2

Light

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Key reading

Further Reading

References

Internet Resources

Understanding Light Microscopy, First Edition. Jeremy Sanderson.

2.1 Introduction

Having emphasised in Chapter 1 that all images are seen by the eye, this chapter considers the start of the imaging process: light. We shall discuss what light is and how it interacts with matter. We shall consider how the velocity of light is altered by materials denser than air, and how rays of light are bent or refracted.

Light is so central to our perception that for much of the time we are unaware of it and take it for granted, except perhaps when viewing a gorgeous sunset or glimpsing crepuscular rays behind clouds. Although light surrounds us and is essential for life, it is anything but ordinary. As one popular science writer has put it: "Light, the universal metaphor for understanding and revelation, is astonishingly opaque" (Brooks, 2012). The Greeks were aware of the importance of light, referring to dying as 'losing the light'. Yet it is only in the last 200 years that man has begun to understand light's unique properties. Indeed, Benjamin Franklin remarked in a letter discussing science to a colleague, written in April 1752 '… I must own that I am much in the *Dark* about *Light*…'

2.2 The Nature of Light

Ancient and medieval scholars made some progress in understanding the nature of light, being aware that it travelled in straight lines (we call this the rectilinear propagation of light; it is the basis of geometrical optics). For our purposes it is convenient to start in the mid-seventeenth century with Robert Hooke, Christiaan Huygens and Isaac Newton. Around 1665 Newton admitted a ray of light though a prism **(Figure 2-1)** and explained the decomosition of white light into spectral colours by refraction. Although Newton investigated some aspects of the wave nature of light, such as the formation of Newton's rings (for an explanation of this phenomenon see Chapter 10, section **xxx**, page **xxx**), his writings emphasised the particulate ('corpuscular') model. Both Robert Hooke and Christiaan Huygens, contemporaries of Newton, came to very different conclusions about the nature of light. Huygens was convinced that regarding light as a series of wave-fronts better explained refraction, but he was unable to extend this to explain the phenomenon of diffraction (Chapter 10, page **xxx**). The corpuscular model failed to explain refraction satisfacorily, but because of Newton's stature, his view was to dominate science until the mid-nineteenth century. Not only that, Newton's disagreement with both Huygens and Hook led to permanent anatagonism[1].

It was the work of the polymath Thomas Young from 1803 onwards, who demonstrated the effects of the interference of coherent light rays, that truly marked the beginning of man's significant understanding of the nature of light. Building upon Young's investigation of diffraction, and also on the earlier work of Christiaan Huygens, Augustin-Jean Fresnel showed that light propagated entirely as a transverse wave, with no longitudinal vibration whatsoever. He also explained both the rectilinear propagation of light and diffraction effects. Later in the mid-19th century, from the data of simple electrical experiments, James Clerk Maxwell formulated his equations to describe electromagnetic waves that could travel at approximately the known speed of light. Maxwell considered this fact more than a coincidence. In a key paper published in 1865 he wrote:

> 'The … results seem to show that light and magnetism are affections of the same substance, and that light is an electromagnetic disturbance propagated … according to electromagnetic laws'.

Towards the end of the nineteenth century, in a successful attempt to prove Maxwell's equations, Heinrich Hertz demonstrated the existence of radio waves. He showed that these belonged to the electromagnetic spectrum, of which light was part.

2.3 The Nature of Waves

Energy is carried from one place to another by two means. Either it is transferred directly by the movement of matter, or is carried by a wave. In the former case, two boats may ram one another, causing a transport, or displacement, of mass. Conversely, a boat may be rocked by an approaching wave. It will bob up and down (or oscillate) in unison with that wave, but not necessarily be displaced. Waves can therefore transfer energy *without* transferring mass. We can also use waves to transmit information.

Mechanical waves are formed when there is a disturbance in a medium from a resting state. Wave motions have the property of periodicity: the disturbance repeats regularly in space and time. This is shown in **Figure 2-2**. For example, sound waves exist in gases or liquids as longitudinal mechanical pressure waves, also called compression waves. This means that the particles of supporting medium (air or liquid) travel back and forth, as successive compression and rarefaction towards and away from the source, *along* the axis of propagation. The motion of the wave can be different from the motion of the medium on which the wave moves. Thus, a wave pulse moves through a medium, while the carrier medium itself does not travel.

In a longitudinal wave (e.g. a mechanical sound wave), the medium vibrates in the same direction as the propagation of the wave **(Figure 2-3)**. Where the motion of the medium is perpendicular to the motion of the wave, is called a transverse wave (e.g. an electromagnetic wave). When travelling through solids, sound waves can exist as both longitudinal and transverse waves. Polarisation effects (Chapter 13, page **xxx**) are associated with transverse waves, but not with longitudinal ones.

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[1] It is thought by some that Newton's famous remark, in a letter to Hooke dated 5th February 1675 damning him with faint praise, in which he concludes:'*If I have seen further it is by standing on ye sholders of Giants*' is a jibe to Hooke and Huygens who were both of short stature.

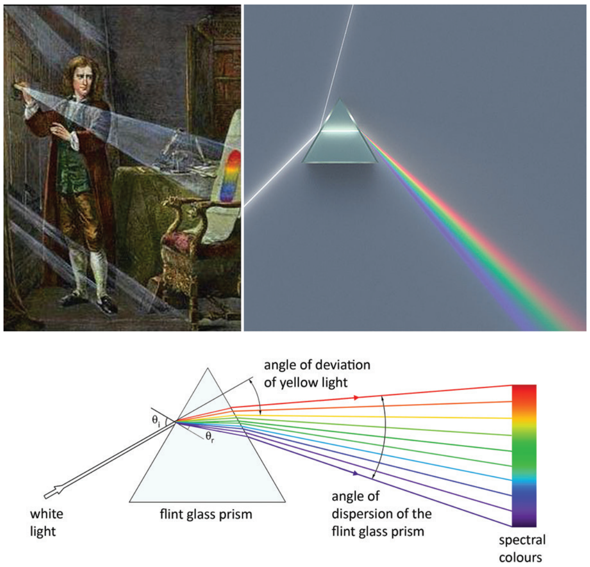

Figure 2.1 **Newton's prism experiment.** *About 1665 Isaac Newton decomposed sunlight into visible spectral colours. An animated gif can be found on Wikipedia (see the Internet Resources listed at the end of the chapter). By using a lens to recombine spectral colours into white light, and also by passing isolated single colours through a second prism (his experimentum crucis), Newton proved that a prism does not 'add' colour to white light per se, but that dispersion is a result of refraction.*

Some books show a second prism recombining the spectrum with a gap between the two prisms, but this is incorrect. Using three prisms, this can be done, but two prisms can only recombine spectral colours to form white light if the second is inverted, and placed very close to the first so that the colours come to a focus and recombine, to form white light, at a particular distance.

