STRUCTURE AND FUNCTION OF CELLS

A text for students in medicine and science

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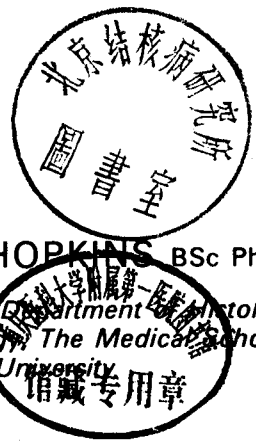

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PREFACE

Over the last decade the rapid, almost explosive growth in our understanding of structure-related function has served to emphasize the central role that cell biology has to play in the education of students in medicine and science. With its recent progress, cell biology has now developed sufficiently to provide a focus in which the surrounding, related areas of interest in biology can be integrated with those in which attention is directed towards the molecular level. The biology of the cell is thus beginning to be taught as a unifying topic in its own right rather than as an appendage to courses in morphology or biochemistry. The purpose of this text is to convey this view to students taking preliminary courses in basic medical science.

The book is intended primarily for students who have a background of school biology and who are taking concurrent courses in biochemistry and physiology. As a brief, contemporary assessment of the field, it should also provide a useful basis for later courses in pharmacology, histopathology, immunology and experimental medicine in general. However, although the approach is clearly directed at the eukaryotic system as typified by the cells of mammalian tissues, it will be read without difficulty by students in life science who lack a strong medical bias. It is with these students in mind that a glossary has been included.

As this book may be the only exposure a student has to the topic of cell biology, I have, in writing it, tried to make the text accessible to first-year university students whilst maintaining something of the momentum and excitement that pervades the subject at the present time. For this reason the more established aspects have often been treated in a condensed and abbreviated manner and the bibliography has been used to provide some indication of historical perspective. The chief dangers of this approach are of course that the generalizations will be too sweeping and the promise of the more recent areas of advance will in time be shown to have been evanescent. Being aware of a danger helps, of course, but is no guarantee that it can always be avoided.

ACKNOWLEDGEMENTS

In my search for suitable illustrative material I have received generous help from workers in many different laboratories. To all of them I am most grateful. For much of the artwork I have been very fortunate in being able to rely upon the talent and patience of Ken Biggs. I am grateful also to Nancy K. Dwyer.

Some of the electron micrographs have been produced in my laboratory and for them I have depended upon the expert technical assistance of Hazel Smith. For the background, often tedious, jobs that have to be done in compiling a text of this kind I am pleased to acknowledge the willing and often innovative help of Annie Pritchard. For much of the typing I am grateful to my secretary, Mrs Doreen Godsell.

To my departmental colleagues and students who over the years have both knowingly and unknowingly made their contributions, I wish to express my thanks.

Finally I thank my wife for her loving support and constant encouragement.

Colin R. Hopkins

LIST OF ILLUSTRATIONS

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PART 1

Techniques in the study of cell structure

Much of our current understanding of cellular structure and function is very closely bound up with a proper appreciation of the strengths and weaknesses of the available technical methods employed in their study. It will, therefore, be instructive for us to preface our discussion of the cell with an account of the most widely used microscopes and their associated preparative and applied techniques.

1

THE MICROSCOPE

MAGNIFICATION AND RESOLUTION

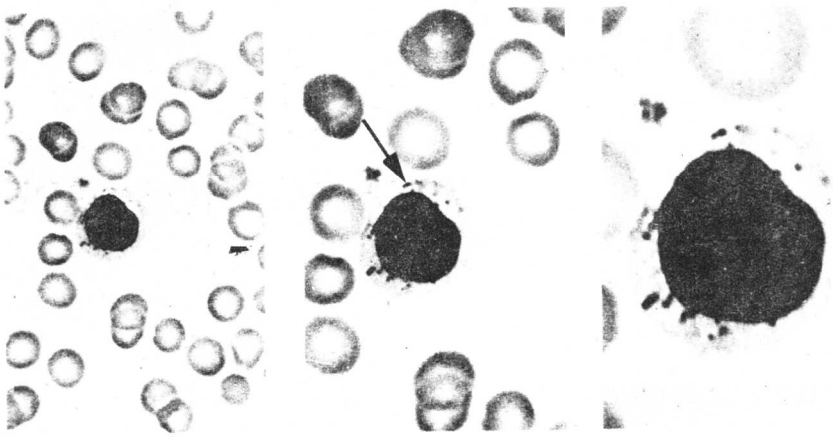
In their most basic essentials all microscopes aim: (a) to magnify the object, and (b) to display the object in greater detail. These aims are interdependent and it is important to realize that to increase magnification without a commensurate improvement in the degree of discernible detail is of little advantage.

