I
Introduction

1 Microbiology: What, Why and How?

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.

Essential Microbiology, Second Edition. Stuart Hogg.

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

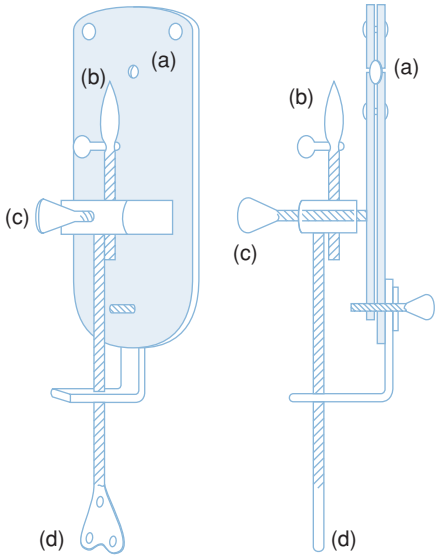


Figure 1.1 Leeuwenhoek's microscope. The lens (a) was held between two brass plates and used to view the specimen, which was placed on the mounting pin (b). Focusing was achieved by means of two screws (c) and (d). Some of Leeuwenhoek's microscopes could magnify up to 300 times. Original source: antoni 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

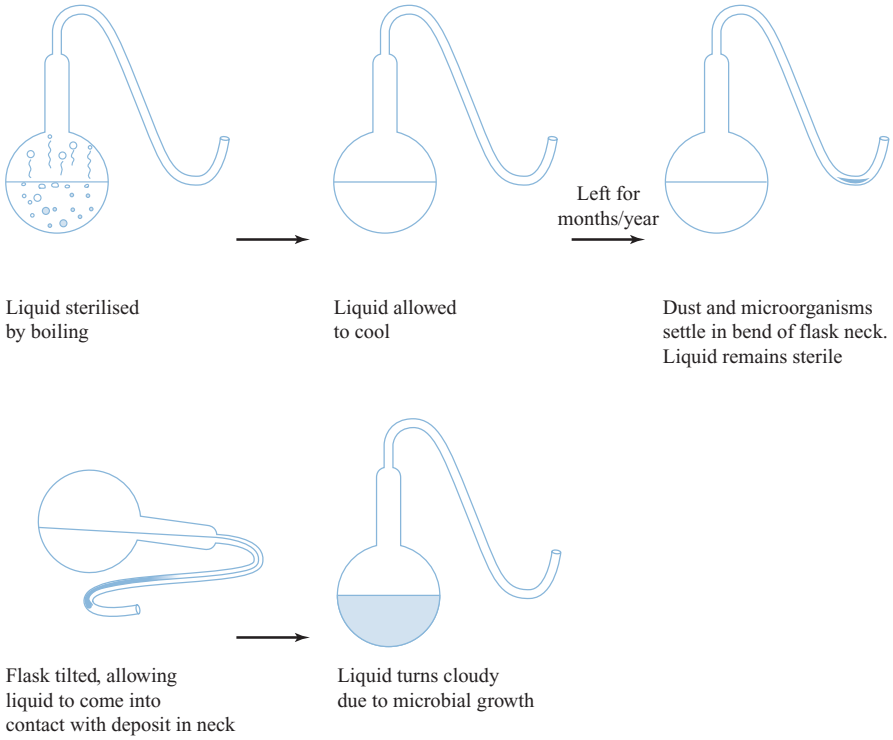


Figure 1.2 Pasteur's swan-necked flasks. Broth solutions rich in nutrients were placed in flasks and boiled. The necks of the flasks were heated and drawn out into a curve, but kept open to the atmosphere. Pasteur showed that the broth remained sterile because any contaminating dust and microorganisms remained trapped in the neck of the flask as long as it remained upright.

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 'pébrine', 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.

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

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

A *pure* or *axenic culture* contains one type of organism only, and is completely free from contaminants.

Table 1.1 The discovery of some major human pathogens

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

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.

Charles Chamberland, a pupil of Pasteur's, invented the *autoclave*, contributing greatly to the development of pure cultures.

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.

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.

Aetiology is the cause or origin of a disease.

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.

The *Human Genome Project* is an international effort to map and sequence all the DNA in the human genome. The project has also sequenced the genomes of many other organisms.