Table 2.1 *Refractive indexes and dispersion of various optical glasses, compared to water and diamond*

	Refractive index at:						
Glass type/material	430.8 nm (G) indigo	486.1 nm (F) blue	589.3 nm (D) yellow	656.3 nm (C) red	686.7 nm (B) red	Dispersion	Abbe No. (V)
Lanthanum dense Flint LaSF9	1.887	**1.869**	1.850	1.843	1.839	0.048	32.7
Dense Flint SF10	1.764	**1.746**	1.728	1.721	1.718	0.046	29.1
Flint F2	1.643	**1.632**	1.618	1.614	1.613	0.030	34.3
Barium Crown BaK4	1.582	**1.576**	1.569	1.566	1.565	0.017	56.9
Borosilicate Crown BK7	1.527	1.522	1.517	1.514	**1.513**	0.010	64.6
Fluorite Crown FK51A	1.494	1.491	1.487	1.485	**1.484**	0.010	81.2
Water	1.338	**1.336**	1.333	1.331	1.331	0.007	66.6
Diamond	2.452	**2.436**	2.417	2.410	2.407	0.044	54.5

Because of their well-defined wavelengths, Fraunhofer lines are often used to characterise the refractive index and dispersion of optical materials. The coefficient of dispersion, or constringence, of a transparent material is the difference between the refractive indexes of the G and B Fraunhofer lines (see Box 6). The Abbe number (Vd) is a commonly-used measure of dispersion. The Abbe number = $(n^D - 1)/ n^F - n^C$, where n^F, n^C and n^D are the refractive indices for the Fraunhofer F, C and D lines (Fraunhofer labelled his lines starting with A in the far red; major lines have upper case letters and minor ones, lower case).

Electromagnetic waves (including light) exist wholly as transverse waves, in which the particles travel up and down, in a plane of vibration perpendicular to the direction of propagation **(Figure 2-4)**. Both water and light waves are examples of transverse waves. When you drop a stone into a pond, the waves propagate radially away from the point of origin, but the actual water droplets travel up in crests and down in troughs while the wave propagates along the surface of the water. A wave has seven characteristics (some of these are illustrated in **Figure 2-5)**:

(a) Phase – two points moving with the same velocity, which also have the same displacement from the undisturbed resting state, are said to be in phase. The phase, or relative position of a point on the wave, can be expressed as an angle. One cycle or wavelength corresponds to 2π radians or 360°. A phase difference occurs when one wave lags behind, or precedes another, in time and space; this difference is often denoted by the Greek letter ϕ.

(b) Wavelength – the shortest distance between two points that are moving in phase. This is often depicted as the distance between two adjacent crests. It is denoted by the Greek letter lambda (λ), with units in nanometers (nm).

(c) Wavenumber – is the reciprocal of the wavelength (i.e. 1/λ), representing the number of wavelengths per unit length. It is useful in spectroscopy, with units of cm^{-1}. The angular wavenumber, k, is equivalent to 2π/λ.

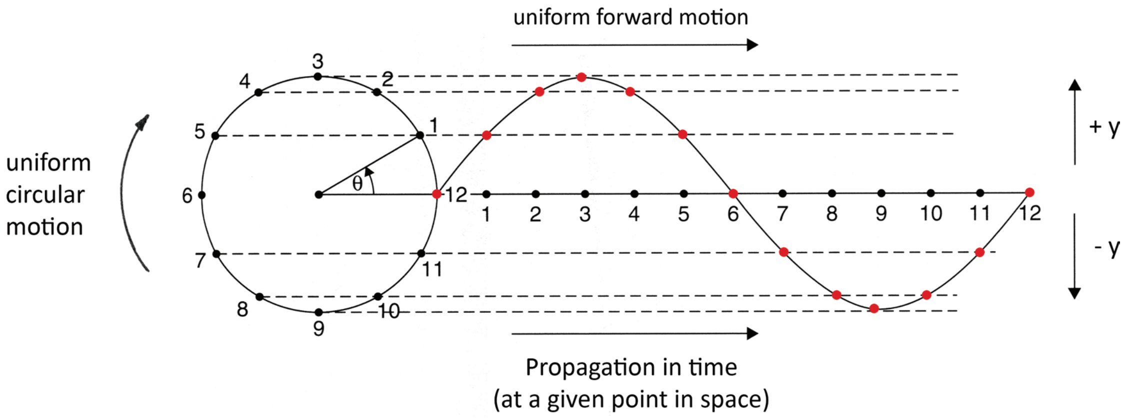

Figure 2.2 **Characteristics of a wave.** *A wave repeats regularly in space and time. A wave may be regarded as the ray trace of a combination of uniform forward motion (shown going from left to right) and uniform circular motion (shown going clockwise). The 'up-and-down' component of a point on the circle moving forward is called simple harmonic motion, the projection of uniform circular motion onto one axis. The resultant (red dots) of a point propagating along a wave vibrating in the plane of the paper is represented as a sine function. The wavelength is given by λ, and the amplitude, A, varies from + y to −y.*

(d) The frequency of the wave (denoted by f or the Greek letter ν) is the number of waves (e.g. measured at their crests) that pass a fixed point every second. A wave whose crests pass a fixed point every second (i.e. one cycle per second) has a frequency of one Hertz (Hz), whose unit is reciprocal seconds (s^{-1}).

(e) The time period of a wave (denoted by T or τ) is the time taken to complete one full cycle, and is the reciprocal of the frequency; its unit is the second.

