Essential Microbiology
Essential Microbiology

Second Edition

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# Contents

<table>
<thead>
<tr>
<th>Preface to Second Edition</th>
<th>vii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface to First Edition</td>
<td>ix</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xi</td>
</tr>
<tr>
<td>About the Companion Website</td>
<td>xiii</td>
</tr>
</tbody>
</table>

## I Introduction
2. Biochemical Principles 19
3. Cell Structure and Organisation 53

## II Microbial Nutrition, Growth and Metabolism
4. Microbial Nutrition and Cultivation 83
5. Microbial Growth 97
6. Microbial Metabolism 115

## III Microbial Diversity
7. Prokaryote Diversity 171
8. The Fungi 203
9. The Protista 217
10. Viruses 243

## IV Microbial Genetics
11. Microbial Genetics 271
12. Microorganisms in Genetic Engineering 319
<table>
<thead>
<tr>
<th>V</th>
<th>Microorganisms in the Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Microbial Associations</td>
</tr>
<tr>
<td>14</td>
<td>Microorganisms in the Environment</td>
</tr>
<tr>
<td>VI</td>
<td>Medical Microbiology</td>
</tr>
<tr>
<td>15</td>
<td>Human Microbial Diseases</td>
</tr>
<tr>
<td>16</td>
<td>The Control of Microorganisms</td>
</tr>
<tr>
<td>17</td>
<td>Antimicrobial Agents</td>
</tr>
<tr>
<td>VII</td>
<td>Microorganisms in Industry</td>
</tr>
<tr>
<td>18</td>
<td>Industrial and Food Microbiology</td>
</tr>
<tr>
<td></td>
<td><strong>Glossary</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Further Reading</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Index</strong></td>
</tr>
</tbody>
</table>
It is now seven years since the first edition of *Essential Microbiology* was published, so it is high time the contents were updated, and I have taken the opportunity to revise the layout in the hope that it will better serve its target readership. The main change to the book from its original incarnation is the inclusion of a chapter on microbial disease in humans. When preparing the content of the first edition, the one major area of doubt I had was whether or not to include a chapter or section on medical microbiology. I was urged to do so by a number of colleagues, but in the end I resisted, feeling it to be too large a topic for inclusion in a general introductory text. The invitation to prepare a second edition, however, has given me an opportunity to reconsider the matter, and comments from several reviewers, together with further reflection on my own part, have persuaded me to change my mind. I have therefore introduced a new chapter on microbial disease in humans, supplementing new material with some expanded and repackaged from other chapters in the first edition. This has resulted in a shuffling and reordering of the second half of the book, which I hope leads to a more logical structure. The new edition no longer features end-of-chapter quizzes; however, these and other forms of self-assessment can now be found on the book’s dedicated website. The other major change that will be noticed by anyone familiar with the original book is the introduction of colour. I feel strongly that a book such as this should be visually attractive as well as instructive, and am grateful to my editorial team at Wiley for allowing me this indulgence, in spite of the additional pressure it creates in trying to keep the selling price to a minimum, which was always one of the principal aims of *Essential Microbiology*.

As always, I should be grateful to receive any comments and suggestions for improvement from students or their tutors.

*Stuart Hogg*

*September 2012*
Every year, in UK universities alone, many hundreds of students study microbiology as part of an undergraduate course. For some, the subject will form the major part of their studies, leading to a BSc degree in Microbiology, or a related subject such as Bacteriology or Biotechnology. For the majority, however, the study of microbiology will be a brief encounter, forming only a minor part of their course content.

A number of excellent and well-established textbooks are available to support the study of microbiology; such titles are mostly over 1000 pages in length, beautifully illustrated in colour, and rather expensive. This book in no way seeks to replace or compete with such texts, which will serve specialist students well throughout their three years of study, and represent a sound investment. It is directed rather towards the second group of students, who require a text that is less detailed, less comprehensive, and less expensive! The majority of the students in my own classes are enrolled on BSc degrees in Biology, Human Biology and Forensic Science; I have felt increasingly uncomfortable about recommending that they invest a substantial sum of money on a book much of whose content is irrelevant to their needs. Alternative recommendations, however, are not thick on the ground. This, then, was my initial stimulus to write a book of ‘microbiology for the non-microbiologist’.

