Light and Video Microscopy

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Second Edition

Randy Wayne

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Dedication

This book is dedicated to
my colorful wife Amy,
the light of my life,
and to
Zachary and Beth,
who shine as kids

Preface to the Second Edition

When power leads men towards arrogance, poetry reminds him of his limitations.
When power narrows the areas of man's concern, poetry reminds him of the richness and diversity of his existence.
When power corrupts, poetry cleanses.

John F. Kennedy
Amherst College October 26, 1963.

In the first sentence on the first page of the first illustrated book on microscopy, Robert Hooke (1665) wrote:

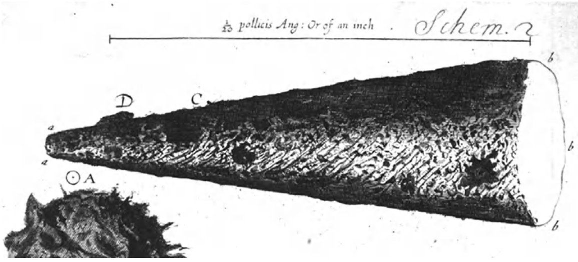
As in Geometry, the most natural way of beginning is from a Mathematical point; so is the same method in Observations and Natural history the most genuine, simple, and instructive. We must first endevour to make letters, and draw single strokes true, before we venture to write whole Sentences, or to draw large Pictures. And in Physical Enquiries, we must endevour to follow Nature in the more plain and easie ways she treads in the most simple and uncompounded bodies, to trace her steps, and to be acquainted with her manner of walking there, before we venture our selves into the multitude of meanders she has in bodies of a more complicated nature; left, being unable to distinguish and judge our way, we quickly lose both Nature our guide, and our selves too, and are left to wander in the labyrinth of groundless opinions; wanting both judgment, that light, and experience, that clew, which should direct our proceedings.

We will begin these our Inquiries therefore with the Observations of Bodies of the most simple nature first, and so gradually proceed to those of a more compounded one. In prosecution of which method, we shall begin with a Physical point; of which the Point of a Needle is commonly reckon'd for one; and is indeed, for the most part, made so sharp, that the naked eye cannot distinguish and parts of it . . . .But if view'd with a very good Microscope, we may find that the top of a Needle . . . appears a broad, blunt, and very irregular end . . . .

There are two lessons here that are just as important today as they were in 1665:

Start at beginning.
A point is not a point.

In this edition of *Light and Video Microscopy*, I continue to present the basics of what is known about light and its role in image formation in the light microscope from the perspective of "how we know what we know." One thing



"The Image we have here exhibited in the first Figure, was the top of a small and very sharp Needle, whose point a a nevertheless appear'd through the *Microscope* above a quarter of an inch broad, not round nor flat, but *irregular* and *uneven*; so that it seem'd to have been big enough to have afforded a hundred armed Mites room enough to be rang'd by each other without endangering the breaking one anothers necks, by being thrust off on either side." From Hooke, 1665.

that we know is that as a consequence of the wave properties of light, a single material point in the specimen is inflated to an ellipsoid of light in the image. The ellipsoid of light in the image has a major axis of about 400 nm and minor axes of about 200 nm. The inflation limits the resolving power of the light microscope. However, new forms of superresolution microscopy discussed in Chapter 12 of this edition allow one to use one's knowledge of light and the interaction of light with matter to remove the light that is out of place and put it back where it would have belonged if light were actually a mathematical point.

There are two lessons here that were not known in 1665:

We are a long way from the beginning.
A point even though it strays can become a point again.

My predecessor in teaching light microscopy at Cornell was Simon Henry Gage, the author of seventeen editions of *The Microscope*. Gage (1941) believed:

"(1) To most minds, and certainly to those having any grade of originality, there is a great satisfaction in understanding principles; and it is only when the principles are firmly grasped that there is complete mastery of instruments, and full certainty and facility in using them. The same is true of the methods of preparing objects for microscopic study, and the interpretation of their appearances when seen under the microscope . . . . (2) Need of

abundant practical work to go with the theoretical part has been shown by all human experience. In all the crafts and in all the fine arts mastery comes only with almost endless effort and repetition, the most common example being the attainment of facility in music . . . .It is also a part of human experience that in successfully going through the manipulations necessary to demonstrate principles, there is acquired not only skill in experiment, but an added grasp of the principles involved."