(f) The velocity (denoted by v; not to be confused with ν for frequency) of the wave is the speed of the wave in a given direction. Velocity gives both the speed *and* direction of propagation. It is the product of frequency and wavelength: $v = f \times \lambda$ (or $v = \nu\lambda$), and has units of meters/second.

(g) Amplitude is the height, or maximum displacement, of the wave crest above the undisturbed position. It is not the distance between the top and bottom of a wave cycle.

Since sound travels as a mechanical wave through a medium, a vacuum will halt the propagation of the sound wave. Electromagnetic waves (e.g. radio waves, microwaves and light), however, do not require the support of a medium, and so are capable of travelling through a vacuum. The self- supporting nature of electromagnetic radiation means that the measured speed of the wave is constant regardless of whether the velocity of the emitter, or detector used to measure it, changes. The frequency of light is related to its speed and wavelength as follows: $\nu = c/\lambda$. When light passes into, or through, a transparent medium the speed is reduced but the frequency remains constant, and so the wavelength changes.

Electromagnetic waves, such as light, are self-propagating, and unlike other wave phenomena (e.g. transverse water waves) is *not* a disturbance of a medium. For many years, light was considered to move through an invisible substance called the 'luminiferous æther'. This was finally proved not to exist by the negative outcome of the Michelson–Morley experiment.

2.4 Wave-Particle Duality

By the end of the nineteenth century, Maxwell's theoretical model of light as continuously oscillating electric and magnetic fields seemed complete. However, several experimental observations (principally black-body radiation and the photoelectric effect) could not be wholly explained by Maxwell's wave model of light. We need only understand the fundamental details of how the

Table 2-2 *Wave-particle duality: explaining the behavior of light*

Phenomenon	Explained in terms of waves	Explained in terms of particles
Reflection	Yes	Yes
Refraction	Yes	Yes
Interference	Yes	No
Diffraction	Yes	No
Polarisation	Yes	No
Photoelectric effect	No	Yes

wave-particle duality of light arose. For those interested, a more detailed explanation can be found in chapter 4 of *Light* (1989) by Michael Sobel, listed under Further Reading.

Max Planck was a very conservative scientist, working in thermodynamics, trying to explain the ‘irregular’ behavior of radiation emitted by an incandescent black-body. It was with reluctance that he proposed the hypothesis that energy is radiated and absorbed as discrete quanta rather than as a continuous stream of energy (see **Figure 2-6** and **Box 2-1**). Planck cautiously insisted that quantum energy transfer was merely an aspect of the itself. Einstein is best known for developing the general theory of relativity, but it is the first of four papers that he published in 1905 on the photoelectric effect, and for which he was later awarded the Nobel Prize, that is important for our purposes. The only reason that the energy flow appears continuous is because the quanta are so small in magnitude.

2.5 Important Properties of Light

Throughout this book we will refer to different properties of light (**Figure 2-8**), which are important for light microscopy:

- **Monochromatic** - waves having the same wavelength (λ) or vibrational frequency (ν). They are therefore perceived to be of the same colour.
- **Coherent** – waves (of a stated wavelength) that maintain the same phase relationship whilst travelling through space and time.
- **Collimated** – waves whose paths of propagation are parallel without convergence or divergence, but not necessarily with the same wavelength, coherence or polarisation.
- **Polarised** – waves whose E vectors vibrate in planes that are parallel to one another. Such polarisation may be linear, circular or elliptical.

Laser light possesses all these qualities: it is coherent, monochromatic, polarised and collimated. As such it is a powerful illumination source in confocal microscopy.

In studying geometrical optics is a helpful to trace the path of a light wave with a single line trace perpendicular to the wave-front. This is called the ray approximation method, and a ray is an idealised narrow beam of light. For this reason, geometrical optics is sometimes called ray optics. Interfaces between materials of different refractive indexes.

When the object is very large compared to the wavelength of light, such as when we use a camera or a single-lens magnifying glass, we can consider light as rays, and use geometric optics (see Chapter 3) to understand how the image is formed. How light forms an image by diffraction is explained in Chapter 10, and the special case of polarised light in Chapters 13 and 14.

2.6 Interaction of Light with Matter

Light interacts with the microscopical specimen in one of six ways:

- Light may pass straight through matter and be partially absorbed. This is how coloured stains work.
- Light may be reflected or refracted by matter.
- Light may be scattered or diffracted (i.e. a regular form of scattering) by matter.
- The light wave may undergo a phase change as a result of passing through matter. A phase-shift may occur in the path of one wave with respect to another.
- Light may be polarised by passing through, or reflecting off, matter.
- Light may excite matter to cause the emission of fluorescence.

To form an image light is diffracted by the matter of the specimen. Part of this diffracted light is then refracted by the objective lens and recombined to form the (primary) image. The controlled manipulation of the interaction of light with matter is the basis of contrast formation in the image. This is described in Chapter 11.

2.9 Refraction of Light

We now consider the propagation of light from a rarer (e.g. air) isotropic medium to a denser isotropic medium (e.g. water or glass). Light incident upon a transparent surface is partially reflected at the surface, but is mostly transmitted. The key physical process which gives rise to the bending, or refraction[2], of the light beam is the slowing down of the light as it enters the denser medium.

For all practical purposes the speed of light in a vacuum (299,792,458 m/s) and in air may be considered equivalent. The speed of light in dry air at a standard temperature of 0 °C and pressure of 1 atmosphere is *very slightly* less than in a vacuum: it is 2.99704764 x 10^8 m/s (RI = 1.0002926).

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[2] The term refraction comes from the Latin *refringere*, meaning to break away. Whilst he used the term ‘refraction’, Newton also used the root word directly, referring to ‘refrangible’ rays.