The facts and principles you will find here are no different from those described elsewhere, but I have tried to select those topics that one might expect to encounter in years 1 and 2 of a typical non-specialist degree in the life sciences or related disciplines. Above all, I have tried to explain concepts or mechanisms; one thing my research for this book has taught me is that textbooks are not always right, and they certainly don’t always explain things as clearly as they might. It is my wish that the present text will give the attentive reader a clear understanding of sometimes complex issues, whilst avoiding over-simplification.

The book is arranged into seven sections, the fourth of which, Microbial Genetics, acts as a pivot, leading from principles to applications of
microbiology. Depending on their starting knowledge, readers may ‘dip into’ the book at specific topics, but those whose biological and chemical knowledge is limited are strongly recommended to read Chapters 2 and 3 for the foundation necessary for the understanding of later chapters. Occasional boxes are inserted into the text, which provide some further enlightenment on the topic being discussed, or offer supplementary information for the inquisitive reader. As far as possible, diagrams are limited to simple line drawings, most of which could be memorised for reproduction in an examination setting. Although a Glossary is provided at the end of the book, new words are also defined in the text at the point of their first introduction, to facilitate uninterrupted reading. All chapters except the first are followed by a self-test section in which readers may review their knowledge and understanding by ‘filling in the gaps’ in incomplete sentences; the answers are all to be found in the text, and so are not provided separately. The only exceptions to this are two numerical questions, the solutions to which are to be found at the back of the book. By completing the self-test questions, the reader effectively provides a summary for the chapter.

A book such as this stands or falls by the reception it receives from its target readership. I should be pleased to receive any comments on the content and style of Essential Microbiology from students and their tutors, all of which will be given serious consideration for inclusion in any further editions.

Stuart Hogg
January 2005
I would like to thank those colleagues who took the time to read over individual chapters of this book, and those who reviewed the entire manuscript. Their comments have been gratefully received, and in some cases spared me from the embarrassment of seeing my mistakes perpetuated in print.

Thanks are also due to my editorial team at John Wiley, Rachael Ballard and Fiona Seymour, and production editor Jasmine Chang for ensuring smooth production of this book.

I am grateful to those publishers and individuals who have granted permission to reproduce diagrams. Every effort has been made to trace holders of copyright; any inadvertent omissions will gladly be rectified in any future editions of this book.

Finally, I would like to express my gratitude to my family for allowing me to devote so many weekends to ‘the book’.
About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/hogg/essentialmicrobiology

The website includes:
- Powerpoints of all figures from the book for downloading
- PDFs of tables from the book
- Short Answer Questions
- Key concepts
- Links for further reading
I

Introduction
Microorganisms (or microbes) inhabit every corner of the globe, and are essential for the maintenance of the world’s ecosystems. They include organisms responsible for some of the most deadly human diseases, and others that form the basis of important industrial processes. Yet until a few hundred years ago, nobody knew they existed! This book offers an introduction to the world of microorganisms, and in this opening chapter, we offer some answers to three questions:

- What is microbiology?
- Why is it such an important subject?
- How have we gained our present knowledge of microbiology?

1.1 What is microbiology?

Things aren’t always the way they seem. On the face of it, ‘microbiology’ should be an easy word to define: the science (logos) of small (micro) life (bios), or to put it another way, the study of living things so small that they can’t be seen with the naked eye. Bacteria neatly fit this definition, but what about fungi and algae? These two groups each contain members that are far from microscopic. On the other hand, certain animals, such as nematode worms, can be microscopic, yet are not considered to be the domain of the microbiologist. Viruses represent another special case; they are most certainly microscopic; indeed, most are submicroscopic, but by most accepted definitions they are not living (why? – see Chapter 10 for an explanation). Nevertheless, these too fall within the remit of the microbiologist.
In the central section of this book you can read about the thorny issue of microbial classification and gain some understanding of just what is and what is not regarded as a microorganism.