I hope this book continues in the tradition of *The Microscope* in helping you to not only use, but to understand and appreciate the relation between the real object and the image formed by the light microscope.

Moving from a point to a line, which is what we use to trace the path that the corpuscles of light take from the object to the image, we get to the word verse. The English word "verse" was originally used to indicate a line or lines of a psalm and later to indicate lines of poetry. Its root is the Latin root *vers*, which means "to turn" and which also gave rise to the word *vertere*, which also means "to turn," just as a farmer turns from one line to another while plowing. Related words, some of which are used in optics, include diverse (turned different ways), inverse (turned upside down), reverse (turned back), converse (turned about), transverse (turned across), adverse (turned against), perverse (turned away from what is right), and universe (turn into one).

I found a lost verse in The Rare & Manuscript Collections of Kroch Library, where I read Hooke's *Micrographia*. They also had a presentation copy of the seventeenth edition of *The Microscope* signed by the author, Simon Henry Gage, and given to the chemical microscopist Émile M. Chamot. Pasted into the book I found the following lost poem by Louis Ginsberg (Wayne, 2013d):

Microscope

With bated breath and buoyant hope,
Man bends above the microscope;
The question, pulsing deep in dark,
Splinters to many a question mark.

He looks upon a point to check
The tiny, faint and finite speck;
And yet the more he stares and broods,
It swells into infinitudes.

The more he peers into the middle
Of particles that shape the riddle,
The lens, for all that he can see,
But magnifies the mystery . . .

The microscope provides a natural interface between poetry and the sciences.

I thank Allan Witztum, my friend, colleague and fellow student of David Bierhorst, for sharing his appreciation for the interface between the humanities and the sciences.

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Preface to the First Edition

I am very lucky. I am sitting in the rare book room of the library waiting for Robert Hooke's (1665) *Micrographia*, Matthias Schleiden's (1849) *Principles of Scientific Botany*, and Hermann Schacht's (1853) *The Microscope*. I am thankful for the microscopists and librarians at Cornell University, both living and dead, who have nurtured a continuous link between the past and the present. By doing so, they have built a strong foundation for the future.

Robert Hooke (1665) begins the *Micrographia* by stating that "... the science of nature has already too long made only a work of the brain and the fancy: It is now high time that it should return to the plainness and soundness of observations on material and obvious things." Today, too many casual microscope users do not think about the relationship between the image and reality and are content to push a button, capture an image, enhance the image with Adobe Photoshop, and submit it for publication. However, the sentence that followed the one just quoted indicates that the microscope was not to be used in place of the brain, but in addition to the brain. Hooke (1665) wrote, "It is said of great empires, that the best way to preserve them from decay, is to bring them back to the first principles, and arts, on which they did begin." To understand how a microscope forms an image of a specimen still requires the brain, and today I am privileged to be able to present the work of so many people who have struggled and are struggling to understand the relationship between the image and reality, and to develop instruments that, when used thoughtfully, can make a picture that is worth a thousand words.

Matthias Schleiden (1849), the botanist who inspired Carl Zeiss to build microscopes, wrote about the importance of the mind of the observer:

It is supposed that nothing more is requisite for microscopical investigation than a good instrument and an object, and that it is only necessary to keep the eye over the eye-piece, in order to be au fait. Link expresses this opinion in the preface to his phytotomical plates: 'I have generally left altogether the observation to my artist, Herr Schmidt, and the unprejudiced mind of this observer, who is totally unacquainted with any of the theories of botany, guarantees the correctness of the drawings.' The result of such absurdity is, that Link's phytotomical plates are perfectly useless; and, in spite of his celebrated name, we are compelled to warn every beginner from using them.... Link might just as well have asked a child about the apparent distance of the moon, expecting a correct opinion on account of the child's unprejudiced views. Just as we only gradually learn to see with the naked eye in our infancy, and often experience unavoidable illusions, such as that connected with the rising moon, so we must first gradually learn to see through the medium of the microscope.... We can only succeed gradually in bringing a clear conception before our mind....

