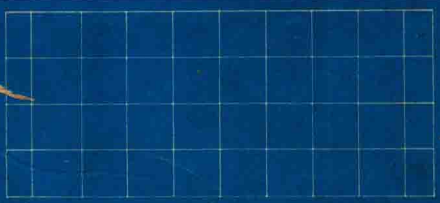
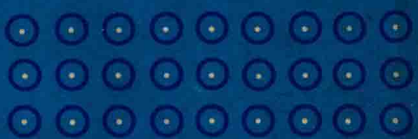
# Quantitative Methods in Biology

M. A. Williams



## Practical Methods in Electron Microscopy

Editor: Audrey M. Glauert



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# QUANTITATIVE METHODS IN BIOLOGY

M. A. WILLIAMS
*Reader in Human Biology and Anatomy*
*University of Sheffield*


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by M.A. Williams
Part II Quantitative methods in biology
by M.A. Williams

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# Practical Methods in ELECTRON MICROSCOPY

Volume 6

*Edited by*
AUDREY M. GLAUERT
*Strangeways Research Laboratory*
*Cambridge*

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# Editor's preface

Electron microscopy is now a standard technique with wide applications in all branches of Science and Technology, and every year a large number of students and research workers start to use the electron microscope and require to be introduced to the instrument and to the techniques for the preparation of specimens. Many books are available describing the techniques of electron microscopy in general terms, but the authors of Practical Methods in Electron Microscopy consider that there is an urgent need for a comprehensive series of laboratory handbooks in which all the techniques of electron microscopy are described in sufficient detail to enable the isolated worker to carry them out successfully. The series of books will eventually cover the whole range of techniques for electron microscopy, including the instrument itself, methods of specimen preparation in biology and the materials sciences, and the analysis of electron micrographs. Only well-established techniques which have been used successfully outside their laboratory of origin will be included.

Great care has been taken in the selection of the authors since it is well known that it is not possible to describe a technique with sufficient practical detail for it to be followed accurately unless one is familiar with the technique oneself. This fact is only too obvious in certain 'one author' texts in which the information provided quickly ceases to be of any practical value once the author moves outside the field of his own experience.

Each book of the series will start from first principles, assuming no specialist knowledge, and will be complete in itself. Following the successful innovation, made by the same publishers in the parallel series Laboratory

Techniques in Biochemistry and Molecular Biology (edited by T. S. Work and E. Work), each book will be included, together with one or two others of the series, in a hardback edition suitable for libraries and will also be available in an inexpensive edition for individual use in the laboratory. Each book will be revised, independently of the others, at such times as the authors and editor consider necessary, thus keeping the series of books continuously up-to-date.

*Strangeways Research Laboratory*
*Cambridge, England*

AUDREY M. GLAUERT, SC. D.
*General editor*

*For those in whose arms I have found myself*

# Author's preface

At the time I commenced writing the first draft of what became the companion volume to this, 'Autoradiography and Immunocytochemistry', the material referred to in the major articles upon which *this* text is based had not been written. The application of quantitative methods is a phase to which all research fields must inevitably come; a phase prior to the building and testing of mechanistic models. Biological electron microscopy has very recently reached its quantitative phase and a detailed laboratory text covering this area was very much called for. With much encouragement from Audrey Glauert, I have written one.

Writing this book has been possible because I have had the great good fortune for the past twelve years to work at number 3 Clarkehouse Road, the annexe to the Department of Human Biology and Anatomy at the University of Sheffield. The friendship, support and skills of all those persons (students, technicians, office staff, researchers and academics) who work, or have worked, at Clarkehouse Road are of immeasurable value to me. The creation of such a vital environment owes much to the Head of Department, Professor Robert Barer.

I have been enriched by friendship with Andrew Rogers and the other teachers helping with a series of Autoradiography Courses held under the auspices of the Anatomical Society of Great Britain and Ireland. The expertise of the teachers on these courses has been made available to me freely, and I have had many instructive opportunities to discuss research problems posed by the participants. However, in so far as opinions are offered in this book, they represent my own.

The aim of this series of books is to provide detailed laboratory guides from which workers, even geographically isolated ones, can carry through

electron microscope techniques with success. The texts, each of which covers a relatively narrow area, are *not* intended as reviews of all the available literature and techniques. Only well-established methods have been included, the criterion being that to merit consideration they have to have been used successfully in at least two independent laboratories. No doubt the application of this rule has caused some excellent methods to be excluded. However, many less than excellent ones have thus been avoided, and new methods of excellence will doubtless find their way in any case into later editions.