1.2 Why is microbiology important?

To the lay person, microbiology means the study of sinister, invisible ‘bugs’ that cause disease. As a subject, it generally tends to impinge on the popular consciousness in news coverage concerning the latest ‘health scare’. It may come as something of a surprise therefore to learn that the vast majority of microorganisms coexist alongside us without causing any harm; indeed, at least a thousand different species of bacteria are to be found on human skin! In addition, many microorganisms are positively beneficial, performing vital tasks such as the recycling of essential elements, without which life on our planet could not continue, as we’ll examine in Chapter 14. Other microorganisms have been exploited by humans for our own benefit, for instance in the manufacture of antibiotics (Chapter 17) and foodstuffs (Chapter 18). To get some idea of the importance of microbiology in the world today, just consider the following list of some of the general areas in which the expertise of a microbiologist might be used:

- medicine
- environmental science
- food and drink production
- fundamental research
- agriculture
- pharmaceutical industry
- genetic engineering

The popular perception among the general public, however, remains one of infections and plagues. Think back to the first time you ever heard about microorganisms; almost certainly, it was when you were a child and your parents impressed on you the dangers of ingesting ‘germs’ from dirty hands or putting things in your mouth after they’d been on the floor. In reality, only a couple of hundred out of the half million or so known bacterial species give rise to infections in humans; these are termed pathogens, and have tended to dominate our view of the microbial world.

In the next few pages we shall review some of the landmark developments in the history of microbiology, and see how the main driving force throughout this time, but particularly in the early days, has been the desire to understand the nature and cause of infectious diseases in humans.

A pathogen is an organism with the potential to cause disease.
1.3 How do we know? Microbiology in perspective: to the Golden Age and beyond

We have learnt an astonishing amount about the invisible world of microorganisms, particularly over the last century and a half. How has this happened? The penetrating insights of brilliant individuals are rightly celebrated, but a great many ‘breakthroughs’ or ‘discoveries’ have only been made possible thanks to some (frequently unsung) development in microbiological methodology. For example, on the basis that ‘seeing is believing’, it was only when we had the means to see microorganisms under a microscope that we could prove their existence.

Microorganisms had been on the Earth for some 4000 million years when Antoni van Leeuwenhoek started his pioneering microscope work in 1673. Leeuwenhoek was an amateur scientist who spent much of his spare time grinding glass lenses to produce simple microscopes (Figure 1.1). His detailed drawings make it clear that the ‘animalcules’ he observed from a variety of sources included representatives of what later became known as protozoa, bacteria and fungi. Where did these creatures come from? Arguments about the origin of living things revolved around the long-held belief in spontaneous generation, the idea that living organisms could arise from non-living matter. In an elegant experiment, the Italian Francesco Redi (1626–1697) showed

Figure 1.1 Leeuwenhoek’s microscope. The lens (a) was held between two brass plates and used to view the specimen, which was placed on the mounting pin (b). Focusing was achieved by means of two screws (c) and (d). Some of Leeuwenhoek’s microscopes could magnify up to 300 times. Original source: antoni van Leeuwenhoek and his little animals by CE Dobell (1932).
that the larvae found on putrefying meat arose from eggs deposited by flies, and not spontaneously as a result of the decay process. This can be seen as the beginning of the end for the spontaneous generation theory, but many still clung to the idea, claiming that while it may not have been true for larger organisms, it must surely be so for minute creatures such as those demonstrated by Leeuwenhoek. Despite mounting evidence against the theory, as late as 1859 fresh ‘proof’ was still being brought forward in its support. Enter onto the scene Louis Pasteur (1822–95), still arguably the most famous figure in the history of microbiology. Pasteur trained as a chemist, and made a lasting contribution to the science of stereochemistry before turning his attention to spoilage problems in the wine industry. He noticed that when lactic acid was produced instead of alcohol in wine, rod-shaped bacteria were always present as well as the expected yeast cells. This led him to believe that while the yeast produced the alcohol, the bacteria were responsible for the spoilage, and must have originated in the environment. Exasperated by continued efforts to substantiate the theory of spontaneous generation, he set out to disprove it once and for all. In response to a call from the French Academy of Science, he carried out a series of experiments that led to the acceptance of biogenesis, the idea that life arises only from already existing life. Using his famous swan-necked flasks (Figure 1.2), he demonstrated that as long as dust particles (and the microorganisms carried on them) were excluded, the contents would remain sterile. This also disproved the idea held by many that there was some element in the air itself that was capable of initiating microbial growth. In Pasteur’s words ‘....the doctrine of spontaneous generation will never recover from this mortal blow. There is no known circumstance in which it can be affirmed that microscopic beings came into the world without germs, without parents similar to themselves’ [author’s italics]. Pasteur’s findings on the role of microorganisms in wine contamination led inevitably to the idea that they may also be responsible for diseases in humans, animals and plants.

