

# Cell Biology Protocols

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# Cell Biology Protocols

# Preface

Cell biology is a rapidly expanding discipline that is dependent upon continual technical development. We have attempted to compile an exciting and broadly useful cell biology techniques book, containing tried-and-tested procedures as well as newly established ones. Thus, this book contains an extensive series of routine and up-to-date protocols of value for those studying diverse aspects of present-day cell biology. The book commences with the presentation of several essential light microscopical procedures and leads on to the basic procedures required for producing a range of different cellular, sub-cellular and macromolecular specimens for transmission electron microscopical study. Then follows a chapter dealing with cell culture and cell separation procedures that are widely used to provide starting material for cellular research. The numerous techniques needed to study subcellular organelles and isolated cellular membranes are presented in the next two chapters, thereby providing the main thrust of the book. A series of 44 more specialist techniques used for *in vitro* studies and reassembly approaches in cell biology appear in the next chapter, each contributed by authors knowledgeable and experienced in their field of study. Finally, a reference chapter contains useful information on chemical hazard/safety aspects, centrifugation and radioisotopes. The book has a strong practical content and is directed to those at all levels who perform research in cell biology.

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*June, 2005*

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# 1

## Basic Light Microscopy

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### Introduction

Light microscopy is an indispensable technique for cell and molecular biologists to study cellular structures and biological processes in both living and fixed cells. This chapter provides an overview of light microscopy, describes the important parts of the microscope and goes on to explain how to set up a standard research microscope for bright field and phase contrast microscopy. There is also a short section on confocal microscopy. More comprehensive descriptions of the different forms of light microscopy are found elsewhere [1–4].

Microscopes are instruments that produce an enlarged image of a specimen. The eyepieces and the objectives are the main components of the magnification system of the microscope, the product of the magnification of the objective lens and the ocular lens give the total magnification of the microscope. The visibility of the magnified specimen depends on contrast and resolution. Contrast is the difference in light intensity between an object and its background. Some biological samples contain coloured compounds, for example pigmented animal cells and chlorophyll-containing chloroplasts in plant cells, but most biological samples are colourless and have to be fixed and stained before observation [5]. Such stained specimens are observed using bright field microscopy. Other kinds of microscope systems are available to enhance contrast in living samples; these include phase contrast, dark field, differential interference contrast (DIC) and fluorescence microscopy (Table 1.1). The flow chart in Figure 1.1 will help in the selection of the appropriate microscopic observation method.



**Table 1.1** Techniques for producing contrast in light microscopy

Type	Mechanism	Requirements	Fixed cells	Live cells	Appearance
Bright field	Absorption of visible light following staining of specimen	Any light microscope; range of histochemical stains	Yes	No	Coloured image depending on stains
Phase contrast	Variations in refractive index within specimen	Phase objective and phase condenser	Yes	Yes	Many shades of grey
Dark field	Scattered light	Dark field stop in condenser	Yes	Yes	Bright objects against dark background
Differential interference contrast	Gradient of refractive index	Special objective lens	Yes	Yes	3D effect
Fluorescence	Excitation and emission of light by fluorophore	An excitation light source and appropriate filters for emission; range of fluorescent probes including naturally fluorescent proteins	Yes	Yes	Bright colours against a dark background

The resolution of the optical system, that is the ability to distinguish objects separated by small distances, determines the degree of detail observable. The limit of resolution of the light microscope is about 0.2  $\mu\text{m}$ . Enlarging the image too much results in 'empty magnification' and the quality of the image deteriorates. The limits of resolution are determined by the quality of the objective and the condenser.

## Key components of the compound microscope

The eyepieces, body tube, nosepiece and objectives are part of the magnification system of the microscope. The condenser, condenser-iris diaphragm, filters, field iris diaphragm and light source are the parts that compose the illumination system of the microscope. To use a microscope properly, and to get the most out of it, it is important to understand the purpose and function of each of the microscope's components (Figure 1.2).

### *The body and lamp*

The binocular body, the arm and the base form the frame of the microscope. This provides the stability and holds the optical and other components rigid and in place. The lamp is in the base of the body; its brightness is controlled by an on/off switch and a rheostat control knob. Just above the lamp is a collector lens with a field diaphragm to control the area of illumination. The field diaphragm also aids focusing and centring of the illumination.