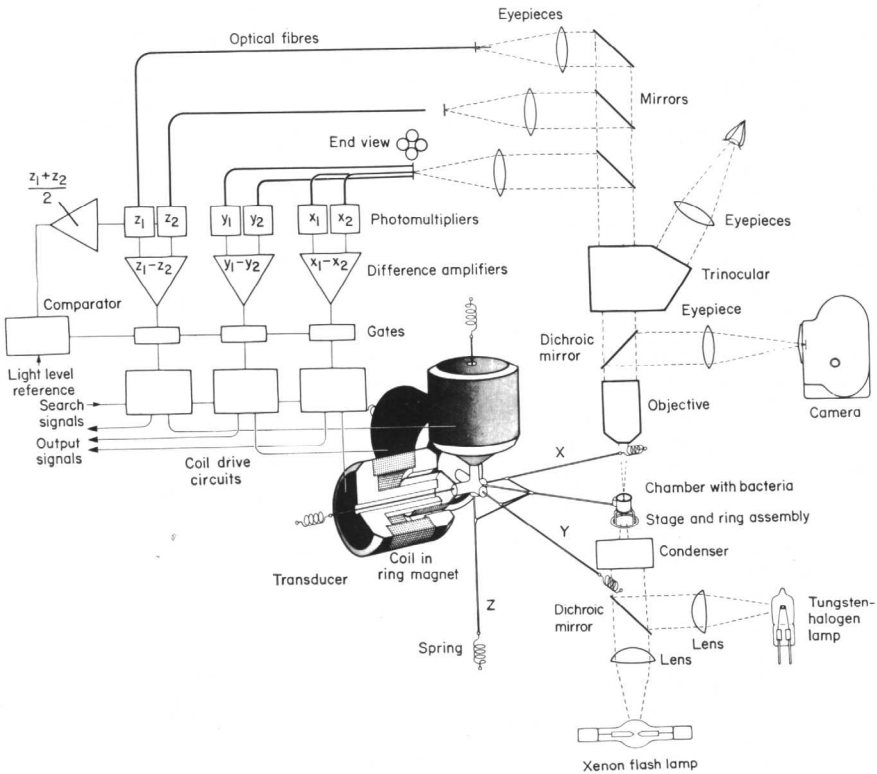

FIG. 1. Schematic diagram of the tracking microscope.

magnetic force, due to the current, and the net elastic force, due to the difference in the lengths of a pair of springs. The currents are bipolar; when the power is off, the stage rests at its centre position; when the power is on, it can be driven about 5×10^{-2} cm in any direction. The servo loop is closed by a comparator when the amount of light falling on the detector exceeds a predetermined value (Fig. 1).

The system is built around a Nikon S-Ke microscope and a Zeiss Optovar (Fig. 2). The transducer is demountable (Fig. 3). The ends of the drive coils can be seen when the transducer is viewed obliquely from below (Fig. 4). Since the springs can move sideways as well as lengthwise, the coils can rotate as well as translate; there are six degrees of freedom. The translational modes have resonant frequencies of about 80 Hz, the rotational modes about 120 Hz. The translational modes are damped electrically, the rotational ones by a ring immersed in an annular pool of oil (Berg, 1971).

FIG. 2. Optical system: (1) Photomultiplier housing (mostly out of view, standing with the microscope on a vibration-isolation table, Barry Controls Serva Bench Mark III-1). (2) Fibre optics bundle. (3) Detector housing (contains mirrors, eyepieces, and input ends of fibre-optics fibres). (4) Trinocular beam splitter. (5) Camera relay lens. (6) Zeiss Optovar (lenses of magnification 1·6 and 2·0 replaced by dichroic mirrors that reflect part of the light to the camera). (7) Beam that supports camera (bolted to the frame of the vibration-isolation table). (8) Transducer. (9) Temperature-controlled housing (for the tracking chamber). (10) Temperature-controlled bucket (for fluids to be transferred to the tracking chamber). (11) Thermistor cable. (12) Condenser cooling coil (covered with tape). (13) Condenser turret. Light sources (not shown): for tracking, a tungsten-halogen lamp, Sylvania FCR, run DC; for photography, a xenon flash lamp, Illumination Industries X-80-2084-6.

