Cell Biology

EDITED BY

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Editor's foreword

The student of biological sciences at Advanced level or in the first year of an undergraduate course is expected to be aware of the changing nature of biology. New research is published in a variety of specialised journals and even if these journals are available they are likely only to confuse students. Review articles often assume a detailed background knowledge of the subject and hence are of limited value. Such articles are written with little knowledge of the information contained in most Advanced level textbooks. In addition, it is no longer possible for a single text to cover the whole field of biology while remaining up to date. Teachers and students at school, college or university who try to keep abreast with advances are thus likely to be faced with a perplexing and often conflicting array of information. The aim of 'Modern Views in Biology' is to review the information contained in Advanced level texts while presenting more current ideas in the fields where significant advances have been made.

The difficulty of dividing a course into a number of volumes is vast and opinion will undoubtedly differ on how it is best done. In devising 'Modern Views in Biology' the amount of overlap has been reduced to a minimum. Each volume consists of a number of articles on closely related topics and can thus be used in isolation or in any sequence with the others. Collectively, the volumes provide an up-to-date coverage of the biology studied at Advanced level and thus are likely to be of tremendous value to teachers and students.

Articles have been written by research workers in association with an experienced Advanced level teacher. In this way the current state of knowledge in a particular field is reviewed while the basic conceptual framework encountered in Advanced level textbooks is not forgotten. In addition, authors have been asked to outline the likely direction of future research together with any possible applied aspects which may result from such work. A concerted effort has been made by authors and editor to maintain a constant level of presentation. However, each author has been encouraged to approach his own subject in his own way in order that the author's enthusiasm might be communicated to the reader.

Barry King

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Abbreviations

A	adenine
ADP	adenosine diphosphate
Ala	alanine
AMP	adenosine monophospate
cAMP	cyclic AMP
Arg	arginine
Asn	asparagine
Asp	aspartate
ATP	adenosine triphosphate
Butyl-PBD	(2-(4-t-butylphenyl)-5-(4″-diphenylyl)-1,3,4-oxadiazole)
C	cytosine
CAP	catabolite gene activator protein diphosphate
CDP	cytosine diphospate
CMP	cytosine monophosphate
CTP	cytosine triphosphate
CoA	coenzyme A
cyclic AMP	adenosine 3′, 5′-cyclic monophosphate
Cys	cysteine
d	2′-deoxyribo
DNA	deoxyribonucleic acid
cDNA	complementary DNA
EM	electron microscope
ER	endoplasmic reticulum
FAD	flavin adenine dinucleotide (oxidised)
$FADH_2$	flavin adenine dinucleotide (reduced)
fMet	formylmethionine
G	guanine
Gln	glutamine
Glu	glutamate
Gly	glycine
GDP	guanosine disphosphate
GMP	guanosine monophosphate
GTP	guanosine triphosphate
His	histidine
HLA	histocompatibility antigen
hnRNA	heterogeneous nuclear RNA
Ile	isoleucine
kdal	a unit of mass equal to 1000 dalton, the terms dalton and molecular weight are interchangeable
Leu	leucine
LH	luteinizing hormone
Lys	lysine
MAP	Microtubule-associated protein
Met	methionine
mRNP	messenger ribonuclearprotein
MTOC	microtubule organising centre

NAD+	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
NADP	nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NTP	nucleoside triphosphate
Phe	Phenylalanine
Pi	for red light absorbing form of phytochrome
PPi	inorganic pyrophosphate
Pfr	inorganic orthophosphate
PPo	2, 5-diphenyloxazole
Pr	red light absorbing form of phytochrome
Pro	proline
RER	rough endoplasmic reticulum
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
Ser	serine
T	thymine
Thr	threonine
TSH	thyroid stimulating hormone
Trp	tryptophan
Tyr	tyrosine
U	uracil
UDP	uridine diphosphate
UDP-glucose	uridine diphosphate glucose
UMP	uridine monophosphate
UTP	uridine triphosphate
Val	valine

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Techniques in cell biology

B. King

MODERN VIEWS IN BIOLOGY

1.1 Introduction

During the past few decades there has been a tremendous growth in the biological sciences. There are many factors underlying this increase in our knowledge of living systems. The development of new techniques in the physical and chemical sciences has been important. It has been argued that the subsequent application of these methods in biology is the single most important factor in the increase of knowledge we have witnessed in the last 30 years.

This chapter is not intended to provide students with a comprehensive treatment of the techniques used in cell biology. It is designed to give the reader an understanding of the methods discussed in the following chapters of this volume.