Hermann Schacht (1853) emphasized that we should "see with intelligence" when he wrote,

But the possession of a microscope, and the perfection of such an instrument, are not sufficient. It is necessary to have an intimate acquaintance, not only with the management of the microscope, but also with the objects to be examined; above all things it is necessary to see with intelligence, and to learn to see with judgment. Seeing, as Schleiden very justly observes, is a difficult art; seeing with the microscope is yet more difficult.... Long and thorough practice with the microscope secures the observer from deceptions which arise, not from any fault in the instrument, but from a want of acquaintance with the microscope, and from a forgetfulness of the wide difference between common vision and vision through a microscope. Deceptions also arise from a neglect to distinguish between the natural appearance of the object under observation, and that which it assumes under the microscope.

Throughout the many editions of his book, *The Microscope*, Simon Henry Gage (1941) reminded his readers of the importance of the microscopist as well as the microscope (Kingsbury, 1944): "To most minds, and certainly to those having any grade of originality, there is a great satisfaction in understanding principles; and it is only when the principles are firmly grasped that there is complete mastery of instruments, and full certainty and facility in using them.... for the highest creative work from which arises real progress both in theory and in practice, a knowledge of principles is indispensable." He went on to say that an "image, whether it is made with or without the aid of the microscope, must always depend upon the character and training of the seeing and appreciating brain behind the eye."

This book is a written version of the microscopy course I teach at Cornell University. I introduce my students to the principles of light and microscopy through lecture−demonstrations and laboratories where they can put themselves in the shoes of the masters and be virtual witnesses to their original observations. In this way, they learn the strengths and limitations of the work, how first principles were uncovered, and, in some respects, feel the magic of discovery. I urge my students to learn through personal experience and to be skeptical of everything I say. I urge the reader to use this book as a guide to gain personal experience with the microscope. Please read it with a skeptical and critical mind and forgive my limitations.

Biologists often are disempowered when it comes to buying a microscope, and the more scared they are, the more likely it is that they will buy an expensive microscope, in essence, believing that having a prestigious brand name will make up for their lack of knowledge. So buying an expensive microscope when a less expensive one may be equally good or better may be more a sign of ignorance than a sign of wisdom and greatness. I wrote this book, describing microscopy from the very beginning, not only to teach people how to use a microscope and understand the relationship between the specimen and the image, but to empower people to buy a microscope based on its virtues, not on its name. You can see whether or not a microscope manufacturer is looking for a knowledgeable customer by searching the web sites to see if the manufacturer offers information necessary to make a wise choice or whether the manufacturer primarily is selling prestige. Of course, sometimes the prestigious microscope is the right one for your needs.

If you are ready to buy a microscope after reading this book, arrange for all the manufacturers to bring their microscopes to your laboratory and then observe your samples on each microscope. See for yourself: Which microscopes have the features you want? Which microscope gives you the best image? What is the cost/benefit relationship? I thank M. V. Parthasarathy for teaching me this way of buying a microscope.

Epistemology is the study of how we know what we know—that is, how reality is perceived, measured, and understood. Ontology is the study of the nature of what we know that we consider to be real. This book is about how a light microscope can be used to help you delve into the invisible world and obtain information about the microscopic world that is grounded in reality. The second book in this series, entitled, *Plant Cell Biology*, is about what we have learned about the nature of life from microscopical studies of the cell.

The interpretation of microscopic images depends on our understanding of the nature of light and its interactions with the specimen. Consequently, an understanding of the nature of light is the foundation of our knowledge of microscopic images. Appendix II provides my best guess about the nature of light from studying its interactions with matter with a microscope.

I thank David Bierhorst, Peter Webster, and especially Peter Hepler for introducing me to my life-long love of microscopy. The essence of my course comes from the microscopy course that Peter Hepler taught at the University of Massachusetts. Peter also stressed the importance of character in doing science. Right now, I am looking through the notes from that course. I was very lucky to have had Peter as a teacher. I also thank Dominick Paolillo, M. V. Parthasarathy, and George Conneman for making it possible for me to teach a microscopy course at Cornell and for being supportive every step of the way. I also thank the students and teaching assistants who shared in the mutual and never-ending journey to understand light, microscopy, and microscopic specimens. I have used the pictures that my students have taken in class to illustrate this book. Unfortunately, I no longer know who took which picture, so I can only give my thanks without giving them the credit they deserve. Lastly, I thank my family: mom and dad, Scott and Michelle, for making it possible for me to write this book.