Box 2.1 Wave-particle duality: Planck, Einstein & de Broglie

According to Planck, each energy element, *E*, is proportional to its frequency, ν:

$$E = h\nu \qquad \text{also} \quad E = hc/\lambda \qquad \text{thus } \lambda = hc/E$$

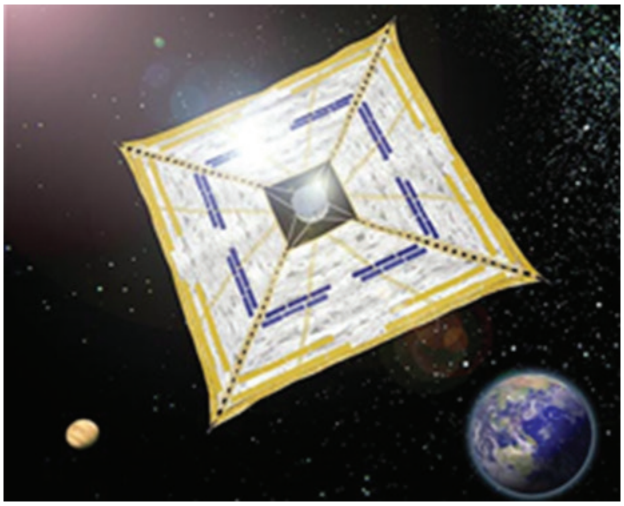
where h is Plank's constant: 6.626×10^{-34} Joule.sec, λ is the wavelength, and c is the velocity of light in a vacuum: 2.998×10^{8} m/s.

Planck's constant can be expressed in several different units. It is 6.626×10^{-27} erg.sec, or 4.136×10^{-15} eV.s. The joule-second is a unit equal to a joule multiplied by a second, used to measure action (as defined in physics) and therefore has the dimensions of energy integrated over time. The joule ($kg.m^2/s$) is the SI unit, the erg the unit of the CGS system. The introduction of Planck's constant in the equations above means that a specific wavelength has a discrete quantum of energy associated with it. For a photon of light to be absorbed or emitted, its energy must exactly match the difference in energy between two energy levels in an atom (or molecule, see Figure 6). The fact that Planck's constant is vanishingly small reflects the fact that everyday objects are made of a *large* number of particles.

Einstein showed that an X-ray photon carries much more energy than a lower frequency, longer wavelength, photon of visible light. Also violet light ($\approx 8 \times 10^{14}$ Hz; 800 THz or $\approx$ 3 eV) has almost twice the energy of a photon of red light ($\approx 4 \times 10^{14}$ Hz; 400 THz or 1.5 eV) where 1eV = 1.602×10^{-19}J. We are all continuously bathed in longer wavelength, low energy, radio waves without ill effect, but even short exposure to short wavelength, high energy, X-rays or γ-rays causes us considerable harm. Thus green light at 550 nm wavelength (from the equations above) has a frequency (ν) of c/λ = 545 THz and an energy of 3.61×10^{-19} Joules, or 0.361 attoJoules.

While Einstein showed that electromagnetic waves are particulate, de Broglie, the French theoretical physicist, turned this concept upon its head. He proposed that since waves could have particle-like properties, then also matter could have wave-like properties: that streams of particles can behave like waves. Thus (as well as a photon in light microscopy) an electron can also act as a wave, with its wavelength given as:

$$\lambda = h/p \qquad (\text{as a particle}) \qquad \text{also} \quad \lambda = h/mv \quad (\text{as a wave})$$



Hence, the momentum, p, of an electron moving with velocity ν equals mv (m is the mass of the electron (9.11×10^{-28} g at rest, or zero velocity). This formula of de Broglie is useful in electron microscopy where electron, rather than photon, waves are used for increased resolving power. Thus p = mv, and by analogy with the equation above, mv = h/λ *and* $\lambda = h/mv$, so $p = h/\lambda$.

We know intuitively that waves exert pressure from observing the power of waves at sea. From Einstein's famous equation, $E = mc^2$, we know that energy and matter are interconvertible. Photons exhibit wave-particle duality - they have the properties of both waves and particles. This means that photons have momentum and can exert a force, albeit very small. For a photon with wavelength λ or energy *E*, this is $h/\lambda c$ or E/c^2.

$E = hc/\lambda$ relates, through the constant *h*, a particle like property (*E*) to a wave-like property (λ or ν). Planck's equation may be combined with Einstein's equation, $E = mc^2$, i.e. $E = hc/\lambda$, hence $mc = h/\lambda$. Now, mc (mass x velocity) represents a momentum, *p*. In short photons carry a momentum and when a beam of light is absorbed by, or reflected from, an opaque object, it exerts a pressure called the *radiation pressure* of light – a phenomenon first predicted by Maxwell.

Thus all moving particles – not just photon or electrons – have wave-like properties, including pieces of chalk that (used to be) thrown at dozing or talkative students in lectures but, as a simple calculation will show, the corresponding wavelengths are very small. If a piece of chalk 20 grammes is thrown at a speed of 50 km/hr at a student, then the wavelength will be:

$\lambda = 4.13 \times 10^{-15}/20 \times 50 = 4 \times 10^{-18}$ m. This is well beyond the wavelength of gamma rays.

Equally, the radiation pressure for a photon will be: $p = h/\lambda$ or 6.63×10^{-34} J.sec/500 $\times 10^{-9}$ m = 1.33×10^{-27} newtons.

The refractive index (denoted by the letter 'n' or the Greek η) is simply defined as:

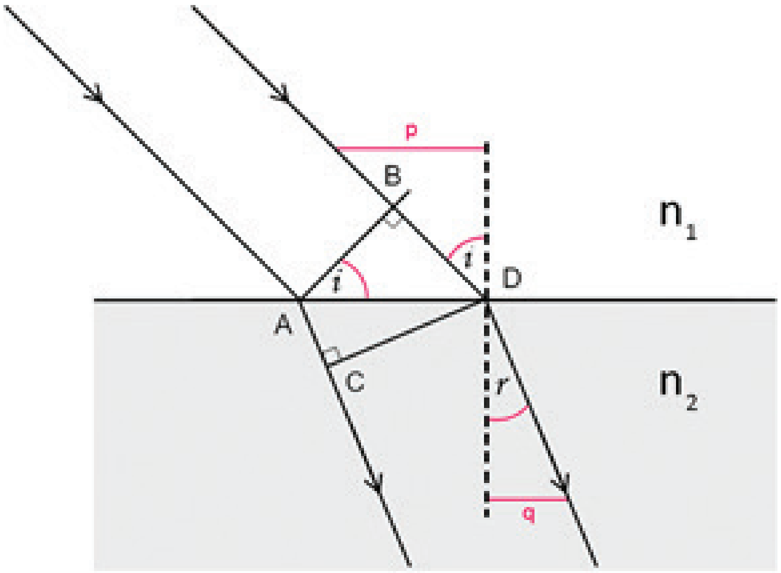
$$n = \frac{\text{speed of light in vacuum (or air)}}{\text{speed of light in the medium}} = \frac{c}{v}$$