The ring is mounted under the stage on three struts and is concentric with the optical axis (Fig. 3). The stage and ring assembly are enclosed by a temperature-controlled housing (Fig. 5) that also holds the pool of oil (oil not shown). The housing is fastened to the top of the condenser via a thin stainless-steel sleeve. Its temperature is sensed by a thermistor and changed

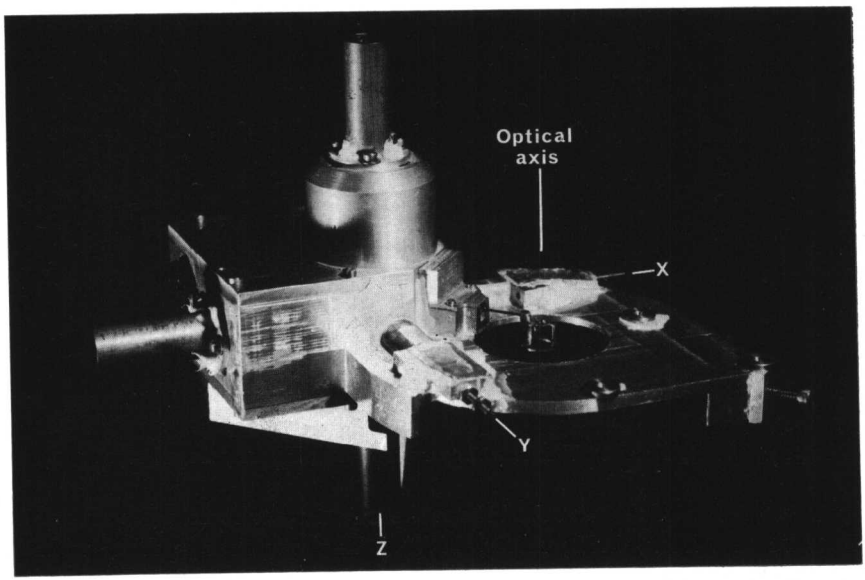

FIG. 3. Transducer. The axes are labeled X, Y, and Z. The stage is mounted on the optical axis at the end of a tungsten strut. The tracking chamber (not shown) sits on the stage. The ring below the stage is immersed in an annular pool of oil (not shown).

by a small heating coil (non-inductively wound). For work below ambient temperature, the body of the condenser is water cooled (see Fig. 2). The chambers (Fig. 6) either sit on the stage or clip on to it. They are made of tantalum, because tantalum has a high thermal conductivity and is chemically inert. They are small, partly for reasons of mass, partly to inhibit convective flow (see below).

IV. OPERATION

A. *Manipulating Bacteria*

The chambers shown in Fig. 6 have been used in studies of the motion of bacteria in spatial (Berg and Brown, 1972) and temporal (Brown and Berg, 1974) gradients of chemical attractants. The spatial gradients are generated

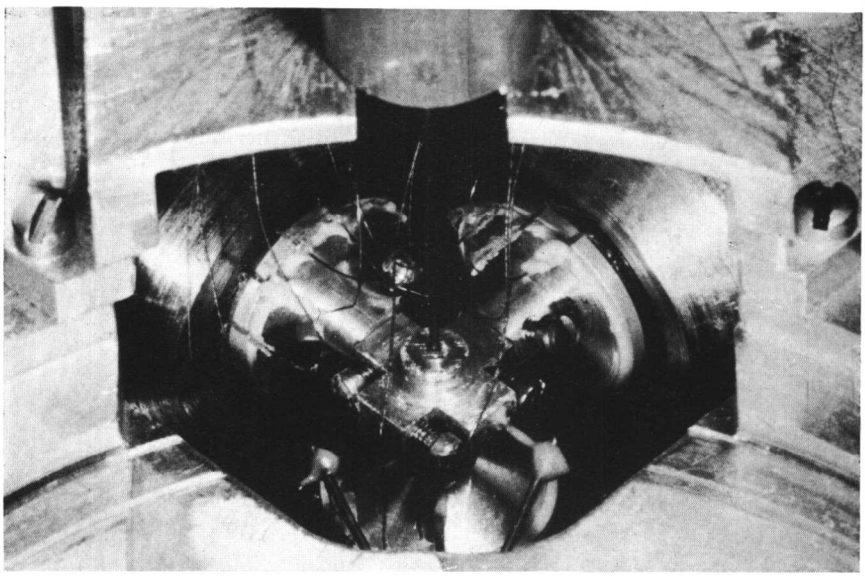

FIG. 4. Transducer drive coils seen projecting from the cylindrical magnets (Z bottom, X left, Y right). The transducer is viewed obliquely from below.