1.2 Light microscopy

1.2.1 MAGNIFICATION AND RESOLUTION

The oldest and still the most widely used instrument for studying the structure of organisms and cells is the light microscope. Students of biology often regard the microscope as no more than a magnifying device and do not really appreciate *the vital property of resolution*. **Magnification** achieved by microscopy can be defined as the ratio of the apparent size of the object to the actual size of the structure. In practice, this can be calculated by multiplying the primary magnification of the objective lens by that of the eyepiece. It is possible to increase the magnifying power of the instrument by increasing the power of the objective or eyepiece or both. Above a certain level, simple enlargement *does not increase the amount of detail but only serves to increase the size of the image.*

The recognition of individual but closely spaced points is termed **resolution**. Each optical instrument has a maximum resolving power; for the eye it is reached when two points come to lie approximately 80 μm apart. Below this distance the eye will just see one point: use of a magnifying glass or microscope would, of course, resolve the points.

The absolute resolving power of the light microscope *is limited by the wave nature of light itself*. A single light ray is scattered by the specimen, the smaller the feature the greater the angle of scatter. The angle of rays gathered by the objective is important in the determination of resolution. This angle is expressed in terms of **numerical aperture**, NA (Fig. 1.1b):

$$\mathrm{NA} = n \sin u$$

where n is the refractive index of the medium between the specimen and the lens, and u is half the angle of light accepted by the objective.

Resolution is given approximately by the formula

$$\text{resolution} = \frac{0.61\lambda}{\mathrm{NA}}$$

where λ is the wavelength of light.

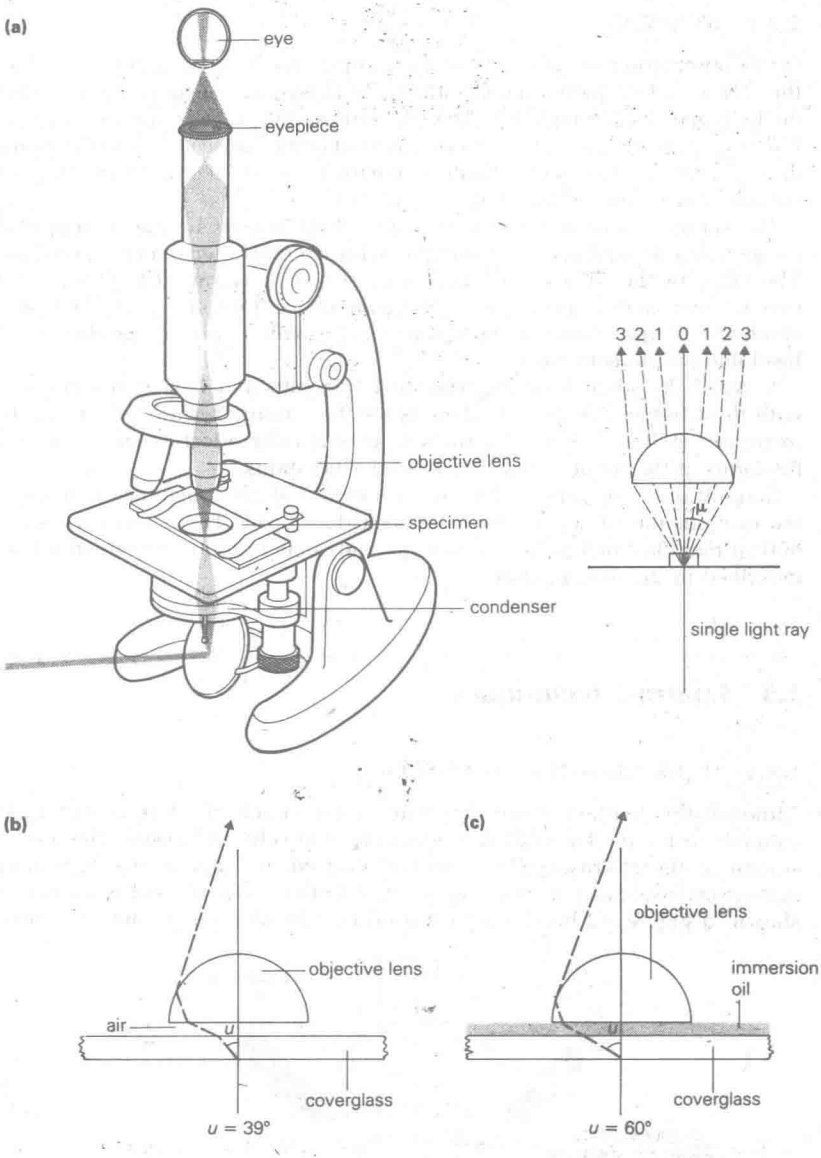

Figure 1.1 Light microscopy: (a) conventional ray pattern in a light microscope together with a single ray; (b) dry objective with a numerical aperture of 0.94; (c) oil immersion objective, numerical aperture increased to approximately 1.2.