The notion that some invisible (and therefore presumably extremely small) living creatures were responsible for certain diseases was not a new one. Long before microorganisms had been shown to exist, the Roman philosopher Lucretius (∼98–55 BC) and much later the physician Girolamo Fracastoro (1478–1553) had supported the idea. Fracastoro wrote ‘Contagion is an infection that passes from one thing to another’ and recognised three forms of transmission: by direct contact, through inanimate objects and via the air; we still class transmissibility of infectious disease in much the same way today (see Chapter 15). The prevailing belief at the time, however, was that an infectious disease was due to something called a miasma, a poisonous vapour arising from dead or diseased bodies, or to an imbalance between the four humours of the body (blood, phlegm, yellow bile and black bile).

During the nineteenth century, many diseases were shown, one by one, to be caused by microorganisms. In 1835, Agostino Bassi showed that a disease
1.3 HOW DO WE KNOW? MICROBIOLOGY IN PERSPECTIVE

**Pasteur's swan-necked flasks.** Broth solutions rich in nutrients were placed in flasks and boiled. The necks of the flasks were heated and drawn out into a curve, but kept open to the atmosphere. Pasteur showed that the broth remained sterile because any contaminating dust and microorganisms remained trapped in the neck of the flask as long as it remained upright.

Liquid sterilised by boiling

Liquid allowed to cool

Dust and microorganisms settle in bend of flask neck. Liquid remains sterile

Flask tilted, allowing liquid to come into contact with deposit in neck

Liquid turns cloudy due to microbial growth

Figure 1.2

of silkworms was due to a fungal infection, and 10 years later, Miles Berkeley demonstrated that a fungus was also responsible for the great Irish potato blight. Joseph Lister’s pioneering work on antiseptic surgery provided strong, albeit indirect, evidence of the involvement of microorganisms in infections of humans. The use of heat-treated instruments and of phenol both on dressings and actually sprayed in a mist over the surgical area, was found greatly to reduce the number of fatalities following surgery. Around the same time, in the 1860s, the indefatigable Pasteur had shown that a parasitic protozoan was the cause of another disease of silkworms called ‘pèbrine’, which had devastated the French silk industry.

The definitive proof of the germ theory of disease came from the German, Robert Koch, who in 1876 showed the relationship between the cattle disease anthrax and a bacillus we now know as *Bacillus anthracis*. This was also the first demonstration of the

*A bacillus* is a rod-shaped bacterium.
Box 1.1  Koch’s postulates

1. The microorganism must be present in every instance of the disease and absent from healthy individuals.
2. The microorganism must be capable of being isolated and grown in pure culture.
3. When the microorganism is inoculated into a healthy host, the same disease condition must result.
4. The same microorganism must be re-isolated from the experimentally infected host.

involvement of bacteria in disease. Koch infected healthy mice with blood from diseased cattle and sheep, and noted that the symptoms of the disease appeared in the mice, and also, crucially, that rod-shaped bacteria could be isolated from their blood. These could be isolated and grown in culture, where they multiplied and produced spores. Injection of healthy mice with these spores (or more bacilli) led them too to develop anthrax, and once again the bacteria were isolated from their blood. These results led Koch to formalise the criteria necessary to prove a causal relationship between a specific disease condition and a particular microorganism. These criteria became known as Koch’s postulates (Box 1.1), and are still in use today.