As a rule of thumb, materials which have higher density have larger refractive indexes. Thus, when a ray of light passes into a transparent, denser, medium, then the ray of light bends towards the normal of the interface, and the angle of refraction, r, is less than the angle of incidence, i (**Figures 2-11 a-c**). Two points are important here:

- The incident and refracted rays are on opposite sides of the normal at the point of incidence, and all three are in the same plane (i.e. coplanar).
- The law of refraction (Snell's law) states that when light passes from one medium to another, the sine of the angle of incidence bears a constant ratio to the sine of the angle of incidence..

Box 2.2 Snell's law and the mathematics of refraction

$$\text{Because } n = \frac{\text{speed of light in vacuum (or air)}}{\text{speed of light in the medium}} = \frac{c}{v} \tag{1}$$



The refractive index, n, of a vacuum is 1 because, in this case, c = v. Now, since $c = \lambda\nu$ (or λf) and $v = \lambda_m \nu$ (where λ_m is the wavelength in the medium) and since the frequency (ν or f) of the wave in a transparent medium is unchanged, we have:

$$n = c / v \text{ and } n = \lambda / \lambda_m \tag{2}$$

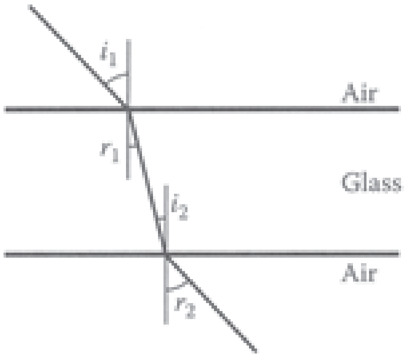
The relationship between the refractive index *n* and the refraction of a light beam passing between a rarer (n_1, e.g. air) and denser medium (n_2, e.g. glass or water) is usually expressed:

$$\sin i / \sin r = v_1 / v_2 = n_2 / n_1 = (n_2).(\sin_2) = (n_1).(\sin_1) = n_r \tag{3}$$

and

$$(n_1).(\sin i) \quad = (n_2).(\sin r) = n_r, \text{ or just n} \tag{4}$$

where nr is the relative refractive index.



$r_1 = i_2$ and so $i_1 = r_2$

The relationship described by equation (4) is called **Snell's Law**. It relates the angle of incidence to the angle of refraction for wave propagation at the boundary between transparent isotropic media. The ratio of the sines of the angles of incidence and refraction are constant for any pair of media. The proof of Snell's Law can be seen from the geometric construction in the figure, above. Here, p/q = sin i/sin r. Where the first medium, n_1, is air (RI = 1), then sin i/sin r = n_2. For a parallel-sided glass plate (e.g. a microscope slide), the ray passing through the glass is refracted in an equal and opposite manner, thus passing through, effectively without deviation.

A ray in air is incident upon a water surface at an angle of 30°. The emergent refracted ray is measured at 22° to the normal. What is the refractive index of water? From Snell's Law in (4), $\sin 30^\circ = n_{water} . \sin 22^\circ$. Therefore $n_{water} = 0.5/\sin 22^\circ = 0.5/0.375 = 1.333$.

Conversely, the angle of the refracted ray can be calculated knowing the refractive index. For glass of RI = 1.5172, $\theta_{glass} = \arcsin [n_{air} . \sin\theta_{air}/n_{glass}] = \arcsin [1.00 \times 0.5/1.5172] = 19.24^\circ$.

We now consider the refraction and reflection of light when the incident ray is within the denser material (e.g. water or glass, so $n_1 > n_2$). At the critical of incidence, where sin i = θ_c, the angle of refraction is 90° to the normal, and thus sin r = 1. Therefore $n_2/n_1 = \sin \theta_c$ and $\theta_c = \arcsin (n_2/n_1)$. The critical angle for any medium can easily be calculated, because the sine of the critical angle is the inverse of the refractive index: $\sin \theta_c = 1/\text{RI}$. For glass, taking $n = 1.5172$, the critical angle is given by: $\sin \theta_c = 1/1.5172 = 0.6591$, and $\theta_c = 41.231^\circ$. Other values are given in Table 2-3.

Thus, when light travelling through a medium of higher refractive index n_1 into a medium with lower refractive index, n_2 (so $n_1 > n_2$), where $\sin i.(n_1)/ n_2 > 1$, and no value of θr fulfills Snell's Law, the light is reflected back into the denser medium, and is totally internally reflected. It does not pass into the medium of lesser refractive index.

2.10 Dispersion

Although the frequency, ν, or the wavelength, λ, of light does not change as it travels from one medium to another, the refractive index of a material will vary according to the wavelength of light passing through it. Thus 'blue bends best' and will have a slightly higher refractive index (e.g. for borosilicate crown glass $RI_{blue}=1.522$ compared to $RI_{red}=1.513$) than red (**Figure 2-18**), since blue light is slowed down more by the glass. This phenomenon is called *dispersion*, and is best known from Newton's experiments[3] with a prism to split white light into its component spectral colours. Dispersion is the rate of variation of refractive index with wavelength. Its effect is manifested as chromatic aberration.

Scientists usually measure the coefficient of dispersion between the Fraunhofer C and F spectral lines. These lines have values of 486.1 nm and 656.3 nm respectively (see **Table 2-4**). The values of the C and F lines lie closer to what our eyes can distinguish than the B and G lines (see **Box 2-4**), which are otherwise used to measure dispersion, but lie in the deep red and indigo part of the spectrum, and so may be hard to see for most humans.