Magnification

The magnification of any optical system is dependent upon the focal length of the lenses in the system and their mutual arrangement. It is usually expressed as the ratio of the length of the final image to that of the object, and for the ordinary class microscope it is usually between ×25 and ×1500.

Resolution

The resolving power of a lens indicates the fineness of detail that it allows to be seen. Thus, if one examines two small objects with a microscope lens, provided the objects are well separated, they will be resolved as separate entities. If, however, they are then gradually moved closer together, a situation will eventually arise in which the two objects, though still separate, can no longer be seen to be distinct from each other. In this situation, only by improving the resolution (i.e. by using a lens with better resolving power) will it again be possible to render the two objects as separate entities (see Figure 1).



(*a*) (*b*) (*c*)

Figure 1. *Resolution and magnification. A leucocyte in a blood smear photographed at increasing magnifications with the same objective lens (×100; NA=1.32). Thus, although in Figures (a) to (c) the final magnification is increased, the resolving power remains the same.*

In (b), at a magnification of ×1150, two granules (arrowed) within the leucocyte are resolved and seen as separate entities, while in (a), at a lower magnification of ×650, although the lens resolves the granules, they are not seen to be separate. In (c), where the magnification is increased to ×2250, the granules are larger than in (b) but there is no more detail to be seen.

Increasing the magnification over (b) without increasing the resolution is thus of no advantage (i.e. it produces 'empty magnification').

Courtesy of J. James, Histologisch Laboratorium Amsterdam, University of Amsterdam.

Factors that determine resolution

1. Numerical aperture

When light rays pass through a specimen containing fine detail they interfere with each other and they are variously diffracted; increasingly fine detail increases their angles of diffraction. Since the resolving power of a lens depends upon its ability to collect these diffracted rays, the wider the angle of rays collected the better is the resolution.

The capacity of a lens to collect rays emerging from an object is defined by its *numerical aperture* (NA), and this depends upon both its *angular aperture* (*u* in Figure 2) and the *refractive index* (*n*) of the medium through which the rays pass.

The relationship is expressed as:

$$NA = n \,.\, \sin u$$

In any given lens, the NA (and thus the resolution) is at its best when the cone of rays emerging from the object just fills the angular aperture. When setting up a microscope this optimum requirement is only obtained by careful focusing of the illumination system (see section below relating to the substage condenser).

In the conventional light microscope the medium between the low-power (less than ×40) objective lenses and the specimen is air (i.e.

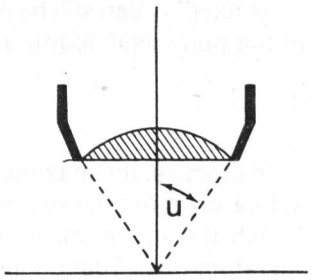


Figure 2. *The angular aperture of an objective lens.*

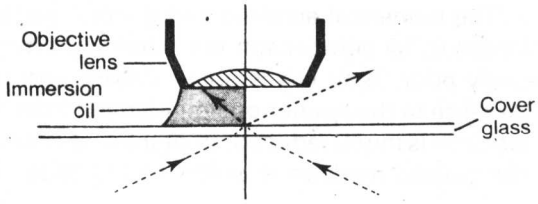


Figure 3. *Oil immersion. Light rays emerging from the coverglass into air (i.e. from a dense to a less dense medium) will be bent by refraction towards the glass. Immersion oils (such as cedar wood oil) have the same refractive index as glass and thus refraction is much reduced.*

the refractive index, $n = 1$). However, for lenses of higher power, where maximum resolution is required, the refractive index may be increased by filling this space with a special 'immersion' oil. The refractive index of the immersion oils used with glass-covered microscope slides is optimally about 1.55. As indicated in the equation above and as shown in Figure 3, this arrangement increases the NA and results in fewer light rays being lost due to refraction. Resolution is thus improved.

2. Wavelength

Resolution also depends upon the wavelength of the transmitted wave form; the smaller the wavelength the better is the resolution. It is primarily for this reason that the electromagnetic lenses of the electron microscope, which depend upon the extremely short wavelength of the electron (0.005 nm at a 60 kV accelerating voltage), can resolve details that are orders of magnitude smaller than those resolved by the light microscope (see below).

The resolution limit (r)

The resolution limit of a lens (which is the converse of its resolving power) can be defined as the minimum separation between two points