As Hermann Schacht wrote in 1853, "Like my predecessors, I shall have overlooked many things, and perhaps have entered into many superfluous particulars: but, as far as regards matters of importance, there will be found in this work everything which, after mature consideration, I have thought necessary."

Randy Wayne

The Relation Between the Object and the Image

And God said, "Let there be light," and there was light. God saw that the light was good, and he separated the light from the darkness.

Gen. 1:3-4

Since we acquire a significant amount of reliable information regarding the real world through our eyes, we often say, "seeing is believing." However, seeing involves a number of processes that take place in space and time as light travels from a real object to our eyes and then gets coded into electrical signals that travel through the optic nerve to the brain. In the brain, neural signals are processed by the visual cortex, and ultimately the brain projects its interpretation of the real object as a virtual image seen by the mind's eye. To ensure that "seeing is not deceiving" requires an understanding of light, optics, the interaction of light with matter, and how the brain functions to create and interpret the relationship between a real object and its image. According to Samuel Tolansky (1964), "There is often a failure in co-ordination between what we see and what we evaluate … .surprisingly enough, we shall find that most serious errors can creep even into scientific observations entirely because we are tricked by optical illusions into making quite faulty judgments." Simon Henry Gage (1941), author of seventeen editions of the classic textbook, *The Microscope,* reminds us that the "image, whether it is made with or without the aid of the microscope, must always depend upon the character and training of the seeing and appreciating brain behind the eye."

The light microscope, one of the most elegant instruments ever invented, is a device that permits us to study the interaction of light with matter at a resolution much greater than that of the unaided eye (Dobell, 1932; Wilson, 1995; Ruestow, 2004; Schickore, 2007; Ratcliff, 2009). Due to the constancy of the interaction of light with matter, we can peer into the would-be invisible world to discover the hidden properties of objects in that world (Appendix I). We can make transparent and invisible cells visible with a dark-field, phase-contrast, or differential interference microscope. We can use a polarizing microscope to reveal the orientation of macromolecules in a cell, and we can use it to determine the entropy and enthalpy of the polymerization process. We can use an interference microscope to weigh objects and to ascertain the mass of the cell's nucleus. We can use a fluorescence microscope to localize proteins in the cytoplasm, genes on a chromosome, and the free Ca^{2+} concentration and pH of the surrounding milieu. We can use a centrifuge microscope or a microscope with laser tweezers to measure the forces involved in cellular motility or to determine the elasticity and viscosity of the cytoplasm. We can use a laser Doppler microscope, which takes advantage of the Doppler effect produced by moving objects, to characterize the velocities of organelles moving through the cytoplasm. We can also use a variety of laser microscopes to visualize single molecules.

I wrote this book so that you can make the most of the light microscope when it comes to faithfully creating and correctly interpreting images. To this end, the goals of this book are to:

- Describe the nature of light.
- Describe the relationship between an object and its image.
- Describe how light interacts with matter to yield information about the structure, composition, and local environment of biological and other specimens.
- Describe how optical systems work so that you will be able to interpret the images obtained at high resolution and magnification.
- Give you the necessary procedures and tricks so that you can gain practical experience with the light microscope and become an excellent microscopist.

LUMINOUS AND NONLUMINOUS OBJECTS

All objects, which are perceived by our sense of sight, can be divided into two broad classes. One class of objects, known as luminous bodies, includes "hot" or incandescent sources such as the sun, the stars, torches,

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oil lamps, candles, coal and natural gas lamps, kerosene lamps, and electric light bulbs, and "cold" sources such as fireflies and glow worms that produce "living light" (Brewster, 1830; Hunt, 1850; Harvey, 1920, 1940). These luminous objects are visible to our eyes. The second class of objects is nonluminous. However, they can be made visible to our eyes when they are in the presence of a luminous body. Thus the sun makes the moon, Earth, and other planets visible to us, and a light bulb makes all the objects in a room or on a microscope slide visible to us. The nonluminous bodies become visible by scattering the light that comes from luminous bodies. A luminous or nonluminous body is visible to us only if there are sufficient differences in brightness or color between it and its surroundings. The difference in brightness or color between points in the image formed of an object on our retina is known as contrast.