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

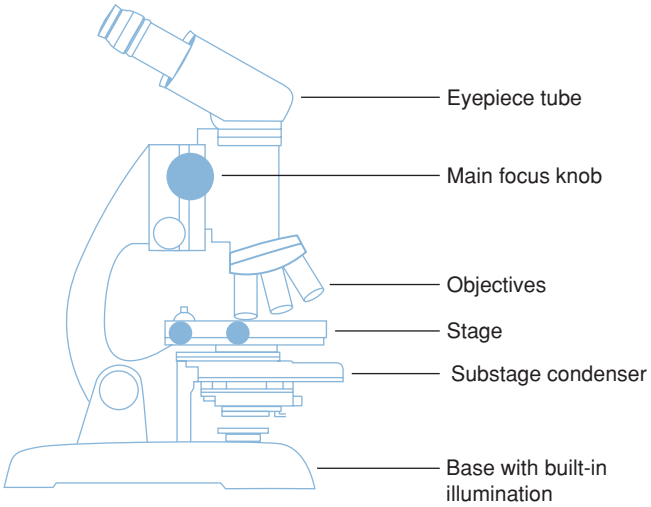


Figure 1.3 The compound light microscope. Modern microscopes have a built-in light source. The light is focused onto the specimen by the condenser lens, and then passes into the body of the microscope via the objective lens. Rotating the objective nosepiece allows different magnifications to be selected. The amount of light entering the microscope is controlled by an iris diaphragm. Light microscopy allows meaningful magnification of up to around 1000 × .

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 *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.

- 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.

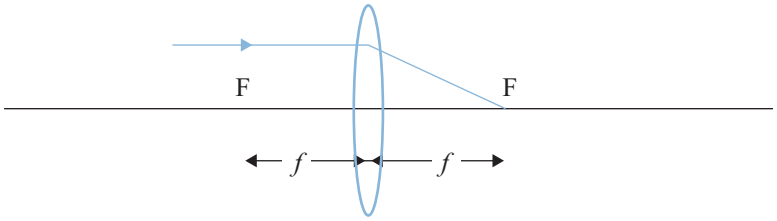


Figure 1.4 Light rays parallel to the axis of a convex lens pass through the focal point. The distance from the centre of the lens to the focal point is called the *focal length* of the lens (f).

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

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.