Despite their value, it is now realised that Koch’s postulates do have certain limitations. It is known for example that certain agents responsible for causing disease (e.g. viruses, prions: see Chapter 10) can’t be grown in vitro, but only in host cells. Also, the healthy animal in Postulate 3 is seldom human, so a degree of extrapolation is necessary – if agent X doesn’t cause disease in a laboratory animal, can we be sure it won’t in humans? Furthermore, some diseases are caused by more than one organism, and some organisms are responsible for more than one disease. On the other hand, the value of Koch’s postulates goes beyond just defining the causative agent of a particular disease, and allows us to ascribe a specific effect (of whatever kind) to a given microorganism.

Critical to the development of Koch’s postulates was the advance in microbial culturing techniques, enabling the isolation of pure cultures of specific microorganisms. These are discussed in more detail in Chapter 4. The development of pure cultures revolutionised

The term in vitro (= ‘in glass’) is used to describe procedures performed outside of the living organism in test tubes, etc. (cf. in vivo).

A pure or axenic culture contains one type of organism only, and is completely free from contaminants.
1.3 HOW DO WE KNOW? MICROBIOLOGY IN PERSPECTIVE

Table 1.1 The discovery of some major human pathogens

<table>
<thead>
<tr>
<th>Year</th>
<th>Disease</th>
<th>Causative agent</th>
<th>Discoverer</th>
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<tbody>
<tr>
<td>1876</td>
<td>Anthrax</td>
<td><em>Bacillus anthracis</em></td>
<td>Koch</td>
</tr>
<tr>
<td>1879</td>
<td>Gonorrhoea</td>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Neisser</td>
</tr>
<tr>
<td>1880</td>
<td>Typhoid fever</td>
<td><em>Salmonella typhi</em></td>
<td>Gaffky</td>
</tr>
<tr>
<td>1880</td>
<td>Malaria</td>
<td><em>Plasmodium spp.</em></td>
<td>Laveran</td>
</tr>
<tr>
<td>1882</td>
<td>Tuberculosis</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Koch</td>
</tr>
<tr>
<td>1883</td>
<td>Cholera</td>
<td><em>Vibrio cholerae</em></td>
<td>Koch</td>
</tr>
<tr>
<td>1883/4</td>
<td>Diphtheria</td>
<td><em>Corynebacterium diphtheriae</em></td>
<td>Klebs and Loeffler</td>
</tr>
<tr>
<td>1885</td>
<td>Tetanus</td>
<td><em>Clostridium tetani</em></td>
<td>Nicolaier and Kitasato</td>
</tr>
<tr>
<td>1886</td>
<td>Pneumonia (bacterial)</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>Fraenkel</td>
</tr>
<tr>
<td>1892</td>
<td>Gas gangrene</td>
<td><em>Clostridium perfringens</em></td>
<td>Welch and Nuttall</td>
</tr>
<tr>
<td>1894</td>
<td>Plague</td>
<td><em>Yersinia pestis</em></td>
<td>Kitasato and Yersin</td>
</tr>
<tr>
<td>1896</td>
<td>Botulism</td>
<td><em>Clostridium botulinum</em></td>
<td>Van Ermengem</td>
</tr>
<tr>
<td>1898</td>
<td>Dysentery</td>
<td><em>Shigella dysenteriae</em></td>
<td>Shiga</td>
</tr>
<tr>
<td>1901</td>
<td>Yellow fever</td>
<td><em>Flavivirus</em></td>
<td>Reed</td>
</tr>
<tr>
<td>1905</td>
<td>Syphilis</td>
<td><em>Treponema pallidum</em></td>
<td>Schaudinn and Hoffman</td>
</tr>
<tr>
<td>1906</td>
<td>Whooping cough</td>
<td><em>Bordetella pertussis</em></td>
<td>Bordet and Gengou</td>
</tr>
<tr>
<td>1909</td>
<td>Rocky Mountain spotted fever</td>
<td><em>Rickettsia rickettsii</em></td>
<td>Ricketts</td>
</tr>
</tbody>
</table>

microbiology, and within 30 years or so of Koch’s work on anthrax, the pathogens responsible for the majority of common human bacterial diseases had been isolated and identified. Not without just cause is this period known as the ‘golden age’ of microbiology! Table 1.1 summarises the discovery of some major human pathogens.