OBJECT AND IMAGE

Each object is composed of many small and finite points composed of atoms or molecules. Ultimately, the image of each object is a point-by-point representation of that object upon our retina. Each point in the image should be a faithful representation of the brightness and color of the conjugate point in the object. Two points on different planes are conjugate if they represent identical spatial locations on the two planes. The object we see may itself be an intermediate image of a real object. The intermediate image of a real object observed with a microscope, telescope, or by looking at a photograph, movie, television screen, or computer monitor should also be a faithful point-by-point representation of the brightness and color of each conjugate point of the real object. While we only see brightness and color, the mind interprets the relative brightness and colors of the points of light on the retina and makes a judgment as to the size, shape, location, and position of the real object in its environment.

What we see, however, is not a perfect representation of the physical world. First, our eyes are not perfect, and our vision is limited by physical, genetic, and nutritional factors (Wald, 1967; Helmholtz, 2005). For example, we cannot see clearly things that are too far or too close, too dark or too bright, or things that emit radiation outside the visible range of wavelengths. Second, our vision is affected by physiological and psychological factors, and we can be easily fooled by our sense of sight (Goethe, 1840; Sully, 1881; Gregory, 1973; Békésy, 1967; Wade, 1998; Russ, 2004). Third, as Goethe learned when he studied the colors of the Italian landscape as they transformed from vibrant to muted and back again as the weather changed (Heisenberg, 1979), or as humankind learned upon the introduction of artificial illumination (Wickenden, 1910; Steinmetz, 1918; Otter, 2008), we must remember to take the source of illumination as well as the environment surrounding the object into consideration.

The architects of ancient Greece knew that the optical illusions that occur under certain circumstances, if not taken into consideration, would diminish the beauty of great buildings such as the Parthenon, which was built in honor of the virgin (parthenos) Athena (Penrose, 1851; Fletcher and Fletcher, 1905; Prokkola, 2011). For example, stylobates, or long horizontal foundations for the classical columns, and architraves, the horizontal beams above doorways, would appear to sag in the middle if they were made perfectly straight. Consequently, the architects used horizontal beams with convex tops to compensate for the optical illusion—the result being a perfectly square-looking structure. The columns of the Parthenon are famous, but they are not identical. The columns that are viewed against the bright Greek sky were made thicker than the columns backed by the inner temple or cella wall, since identical columns viewed against a bright background appear thinner than those viewed against a dark background. By compensating for the optical illusion, the columns appear identical and magnificent.

The sculptors of ancient Greece also knew about optical illusions, as evidenced by an apocryphal legend concerning two sculptors, Phidias, the teacher, and his student, Alkamenes (Anon, 1851). They were contenders in a contest to produce a sculpture of Athena that would stand upon a pedestal. Alkamenes sculpted a beautiful and well-proportioned figure of Athena, while Phidias, using his knowledge of geometry and optics, fashioned a grotesque and distorted figure. While the two sculptures were on the ground, the judges marveled at the one created by Alkamenes and laughed at the one created by Phidias. However, once the sculptures were put on top of the column, the perspective changed, and Phidias's sculpture assumed great beauty while Alkamenes sculpture looked distorted. Knowing that the angles subtended by each feature of the object become proportionally smaller as the height and distance of the feature increased, Phidias formed the facial features proportionately larger and the lower features proportionately smaller so that the sculpture of Athena would look normal and beautiful atop its final location. As Alexander Pope (1711) wrote in *An Essay on Criticism*,

Some Figures monstrous and mis-shap'd appear,
Consider'd singly, or beheld too near,
Which, but proportion'd to their Light, or Place,
Due Distance reconciles to Form and Grace.

Leon Battista Alberti, perhaps the original Renaissance man, restored the union of the arts and sciences that had been lost during the Middle Ages. He combined his love of nature with Euclidean geometry and