As light travels through objects its speed may be slowed according to the refractive index of the medium. The use of immersion oil, which has an index of 1.5 (similar to that of glass), increases the numerical aperture of the objective lens (Fig. 1.1c). In addition, the distance between the specimen and the objective is reduced. In simple terms these two changes are effective in allowing a greater degree of information to be collected from the specimen, and hence a higher resolving power can be achieved.

1.2.2 STAINING

Direct observation of cells reveals a certain degree of information. However, the relatively transparent nature of cytoplasm means that there is little effect on light passing through the cells or tissues. The use of specific staining techniques greatly increases the usefulness of any microscope. In recent years, developments in the field of histochemistry have enabled scientists to stain specific molecules and hence cell organelles.

The staining of deoxyribonucleic acid (DNA) in cells by the technique of Feuglen and Rossenbeck is an example with which students may be familiar. The DNA in the nuclear material is treated with warm HCl. This causes partial hydrolysis, producing *deoxyribose components with exposed aldehyde groups*. These in turn react with Schiff's reagent, producing an insoluble purple compound.

Many of the stains used are toxic to living cells and hence only effective with dead tissue. Fortunately, there is another group of stains which can be incorporated into living cells without dramatically affecting the necessary functions of the tissue. These are termed **vital stains**.

In addition to staining techniques, a number of other methods that allow the examination of specimens have been developed. These can necessitate optical modifications to the microscope and some of the more important are described in the next section.

1.3 Contrast techniques

1.3.1 DARK GROUND MICROSCOPY

Although this method is not commonly used in schools it is an extremely valuable technique for studying certain types of cells and tissue. The optical system of the microscope is virtually reversed, *a bright image appearing against an essentially dark background*. A hollow cone of light is created as shown in Figure 1.2 by placing a central circular stop below the condenser.

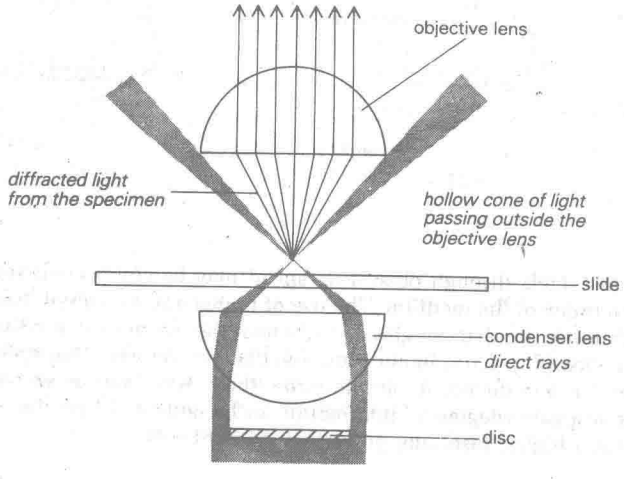

Figure 1.2 Optical arrangement used in dark ground illumination. The insertion of an opaque disc below the condenser prevents any of the direct light from the specimen entering the objective lens.

No direct light rays are gathered by the objective lens; the light rays diffracted from the specimen create a bright image on a black background. The use of dark ground illumination enables examination of details of the structure of aquatic organisms such as protozoa, rotifers and small crustaceans.

1.3.2 PHASE CONTRAST

In order to appreciate the usefulness of the technique of phase contrast a certain knowledge of the wave nature of light is required. The electromagnetic theory considers light to be associated with variations in electric and magnetic fields. This idea is illustrated in Figure 1.3a, together with several of the important parameters of light. The amplitude of a light wave is important as it determines the intensity of the light: the intensity is proportional to the square of the amplitude. The colour of the light is determined by its wavelength (λ). Light with a wavelength of 450 nm is detected as blue while a wavelength of around 660 nm gives red light.

Another important property of light is its **phase**. If two light waves are completely in phase (Fig. 1.3b), interference occurs between the waves; the resultant amplitude is greater and a much brighter light is seen. At the other extreme, if two waves are out of phase, the resultant wave has an amplitude of zero (Fig. 1.3c). In this case nothing is seen. When cells are viewed using normal light optics with no staining techniques, the indirect light waves passing through the cytoplasm are likely to be retarded. This amounts to around $\lambda/4$ relative to the direct rays (Fig. 1.3d) passing through the aqueous medium. Although interference has occurred it is usually insufficient to enable the eye to detect the detail within the cell, and the cytoplasm appears transparent.