We noted at the beginning of this chapter that waves can be used to carry information. Electromagnetic waves are particularly good for this, as the use of radio (mobile phones), microwaves (satellites) and light (fibre optics) attest. Modern materials are now used in fibre optic cables to with minimal dispersion. However, dispersion is not all bad: high dispersion is valued in gemstones, giving diamond[4] its characteristic 'fire', or flashes of colour.

2.11 Refraction and the Action of Lenses

In its simplest form a lens can be regarded as a continuous series of prisms (**Figure 2-19**), aberration in objectives and other optical components, as discussed in Chapter 6, section **xxx** on page **xxx**. A graph showing the dispersion curve for borosilicate glass is shown in **Figure 2-18a**.

2.12 Chapter Summary

At the end of this chapter you should understand the wave-particle dual nature of light and how waves propagate, carrying photons, or 'wave-packets', of energy. The ray approximation of light is useful for studying geometrical optics and refraction, while consideration of light as a wave is essential for explaining the effects of diffraction. Lenses refract light, and so form images.

1. Newton investigated the dispersion of light, and some wave-like characteristics, but emphasized the corpuscular nature of light. This view dominated for about 100 years.
2. The work of Huygens, Young, Fresnel, Maxwell and Hertz established the wave theory of light during the 19th century. Einstein described light as also being composed of discrete quanta.
3. Energy is transferred directly between, or is carried by, a wave. Waves can therefore transfer energy *without* transferring mass.
4. Electromagnetic waves are transverse waves, with a velocity of 3×10^8 m/s *in vacuo*. The wave-front is perpendicular to the direction of propagation.

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[3] See the Wikipedia animation given in the list of Internet Resources at the end of the chapter.

[4] Usually RI values are given with any reference to wavelength, but since RI values are wavelength dependent, for the refractive index value to have any meaning a particular wavelength should be cited.meaning to break away. Whilst he used the term 'refraction', Newton also used the root word directly, referring to 'refrangible' rays.

5. Light possesses no actual colour; the division of the visible spectrum into colours is arbitrary.
6. Laser light is coherent, monochromatic, polarised and collimated.
7. When the object is very large compared to the wavelength of light, we can consider light as rays, and use geometric optics to understand how the image is formed.
8. When the object is very small, about the same size as the wavelength of light, diffraction dominates, and we must consider light as waves.
9. Light interacts with the microscopical specimen in one of six ways:
    - By absorption
    - Through reflection or refraction
    - By scattering or diffraction
    - Through a change in phase
    - By polarisation while being absorbed or reflected
    - Through excitation to re-emit fluorescent light
10. A ray can be specularly reflected off a polished, shiny, surface and diffusely reflected off a non-homogeneous, rough, surface.
11. A ray of light slows down as it enters a denser transparent medium. This causes refraction, of the light towards the normal with the interface of the two states of matter $(\theta_i > \theta_r)$.
12. A ray of light incident upon a surface at 90° (i.e. normal to the surface) will pass straight through without deviation, although it will be slowed down.
13. When passing from a denser to a lighter medium, a ray of light not normal to the surface (or interface) of the two media will be refracted away from the normal $(\theta_i < \theta_r)$.

Key Reading

Brooks, M (2012) What is light? Chapter 15, pages 201 – 213 in: *Can we travel through time? The 20 Big questions of Physics* Quercus Publishing, London ISBN = 978-1-78087-589-7

- A short and very readable summary of the duality of light, the photo-electric effect and the importance of lasers.

Johnsen, S (2012) *The Optics of Life: a Biologist's Guide to Light in Nature* Princeton University Press, Princeton & Oxford ISBN = 978-0-691-13991-3

- A 300-page text explaining optics for biologists in non-mathematical fashion. A unified and sufficiently-detailed account of the emission of light, absorption, scattering and interference is given together with chapters explaining fluorescence and polarisation. A further two chapters explain simply the units employed for measuring light and how to measure light flux. The final chapter introduces the concept of quantum mechanics.

Reference

Silfvast, WT (2005) *Lasers*, Module 5/10 in: SPIE Fundamentals of Photonics series (eds) Guenther, A; Pedrotti, LS & Roychoudhuri, C. URL: https://goo.gl/G1bg0p (series) and https://goo.gl/Iq1zg1 (*Lasers*)

Further Reading

Falk, D; Brill, D & Stork, D (1986) *Seeing the Light* John Wiley & Sons, New York ISBN = 978-0-471-60385-6
Nature Milestones, Photons URL: http://goo.gl/z1klWe and: http://goo.gl/C7EHYC
Saxby, G (2002) *The Science of Imaging: an introduction* 2nd Edition. CRC Press, Boca Raton ISBN = 978-1-4398-1286-0
Sobel, MI (1989) *Light* The University of Chicago Press, Chicago & London ISBN = 0-226-76751-5
Bahaa, EAS & Teich, MC (2007) *Fundamentals of Photonics* 2nd Edn, Wiley, New York ISBN = 978-0-471-35832-9

Internet Resources

1. Dispersion of light through through a prism, animated gif: http://goo.gl/nPbUQm
2. Dispersion factsheet (Bellingham & Stanley): http://goo.gl/eoeV5G
3. Demonstration of Newton's *Experimentum Crucis* by Dr Jonathan Hare (M4V file): http://goo.gl/VgV2rV
4. The Institute of Physics teaching webpages: http://goo.gl/ixpWtg
5. Simple harmonic motion describing a wave: http://goo.gl/VXQxN0
6. Refractive Index database: http://refractiveindex.info
7. Proof of Snell's Law using Fermat's principle: http://goo.gl/mvEBRU
8. Lasers: (Phoenix Laser Safety LLC): http://goo.gl/SxBzel