Koch’s greatest achievement was in using the advances in methodology and the principles of his own postulates to demonstrate the identity of the causative agent of tuberculosis, which at the time was responsible for around one in every seven human deaths in Europe. Although it was believed by many to have a microbial cause, the causative agent had never been observed, either in culture or in the affected tissues. We now know this is because *Mycobacterium tuberculosis* (the tubercle bacillus) is very difficult to stain by conventional methods due to the high lipid content of the cell wall surface. Koch developed a staining technique that enabled it to be seen, but realised that in order to satisfy his own postulates, he must isolate the organism and grow it in culture. Again, there were technical difficulties, since even under favourable conditions, *M. tuberculosis* grows slowly, but eventually Koch was able to demonstrate the infectivity of the cultured organisms towards guinea pigs. He was then able to isolate them again from the diseased animal and use them to cause disease in uninfected animals, thus satisfying the remainder of his postulates.

Charles Chamberland, a pupil of Pasteur’s, invented the autoclave, contributing greatly to the development of pure cultures.
Although most bacterial diseases of humans and their aetiological agents have now been identified, important variants continue to evolve and sometimes emerge; examples in recent decades include Lyme disease and legionellosis (legionnaire’s disease); the latter is an acute respiratory infection caused by the previously unrecognised genus, *Legionella*. Also, *Helicobacter pylori*, only discovered in the 1980s, has been shown to play an important (and previously unsuspected) role in the development of stomach ulcers. There still remain a few diseases that some investigators suspect are caused by bacteria, but for which no pathogen has been identified.

Another cause of infectious diseases are viruses, and following their discovery during the last decade of the nineteenth century, it was soon established that many diseases of plants, animals and humans were caused by these minute, non-cellular agents.

The major achievement of the first half of the twentieth century was the development of antibiotics and other antimicrobial agents, a topic discussed in some detail in Chapter 17. Infectious diseases that previously accounted for millions of deaths became treatable by a simple course of therapy, at least in the affluent West, where such medications were readily available.

If the decades either side of 1900 have become known as the golden age of microbiology, the second half of the twentieth century will surely be remembered as the golden age of molecular genetics. Following on from the achievements of others such as Griffith and Avery, the publication of Watson and Crick’s structure for DNA in 1953 heralded an extraordinary period of achievement in this area, culminating at the turn of the twenty-first century in the completion of the Human Genome Project.

You may ask, what has this genetic revolution to do with microbiology? Well, all the early work in molecular genetics was carried out on bacteria and viruses, as you’ll learn in Chapter 11, and microbial systems have also been absolutely central to the development of the techniques of genetic engineering. In addition, as part of the Human Genome Project, the genomes of many microorganisms have been decoded, something that has now become almost routine, thanks to methodological advances made during the project. Having this information will help us to understand in greater detail the disease strategies of microorganisms, and to devise ways of countering them.

As we have seen, a recurring theme in the history of microbiology has been the way that advances in knowledge have followed on from methodological or technological developments, and we shall refer to a number of such developments during the course of this book. To conclude this introduction to microbiology, we shall return to the instrument that, in some respects, started it all. In any microbiology course, you are sure to spend some time
Modern microscopes have a built-in light source. The light is focused onto the specimen by the condenser lens, and then passes into the body of the microscope via the objective lens. Rotating the objective nosepiece allows different magnifications to be selected. The amount of light entering the microscope is controlled by an iris diaphragm. Light microscopy allows meaningful magnification of up to around $1000 \times$.

looking down a microscope, and to get the most out of the instrument it is essential that you understand the principles of how it works. The following pages attempt to explain these principles.

### 1.4 Light microscopy

Try this simple experiment. Fill a glass with water, then partly immerse a pencil and observe from above; what do you see? The apparent ‘bending’ of the pencil is due to rays of light being slowed down as they enter the water, because air and water have different refractive indices. Light rays are similarly retarded as they enter glass, and all optical instruments are based on this phenomenon of refraction.