The principle behind phase contrast microscopy lies in the *further retardation of these indirect waves*. If the difference between these two different waves can be increased to approximately $\lambda/2$ then the interference created between the two sets of waves will be sufficient to reveal details of the cytoplasm. This is achieved by the insertion of a phase plate within the objective lens (Fig. 1.4a). The plate is a glass disc in which an annular groove has been cut. The light waves which have been diffracted by the specimen are forced through the central region of the phase plate (Fig. 1.4b). This area is thicker and retards these light rays by $\lambda/4$. Since the waves are already out of phase with the direct rays, a total phase shift of $\lambda/2$ is achieved. This phase shift is ensured by placing an annular diaphragm before the condenser, which forces the majority of the direct light waves through the inner part of the phase plate.

Since the separation of direct and diffracted light rays can never be perfect, a small proportion of the diffracted light will in fact pass through the groove in the phase plate. This leads to a 'halo' appearing around the object. This effect can be seen quite clearly in Figure 7.15a. The use of phase microscopy has enabled such processes as cell division and endocytosis to be studied in living cells.

1.3.3 FLUORESCENCE MICROSCOPY

Certain compounds absorb short wavelength radiation and then re-emit energy as light of a longer wavelength. This phenomenon is known as **fluorescence** and is now routinely used in microscopy. The fluorescent microscope (Fig. 1.5) is essentially an ordinary optical instrument which has