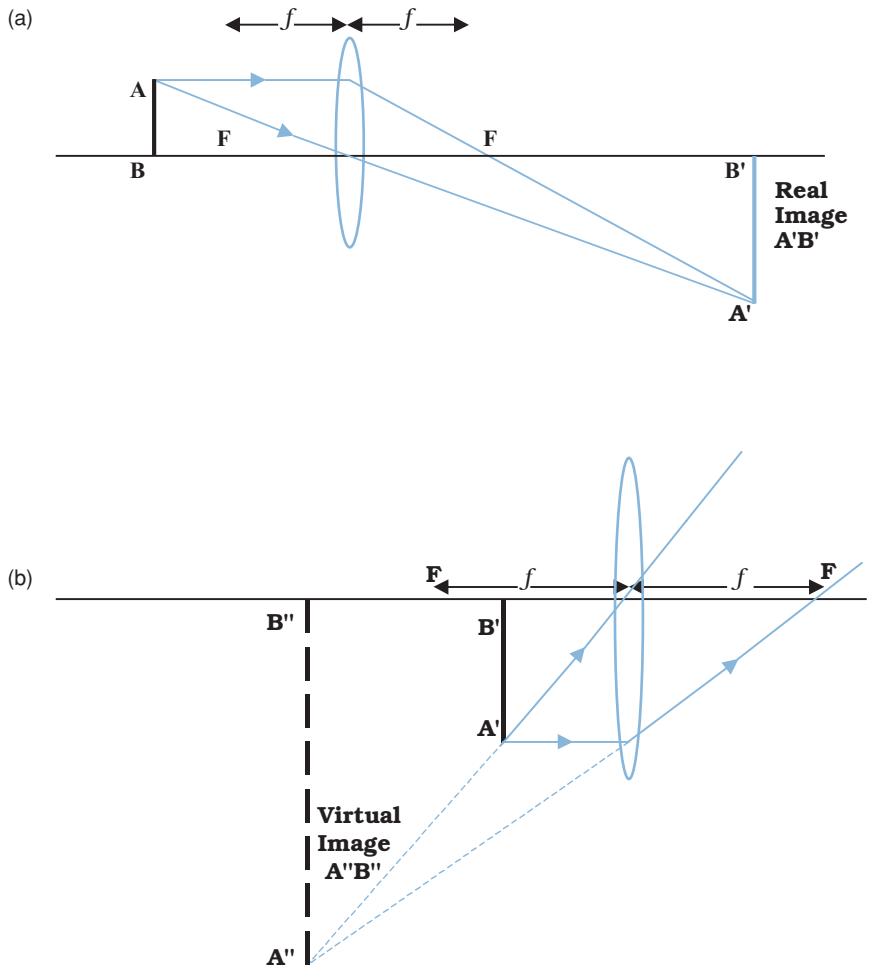


Figure 1.5 The objective lens and eyepiece lens combine to produce a magnified image of the specimen. (a) Light rays from the specimen AB pass through the objective lens to give a *magnified*, *inverted* and *real* primary image, A′B′. (b) The eyepiece lens magnifies this further to produce a *virtual* image of the specimen, A″B″.

eventually converge is actually 'further back' than the original object! What does this mean? The *secondary image* only *appear*s 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:

Immersion oil is used to improve the resolution of a light microscope at high power. It has the same refractive index as glass and is placed between the high-power objective and the glass slide. With no layer of air, more light from the specimen enters the objective lens instead of being refracted outside of it, resulting in a sharper image.

$$d = \frac{0.61\lambda}{n \sin\theta}$$

where:

λ= the wavelength of the light source;
n = the refractive index of the air or liquid between the objective lens and the specimen;
θ = 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:

$$d = \frac{0.61 \times 400}{1.4} = 0.17\ \mu\text{m}$$

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.

A *nanometre* (nm) is one-millionth of a millimetre. There are 1000 nanometres in 1 *micron* (μm), which is therefore one-thousandth of a millimetre.
1 mm = 10^{-3} metre
1 μm = 10^{-6} metre
1 nm = 10^{-9} metre

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*

procedures prior to viewing. The introduction of coloured dyes, which bind to certain structures, enables the viewer to discern more detail.

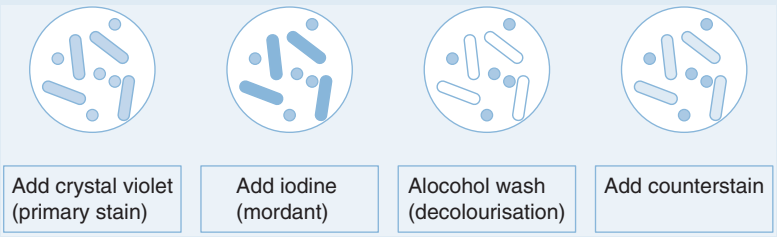
Since staining procedures involve the addition and washing off of liquid stains, the sample must clearly be immobilised or *fixed* to the slide if it is not to end up down the drain. The commonest way of doing this is to make a *heat fixed smear*; this kills the cells and attaches them to the glass microscope slide. A thin aqueous suspension of the cells is spread across the slide, allowed to dry, then passed (sample side up!) through a flame a few times. Excessive heating must be avoided, as it would distort the natural structure of the cells.

Using simple stains, such as methylene blue, we can see the size and shape of bacterial cells, for example, and their arrangement, while the binding properties of *differential stains* react with specific structures, helping us to distinguish between bacterial types. Probably the most widely used bacterial stain is the *Gram stain* (see Box 1.2), which for more than 100 years has been an invaluable first step in the identification of unknown bacteria.

The Gram stain is a differential stain, which only takes a few minutes to carry out, and which enables us to place a bacterial specimen into one of two groups – Gram-positive or Gram-negative. The reason for this differential reaction to the stain was not understood for many years, but is now seen to be a reflection of differences in cell wall structure, discussed in more detail in Chapter 3.

Box 1.2 The Gram stain

The Gram stain involves the sequential use of two stains. The critical stage is step 3; some cells will resist the alcohol treatment and retain the crystal violet, while others become decolourised. The counterstain (safranin or neutral red) is weaker than the crystal violet, and will only be apparent in those cells that have been decolourised.



Specialised forms of microscopy have been developed to allow the viewer to discern detail in living, unstained specimens; these include *phase-contrast* and *dark-field* microscopy. We can also gain an estimate of the number of microorganisms in a sample by directly counting them under the microscope. This is discussed along with other enumeration methods in Chapter 5.

Phase-contrast microscopy exploits differences in thickness and refractive index of transparent objects such as living cells to give improved contrast.