Notes

1. It is thought by some that Newton's famous remark, in a letter to Hooke dated 5th February 1675 damning him with faint praise, in which he concludes:'*If I have seen further it is by standing on ye sholders of Giants*' is a jibe to Hooke and Huygens who were both of short stature.
2. All objects above absolute zero emit electromagnetic radiation. A spectroscope is chiefly used by astronomers and chemists to determine the nature of materials. When heated to incandescence many materials emit light that is a characteristic signature of the elements that make up their atomic structure. The absorption spectra of materials can also be studied. Joseph Fraunhofer developed the spectroscope to test the refractive power of optical glasses and lenses. A spectroscope disperses light into a very wide spectrum of colors using a prism or (nowadays) a diffraction grating. As such, it is another instrument – apart from the microscope – used to study the interaction of light with matter.
3. The wavelength of light emitted can be determined by the formula $\lambda = hc/\Delta E$, where ΔE equals the discrete transitional energy difference between the excited and ground state electron orbitals in Joules (also see Box 2-1).
4. A wave-packet, a term coined by Einstein in order to express the idea that light travels with discrete 'packets' of Energy, E, may generally be (but not wholly) identified with a photon. The term photon was widely adopted from the late 1920s.
5. Since 1983 the metre has been defined by international agreement as the length of the path travelled by light in a vacuum during a time interval of 1/299,792,458 of a second. This makes the speed of light exactly 299,792.458 km/s.
6. X-rays and γ-rays should, strictly, be called X-*waves* and γ -*waves*.
7. The term normal comes from the Latin *norma*, describing a carpenter's try square
8. The term refraction comes from the Latin *refringere*, meaning to break away. Whilst he used the term 'refraction', Newton also used the root word directly, referring to 'refrangible' rays.
9. The proof is given in optics textbooks; also see the URL links under Internet Resources. However, the significance of Fermat's principle may be better understood by a 'real life' analogy. of deviation from a straight line being a measure of the difference in speed of walking across the sand and rocks, i.e. the refractive index, air to glass.
10. See the Wikipedia animation given in the list of Internet Resources at the end of the chapter.
11. Usually RI values are given with any reference to wavelength, but since RI values are wavelength dependent, for the refractive index value to have any meaning a particular wavelength should be cited.

3

Basic Microscope Optics

3.1 Introduction

We have seen in Chapter 1 how light waves radiate from a point source, and in Chapter 2 how a lens can be regarded as a continuous series of prisms which will refract light and bring it to a focus. We have also assumed, whilst discussing the refraction of light, that it travels in straight lines, a phenomenon known as the rectilinear propagation of light. These phenomena are illustrated in Figure 3-1. Later, in Chapter 10 we shall see how light is 'bent' by diffraction, and indeed how image formation (at any scale) depends fundamentally upon diffraction by the object and the interference of light. However, where the size of the object is very much larger than the wavelength of light illuminating it (e.g. when using cameras or telescopes), we can invoke Fermat's Principle, which states that a ray of light takes the shortest time to travel between two points, and so we can therefore discount the effect of diffraction.

When ray tracing, analysing or designing an optical system, the usual convention is to show a ray of light as travelling from left to right. The Cartesian sign convention is adopted, whereby all distances are measured from the vertex of the lens surface; the numerical value of a distance which is measured in the same direction as that in which light is travelling (left to right) is given a positive sign. Conversely, the numerical value of a distance which is measured in the opposite direction to that in which light is travelling is given a negative sign. All heights measured above the optical axis are positive, and those below are negative. The focal length of a converging lens is positive, and that of a diverging lens is negative

Each lens has two focal points, front and rear, and the distance from the centre of the lens to the focal point is the focal length of that lens. The plane passing through the focal point normal to the axis is the focal plane of the lens. Three properties enable us to construct a ray diagram for a particular lens:

1. When parallel rays of light (i.e. from infinity) enter a lens, they come to a focus at the rear focal point of that lens.
2. Conversely, rays passing from the object, through a focal point will be refracted by the lens and leave it parallel to the optical axis.
3. Rays that pass through the centre of the lens pass through undeviated.

Therefore, if you know the focal length of a lens and the position of the object, you can construct a ray trace using these just two of these three conjugate ray pairs (compare **Figure 3-5a and Figure 3-7**), and determine the position of the image formed by a thin lens.

Both the objectives and condenser contain multiple lens elements, and perform close to their theoretical limits. The three essentials for microscopy are:

1. **Resolving power**
2. **Contrast** or visibility
3. **Magnification**, in that order

As we noted in the introduction, the primary function of the microscope is not to magnify but resolve fine detail. Clearly the fine detail in an image cannot be made visible without sufficient contrast. Not every microscope will have all the following components listed, but most will be found on the microscope in your possession.

A. Stand and Light Source

Trace the power supply from the mains supply to the light source or entry into the microscope stand. Identify the on-off switch, and any rheostat controls. There may be a separate control to pre-set the lamp intensity (colour temperature) for photomicrography.

The significance of this is explained in Chapter 7, section **7.6** on page **xxx**.

Understanding Light Microscopy, First Edition. Jeremy Sanderson.

- Never drop or otherwise strain objectives or other optical components of the microscope
- Never force the focus controls of the objective and condenser, and always watch the lens surface as it approaches the specimen, to avoid damage
- Never touch the anti-reflection coatings on the front of lenses or filters
- DO keep the objectives clean!
- Clean objectives only with the correct soft fibre lens-cleaning tissues and correct solvent

B. Eyepieces

The eyepieces will have their magnification engraved on them. It will always be e.g. x10 or 15x, never a ratio. The common values are 10x; sometimes 8x or 12x or 6.3x eyepieces are found.

The inscription 'WF' may be present to indicate a wide field of view. The field-of-view number (FVN) may be given as a single figure: 18, 20 22. The field of view number is the diameter in millimeters of the field diaphragm.Using the formula in Chapter 8, section 8.10, on page xxx, this can be used to calculate the diameter of the object field in millimeters, and so give an approximate indication of the size of the image.

Some eyepieces are made with high exit pupils (e.g. Figure 8-10 on page xxx), to enable spectacle wearers to use these comfortably. In this case a little spectacles symbol is usually seen on the rim. If the eyepieces are compensating types to be used with apochromats, this may be indicated by the abbreviation 'Comp' or 'Komp'.

If the eyepiece is designed as a measuring eyepiece, to hold a graticule at the primary image plane, it will have a helical or push-pull focusing capability which will be independent of the dioptre control on the eyepiece holder on the binocular head.