The subject matter of this book is intended to fit into the series *Practical methods in electron microscopy*. Nevertheless, the treatment of the subject matter reflects the excitement I personally find in the emergence of quantitative data and conclusions from visual images. The presentation further asserts my personal conviction of the importance of investing effort in quantitative methods for promoting and developing objectivity in structural biology.

What solace here for a laborious mind!
What a redoubtable and single task
One might attempt here:

Threading a logic between wall and wall,
Ceiling and floor, more accurate by far
Than the cob-spider's.

Truth captured without increment of flies –
Spinning and knotting till the cell became
A spacious other head

In which the emancipated reason might
Learn in due time to walk at greater length
And more unanswerably.

Robert Graves, *The Philosopher*

# Acknowledgements

I am particularly grateful to the following persons and holders of copyright who have kindly given permission to reproduce material. Dr. N. T. J. Bailey and English Universities Press kindly allowed me to republish the statistical tables given in Appendix 2. These were previously published in *Statistical Methods in Biology* (1959). Lines from *The Philosopher* are printed by permission of Robert Graves.

Professor R. Barer and Mr. K. Robinson have generously allowed me to publish their account of data tabulators designed and built by them in the Department of Human Biology and Anatomy at the University of Sheffield. Dr. N. M. Blackett and Miss D. M. Parry have kindly given me a copy of their computer programme for the hypothetical grain distribution technique (§ 3.4.2).

*Sheffield, September 1976* MIKE WILLIAMS

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Chapter 1

# Introduction

Electron microscopy has played a major formative role in the development of modern cellular biology. Hardly any area of the subject has been left uninfluenced. Standard textbooks in Anatomy, Biochemistry, Botany, Microbiology, Physiology and Zoology are all found to be illustrated by electron micrographs of cells, subcellular particulates or macromolecules. Many areas of applied biology in agricultural, veterinary or medical science also rely heavily on electron microscopy as witnessed by texts in these subjects. Research and review papers refering to electron microscopy in these areas together number many thousands.

This mass of published work relies very largely on descriptions in words and on 'representative' micrographs to depict experimental results. However, during the last 5–10 years a more numerical and quantitative approach to the determination and description of ultrastructure has emerged. For some investigations, this has taken the form of instrumental methods applied to micrographs (optical diffraction for example); for others this has consisted of measurements on the sections themselves. X-ray microanalysis is such an example. In many further experiments, the quantitation has taken the form of collecting data from micrographs by means of counts or measurements made with the human eye, aided only by the simplest of equipment. The estimation of surface areas, volumes, numbers and sizes of cells and organelles by stereological techniques is of this type. The evaluation techniques for autoradiographs and for some immunocytochemical preparations are of this type also. Although autoradiography, immunocytochemistry and stereology differ profoundly in their fundamental roots and in their detailed methodology, the three have much in common in their analytical and interpretational stages, whilst each also ultimately

yields data that in essence relate to biochemical matters. This book is about the role of counting and measuring methods in autoradiography, immunocytochemistry and stereology and about the practical and theoretical interrelations between these three areas of technique.

## 1.1 The demand for quantitative methods

The demand for autoradiographic and immunocytochemical methods develops out of the growing area of common ground lying between the various regions of biological science. These methods in essence represent the laying of biochemical observations on to a prepared anatomical substratum. They are, therefore, along with enzyme cytochemistry and staining methods for various classes of chemical or substituent chemical groups (see Lewis and Knight 1977), part of a logical chemical development of the ultrastructural approach.

Stereological techniques (described in chapter 2) represent a different and vital direction of development. They place description of cellular structure on a numerical basis – a logical and necessary progression. The effects of experimental treatments on cell structure and the time course of morphological events can now be precisely charted on the basis of cell or organelle volume, membrane area, organelle number or size. Subjectivity is thereby greatly reduced and firm kinetic data can result. Mechanistic hypotheses may then follow. Stereological methods represent, therefore, a major advance in microanatomy.

Quantitative microanatomical estimations, furthermore, provide an improved framework on which to found cytochemical observations. Immunocytochemical reactions are greatly enhanced in interest if, for example, a statement in words about the site of antibody binding can be upgraded to a figure stating the number of antigenic sites that occur per unit area of a particular species of membrane.

EM autoradiographs (see Williams 1977 for methods of preparation) are notoriously difficult to evaluate merely by looking. The silver grain numbers are generally low and hence sampling errors from this source are usually large. Furthermore, the cells themselves have to be sampled adequately in an ultrastructural sense. The 'image spread' of EM autoradiographs is greater than many people realise and attempts to interpret the origins of individual silver grains are mostly futile. For these reasons, quantitative objective methods have to be constructed (see Chapter 3). EM autoradiography can have very little significance without them. The