Dark-field microscopy employs a modified condenser. It works by blocking out direct light, and viewing the object only by the light it diffracts.

1.5 Electron microscopy

From the equation shown in the previous section, you can see that if it were possible to use a shorter wavelength of light, we could improve the resolving power of a microscope. However, because we are limited by the wavelength of light visible to the human eye we are not able to do this with the light microscope. The *electron microscope*, however, is able to achieve greater magnification and resolution because it uses a high-voltage beam of electrons, whose wavelength is very much shorter than that of visible light. Consequently we are able to resolve points that are much closer together than is possible even with the very best light microscope. The resolving power of an electron microscope may be as low as 1–2 nm, enabling us to see viruses, for example, or the internal structure of cells in considerable detail. The greatly improved resolution means that specimens can be meaningfully magnified over 100 000 × .

Electron microscopes, which were first developed in the 1930s and 40s, use ring-shaped electromagnets as 'lenses' to focus the beam of electrons onto the specimen. Because the electrons would collide with, and be deflected by, molecules in the air, electron microscopes require a pump to maintain a vacuum in the column of the instrument. There are two principal types of electron microscope: the *transmission electron microscope* (TEM) and the *scanning electron microscope* (SEM).

Figure 1.6 shows the main features of a TEM. As the name suggests, in TEM, the electron beam passes *through* the specimen and is scattered according to the density of the different parts. Due to the limited penetrating power of the electrons, extremely thin sections (<100 nm, or less than one-tenth of the diameter of a bacterial cell) must be cut, using a diamond knife. To allow this, the specimen must be fixed and dehydrated, a process that can introduce shrinkage and distortion to its structure if not correctly performed.

After being magnified by an objective 'lens', an image of the specimen is projected onto a fluorescent screen or photographic plate. Denser areas, which scatter the beam, appear dark, and those which allow it to pass through are light. It is often necessary to enhance contrast artificially, by means of

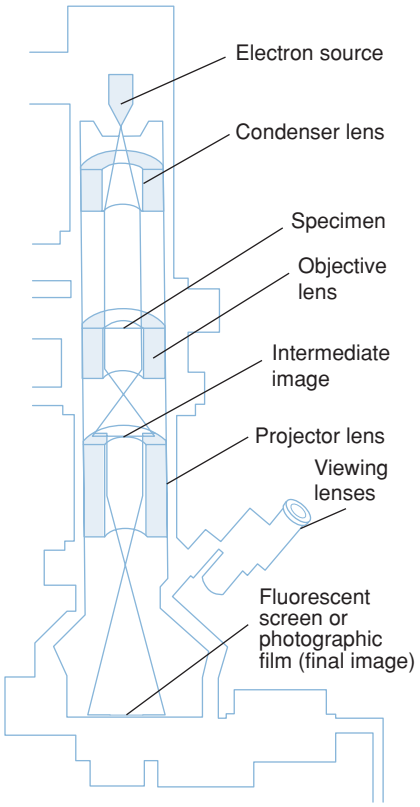


Figure 1.6 The transmission electron microscope (TEM). Electrons from a tungsten filament pass through a vacuum chamber and are focused by powerful electromagnets. Passage through the specimen causes a scattering of the electrons to form an image that is captured on a fluorescent screen. Reproduced from Black, JG (1999) Microbiology: Principles and Explorations, 4th edn, with permission from John Wiley & Sons.

'staining' techniques that involve coating the specimen with a thin layer of a compound containing a heavy metal, such as osmium or palladium. It will be evident from the foregoing description of sample preparation and use of a vacuum that electron microscopy cannot be used to study living specimens.

The TEM has been invaluable in advancing our knowledge of the fine structure of cells, microbial or otherwise. The resulting image is, however, a flat, two-dimensional one, and of limited use if we wish to learn about the surface of a cell or a virus. For this, we turn to SEM. The scanning electron microscope was developed in the 1960s and provides vivid, sometimes startling, three-dimensional images of surface structure. Samples are dehydrated and coated with gold to give a layer a few nanometres thick. A fine beam of electrons probes back and forth across the surface of the specimen

and causes secondary electrons to be given off. The number of these, and the angle at which they are emitted, depends on the topography of the specimen's surface. SEM does not have quite the resolving power of the TEM, and therefore does not operate at such high magnifications.

Between them, SEM and TEM have opened up a whole new world to microbiologists, allowing us to put advances in our knowledge of microbial biochemistry and genetics into a structural context.