Al-Haytham's optics to introduce *perspective* into painting as a way of producing the illusion of depth intrinsic to the three-dimensional natural world on a two-dimensional surface. The perspective techniques used for creating the illusion of depth in a painting were also used to create the illusion of great distances in cathedrals and theatrical scenery (Alberti, 1755; Hopkins, 1836; da Vinci, 1970; Gill, 1974; Veaner, 1984; Summers, 2007). While perspective techniques are a device to restore the illusion of the third dimension onto a two-dimensional surface, anamorphosis is a technique devised by Leonardo da Vinci to obscure realistic-looking images in such a way that we can "see" them only if we know the laws of perspective and view them from a particular angle (Leeman, 1976; Baltrusaitis, 1983; Kemp, 1990). Erhard Schön used anamorphosis in his woodcut of Emperor Ferdinand, and William Scrots used it in his painting of Edward VI. It was also used to disguise erotic images (Leeman, 1976). Anamorphosis was most famously used in the sixteenth century by Hans Holbein the Younger in his painting, entitled *The Ambassadors*, to hide a human skull that can only be seen when the painting is viewed obliquely (Hervey, 1900; Foister et al., 1997; North, 2002). The life-sized painting was meant to be observed from two perspectives: when viewed straight on, one sees signs of the achievements made in the arts and sciences, but when looked at obliquely from above, as if the painting was meant to be viewed while the observer descended a stairway, one sees a human skull, an indication that all earthly accomplishment is vain illusion (Ecclesiastes 1:2). Another optical illusion can be found in the portrait of Lisa Gherardini, known as da Vinci's *Mona Lisa*. Since the direction of a gaze depends on the position of the irises and the head, we think that a figure's gaze follows us when the head is painted to face one way and the irises are painted to face the other (Wollaston, 1824; Brewster, 1835; Todorović, 2006).

Many examples of optical illusions occur in the natural world—particularly when it comes to animals using camouflage and mimicry to fool their predators (Cott, 1940; Winckler, 1968; Hooper, 2002; Ruxton et al., 2004; Forbes, 2009). Peppered moths hide from predators by mimicking tree bark; katydids, mantises, and walkingsticks hide from predators by mimicking leaves and twigs; and the Malaysian orchid mantis not only hides from its predators by mimicking flowers, but it also uses its camouflage to surprise its prey. The "Moon Illusion" is another natural illusion. The moon rising on the horizon looks bigger than the moon at its zenith, yet we can easily see that they are the same size by holding a quarter at arm's length and observing that in both cases the quarter just obscures the moon (Molyneux, 1687; Wallis, 1677; Berkeley, 1709; Schleiden, 1849; Kaufman and Rock, 1962; Tolansky, 1964; Ross, 2000).

Do you remember looking through the microscope at a drop of pond water teeming with life? The animalcules seemed to be moving around with incredible speed, right? This too is an illusion. It occurs because the microscope magnifies space but not time (Tolansky, 1964). If we observe an organism that is swimming at 100 μm/s through a microscope that magnifies one thousand times, it will appear to be swimming with a speed of 10 cm/s, which would be quite fast for an organism that might only be 10 μm long—ten thousand body lengths per second instead of ten body lengths per second! At ten body lengths per second, the actual speed, it would take the animalcule one thousand seconds to travel ten centimeters.

Look at the following optical illusions and ask yourself, is seeing really believing (Figure 1.1)? To further test the relationship between seeing and believing, look at the following books on optical illusions—Tolansky, 1964; Luckiesh, 1965; Joyce, 1995; Fineman, 1981; Seckel, 2000, 2001, 2002, 2004a, 2004b; Del-Prete, 2008—then ask yourself, "When I look at an unknown object in the microscope, how do I know what is real and what is an illusion?"

THEORIES OF VISION

In order to appreciate the relationship between an object and its image, the ancients developed several theories of light and vision that can be reduced into two classes (Priestley, 1772; Vavilov, 1955, 1965; Lindberg, 1976; Burkert, 1977; Sabra, 1989; Ronchi, 1991; Zajonc, 1993; Park, 1997; Gross, 1999; Summers, 2007; Darrigol, 2012):

- Theories that state that vision results from light in the form of a minute replica (eidola) or a thin film (simulacra) of atoms that is emitted from the object and enters the eye (intromission theory). Intromission theory was championed by Lucretius and Democritus, who said that he would rather find one causal reason than become King of Persia.
- Theories that state that vision results from the emission of visual rays from the eye to the object being viewed (extramission theory). Extramission theory was championed by Euclid and Ptolemy.

Both theories relate the sense of vision to the sense of touch. However, the extramission theory is analogous to the act of touching, while the intromission theory is analogous to the act of being touched. Extramission theory, which was based in part on the belief that the gods endowed us with the "fire in the eye," explained why we see "stars" when someone strikes our head, why we see light or phosphenes when we rub our closed eyes, why we see images when we sleep in the dark, why we "feel" it when someone stares at us, and why we see only the surface of objects. The intromission theory, by contrast, explained why we cannot see in the dark.