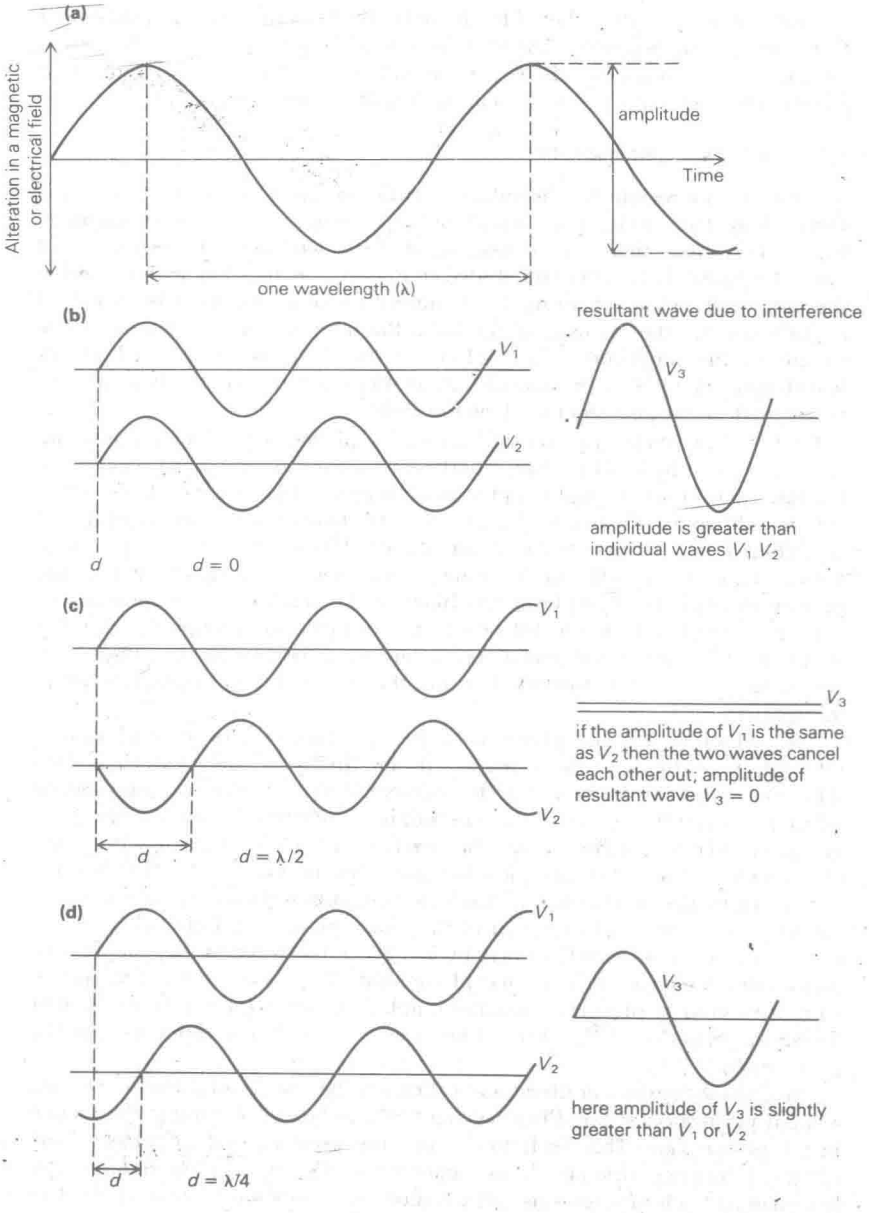

Figure 1.3 (a) Wave form of light. (b), (c) and (d) Resultant waves produced after interference between waves. The degree to which the waves are cut of phase (d) affects the amplitude of the resultant wave.

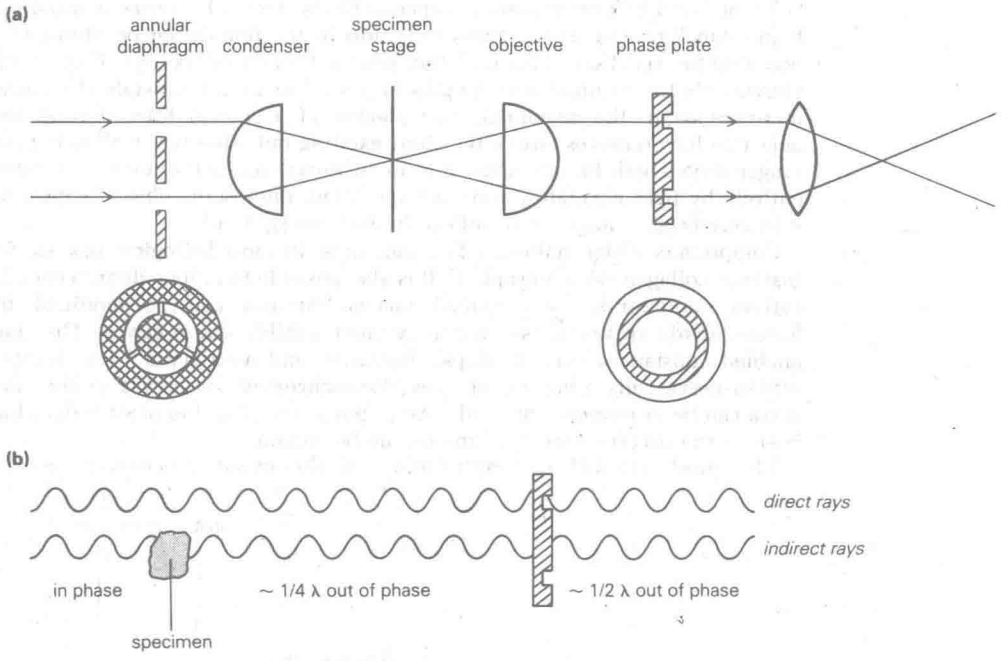

Figure 1.4 (a) Optical system of a phase contrast microscope. (b) Enhanced contrast ensured by the phase plate.

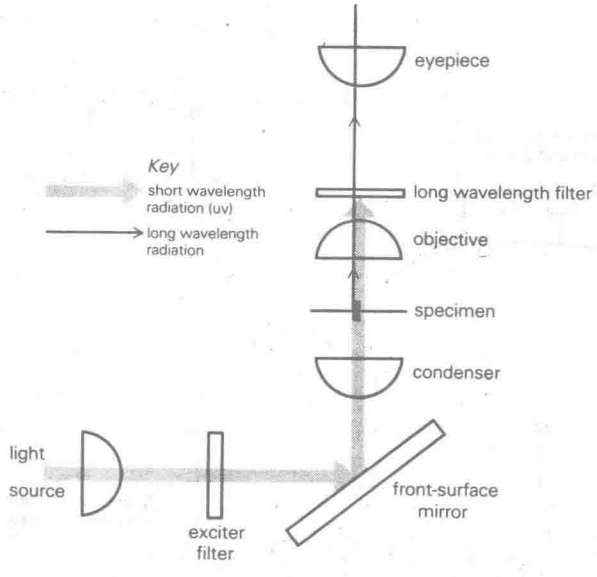

Figure 1.5 Fluorescent light microscopy. The pathway of the short wavelength light is shown by the thick line. Longer wavelength fluorescence produced by the specimen is shown by the thinner line.