by the diffusion of the chemical from the capillary tube into the chamber (Futrelle and Berg, 1972; Adler, 1973).‡ The chamber is filled with a suspension of bacteria, the capillary tube with a solution of the chemical; the capillary tube is inserted, and the top window is sealed on. The gradient changes with time, but not rapidly; the bacteria are stimulated as they swim from place to place. The temporal gradients are generated by enzymatic reaction. The inlet pipe (Fig. 6) is connected with polyethylene tubing to a vial containing a suspension of bacteria and substrates for the reaction; the outlet pipe is connected to a valve on a vacuum line; the chamber is purged of air, and the top window is sealed on. Enzyme is added to the vial, and the mixture is drawn into the chamber. Subsequent changes in concentration are strictly temporal; the bacteria are stimulated regardless of how they swim.

Convective flow can introduce serious errors, particularly with cells that swim slowly. This is not a problem with the chambers shown in Fig. 6 (flow rates 10^{-5} cm/sec or less), but it is with chambers that have four windows (additional windows in front and back) used for stimulation of cells that respond to light (flow rates 10^{-4} cm/sec or more, higher when the

‡ In the former reference, for $\sqrt{Dt}$ read $\sqrt{(Dt)}$; for γ^2 in the argument of *erfc* read 2γ. For a solution of the diffusion equation with different boundary conditions, see Brokaw (1958).

stimulus light is on). The convection can be eliminated as follows: First, a suspension of bacteria is drawn into the chamber (via inlet and outlet pipes, as in Fig. 6). Then the lower fourth of this suspension is displaced by an identical one containing, in addition, 0·1 M stachyose. The diffusion constant and specific gravity of this tetrasaccharide are such that a vertical density gradient is established in a few minutes that stabilizes the fluid against convective flow for an hour or more. A procedure of this kind is required in any study of the motion of inert particles.

The motile behaviour of bacteria can be monitored in another rather novel way. The helical filaments that propel bacteria are driven at their base by rotary motors (Berg and Anderson, 1973). A cell tethered to a glass

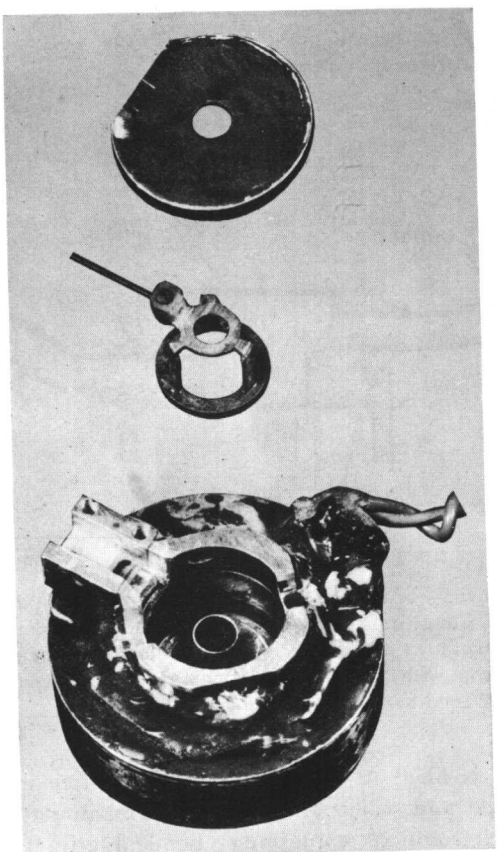

FIG. 5. Stage and ring assembly and temperature-controlled housing (expanded view; oil and thermistor not shown).