3.2 Different Types of Microscope Design

3.2.1 Transmitted-Light Microscopes

The traditional microscope is the one generally used for biological work and configured for transmitted light (light which passes through a specimen). Most explanations in this book will centre on this design, partly because it is so common, but also because with the optical components are arranged one after the other, it is the easiest to explain and also to understand.

3.2.2 Reflected-Light Microscopes

Microscopes designed for use in materials science are configured to examine opaque specimens by reflected light, which illuminates the object through the objective lens. The objective therefore also acts as its own condenser. These microscopes are generally rather easier to use than the transmitted-light design, because whenever the objective lens is changed, the condenser is also changed appropriately, and no major readjustment or centring are required. Their use is discussed in Chapter 12, from page xxx onwards.

The magnification of an objective is very much secondary to its aperture, but it is nonetheless important to consider it. Objectives of larger aperture generally have higher magnification so that the eye can more easily see the finer image detail resolved; conversely, low magnification objectives cannot take advantage of the fine resolution provided by large aperture. There is thus a relationship between aperture and magnification, but it is only an approximate one. It is the aperture that you pay for, the magnification is free; indeed a 40x/1.4 objective will be more expensive than a 100x/1.4, most likely because the 40x NA 1.4 objective has a greater NA : magnification ratio (optical index; Chapter 31, section 31.7, page xxx) and a longer working distance.Figure 7-9 shows two objectives of similar numerical aperture, but which have different magnification. The 20x apochromatic objective is of higher quality than the 40x objective, and resolves the same detail into a much better-corrected image having a larger field of view.

The images of each diatom will both be magnified to approximately the same extent: 240 times. However, more fine detail will be resolved in the image of the diatom viewed with the NA 0.65 objective than with the NA 0.25 objective. In the latter case, it should be impossible to resolve the striae in the images of each diatom. This should underline the message that of the three essentials for microscopy: resolving power, contrast and magnification - resolving power is more important than magnification. The purpose of the objective is to resolve in the image the fine detail present that is in the object. Magnification is only required to make the image large enough for the eye, or camera, to see the resolved detail.

Aperture (illumination) set	Field (imaging) set
1. Lamp filament	
2.	Illuminated Field diaphragm (IFD)
3. Front focal plane of the condenser	
4.	Specimen plane
5. Back focal plane of the objective (BFP)	
6.	Primary Image Plane (PIP)
7. Exit pupil of the eyepiece (Ramsden disc)	
8.	Retina/camera

Exercise 1 Comparison of magnification and resolving power

If you have a pair of 25x eyepieces, and a pair of 6x eyepieces – although these are not common, they are useful for this exercise.

1. Set up two microscopes, each with a diatom test plate as the specimen. Set up one microscope with the 25x eyepieces and a 10x NA 0.25 or NA 0.30 objective, and the other microscope with the 6x eyepieces and a 40x NA 0.65 or 0.7NA objective.
2. Focus on the *Stauroneis phoenocenteron* or *Pleurosigma angulatum* diatoms (refer to Table 10.3, **page xxx** and Box 10-7 **page xxx**). Record what you see in each case.

The lamp is deliberately placed in the aperture set of planes. It is imaged by the lamp collector lens into the front focal plane of the condenser. Thus the structure of the illuminating lamp filament, in the aperture set of conjugate planes, cannot disturb the illumination of the object or its images, all of which lie in the field set of conjugate planes.

3.2.3 Darkfield Microscopy

Darkfield microscopy is also referred to as dark-ground microscopy. For consistency, the term darkfield is used throughout this book. Because of inherently high contrast, and because very weak diffracted rays are captured over the full aperture of the objective, darkfield microscopy (and also fluorescence microscopy) allows us to detect structures far below the resolution limit of the light microscope, although we cannot truly discriminate their size (which will be diffraction-limited). The theoretical aspects are discussed by Françon (1961). Darkfield microscopy is particularly suited to the examination of minute refractile structures, which scatter light well. These include the silica of the frustules (i.e. shells) of diatoms (Figure 11-9), bacteria, aquatic organisms, small inclusions in cells and polymer materials. Darkfield illumination has been known since the beginning of microscopical science. Leeuwenhoek almost certainly knew of the method , and ChristiaanHuygens wrotein 1678 in his journal:

> 'I look at these animals not directly against the light but on turning the microscope a little which makes them appear on a black ground. One can best discover by this means the smallest animals living and can also distinguish best the parts of larger ones…'

All that is required for darkfield microscopy is to exclude the zero order (or direct) illuminating light from entering the acceptance angle of the objective. The background will then be dark, and the image will appear self-luminous, shining with high contrast against the background. Unlike fluorescence microscopy, where similar levels of contrast enhancement can occur, the object is not self-luminous, but diffracts the image-forming light into the objective. This is akin to dust particles suspended in the air made visible by a shaft of direct sunlight into an otherwise shaded room. Although darkfield is an extension of oblique illumination, and darkfield contrast can also be produced by single beam, laterally illuminating at an angle beyond the acceptance angle of the objective lens, the illuminating rays are normally symmetrical about the optical axis rather than occupying a single azimuth. The light from a darkfield condenser forms a hollow cone with the specimen.

3.3 Brightfield reflected-light microscopy

When an objective in reflected-light or epi-illumination mode (Figure 12-11), directs a cone of incident light down onto a perfectly flat, equally-reflecting featureless surface, the surface acts as a mirror or speculum. All the light is reflected back into the objective. This is the equivalent, in transmitted-light microscopy, of no specimen present.

Protocol for reflected-light brightfield microscopy

Required:
- A specimen with well-defined surface features (e.g. a piece of old CD observed from the non-printed side; minature electronic circuit component or polished, etched, metal).
- An epi-illuminator with adjustable illuminated field diaphragm

Procedure
1. Place the specimen slide onto the stage of the microscope.
2. Select a low magnification objective (e.g. 10x). Look sideways on, and raise the stage until the specimen lies within the position of focus for the objective. Lower the stage and bring the specimen into focus.
3. Open the IFD to just outside the field of view. This is more critical than with transmitted-light microscopy, in order to minimise unwanted reflections and glare in the image.