The compound light microscope consists of three sets of lenses (Figure 1.3):

- the **condenser** focuses light onto the specimen to give optimum illumination;
- the **objective** provides a magnified and inverted image of the specimen;
- the **eyepiece** adds further magnification.

The **refractive index** of a substance is the ratio between the velocity of light as it passes through that substance and its velocity in a vacuum. It is a measure of how much the substance slows down and therefore refracts the light.
Figure 1.4  Light rays parallel to the axis of a convex lens pass through the focal point. The distance from the centre of the lens to the focal point is called the focal length of the lens \( f \).

Most microscopes have three or four different objectives, giving a range of magnifications, typically from 10× to 100×. The total magnification is obtained by multiplying this by the eyepiece value (usually 10×), thus giving a maximum magnification of 1000×.

In order to appreciate how this magnification is achieved, we need to understand the behaviour of light passing through a convex lens:

- rays parallel to the axis of the lens are brought to a focus at the focal point of the lens (Figure 1.4);
- similarly, rays entering the lens from the focal point emerge parallel to the axis;
- rays passing through the centre of the lens from any angle are undeviated.

Because the condenser is not involved in magnification, it need not concern us here. Consider now what happens when light passes through an objective lens from an object AB situated slightly beyond its focal point (Figure 1.5a). Starting at the tip of the object, a ray parallel to the axis will leave the lens and pass through the focal point; a ray leaving the same point and passing through the centre of the lens will be undeviated. The point at which the two rays converge is an image of the original point formed by the lens. The same thing happens at an infinite number of points along the object’s length, resulting in a primary image of the specimen, A′B′. What can we say about this image, compared to the original specimen AB? It is magnified and it is inverted (i.e. it appears upside down).

This primary image now serves as an object for a second lens, the eyepiece, and is magnified further (Figure 1.5b); this time the object is situated within the focal length. Using the same principles as before, we can construct a ray diagram, but this time we find that the two lines emerging from a point don’t converge on the other side of the lens, but actually get further apart. The point at which the lines do

A real image is one that can be projected onto a flat surface such as a screen. A virtual image does not exist in space and cannot be projected in this way. A familiar example is the image seen in a mirror.
eventually converge is actually ‘further back’ than the original object! What does this mean? The secondary image only appears to be coming from $A''B''$, and isn’t actually there. An image such as this is called a virtual image. Today’s readers, familiar with the concept of virtual reality, will probably find it easier to come to terms with this than some of their predecessors! The primary image $A'B'$, on the other hand, is a real image; if a screen was placed at that position, the image would be projected onto it. If we compare $A''B''$ with $A'B'$, we can see that it has been further magnified, but not further inverted, so it is still upside down compared with the original. The rays of light
emerging from the eyepiece lens are focused by the lens of the eye to form a real image on the observer’s retina.

So a combination of two lens systems allows us to see a considerably magnified image of our specimen. To continue magnifying an image beyond a certain point, however, serves little purpose, if it is not accompanied by an increase in detail. This is termed empty magnification, since it does not provide us with any more information. The resolution (resolving power, \( d \)) of a microscope is its capacity for discerning detail. More specifically, it is the ability to distinguish between two points a short distance apart, and is determined by the equation:

\[
d = \frac{0.61 \lambda}{n \sin \theta}
\]

where:

\( \lambda = \) the wavelength of the light source;
\( n = \) the refractive index of the air or liquid between the objective lens and the specimen;
\( \theta = \) the aperture angle (a measure of the light-gathering ability of the lens).

The expression \( n \sin \theta \) is called the numerical aperture and for good quality lenses has a value of around 1.4. The lowest wavelength of light visible to the human eye is approximately 400 nm, so the maximum resolving power for a light microscope is approximately:

\[
d = \frac{0.61 \times 400}{1.4} = 0.17 \text{ \( \mu m \)}
\]

that is, it cannot distinguish between two points closer together than about 0.2 microns. For comparison, the naked eye is unable to resolve two points more than about 0.2 mm apart.

For us to be able to discern detail in a specimen, it must have contrast; most biological specimens, however, are more or less colourless, so unless a structure is appreciably denser than its surroundings, it will not stand out using conventional light microscopy. This is why preparations are commonly subjected to staining.