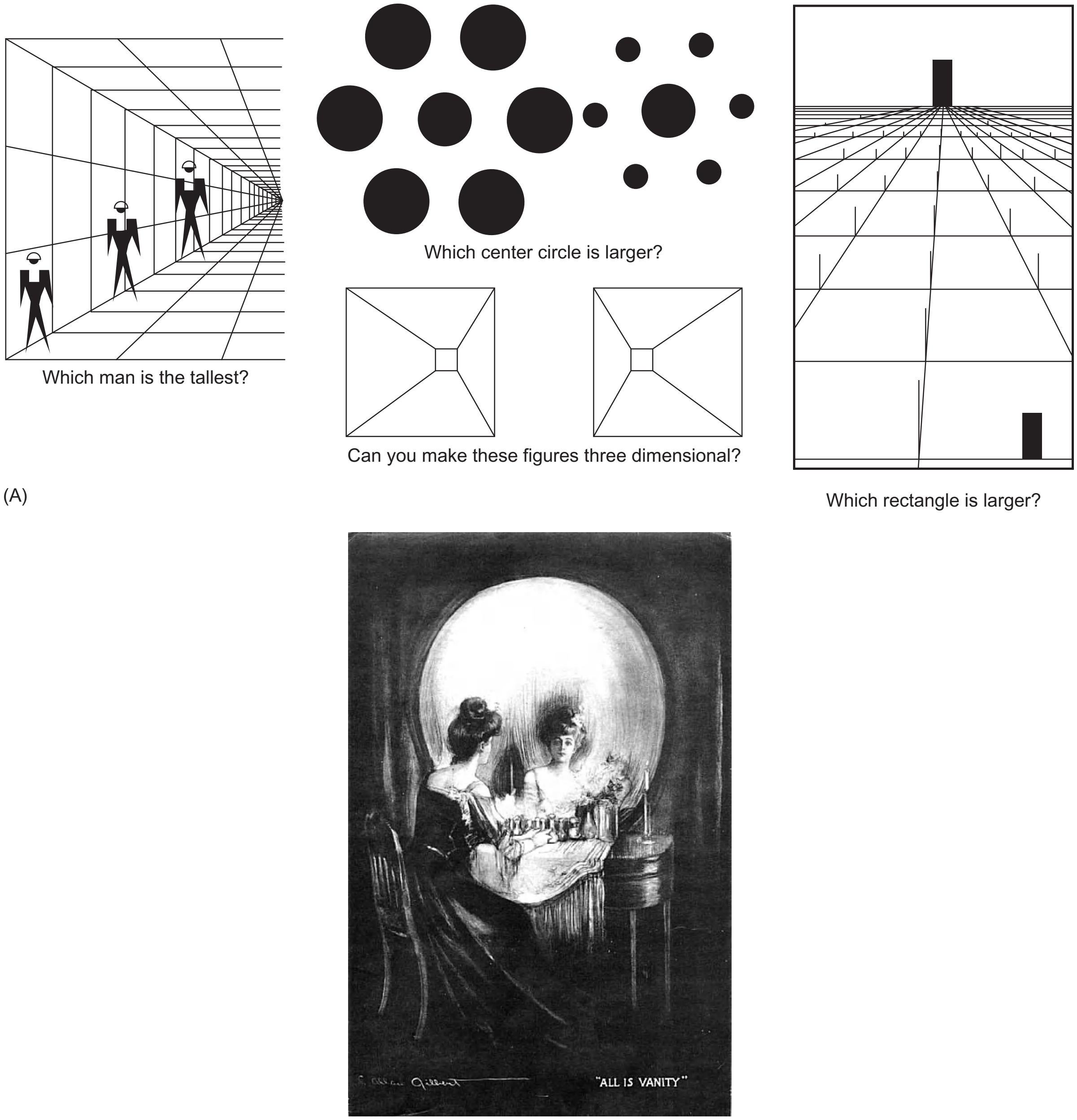

FIGURE 1.1 (A) Optical illusions. Is seeing believing? (B) "All is Vanity" by Charles Allan Gilbert (1892). When we look at this ambiguous optical illusion, our mind forms two alternative interpretations, each of which is a part of the single reality printed on the page. Instead of seeing what is actually on the page, our mind produces two independent images, each of which makes sense to us and each of which has meaning. When we look at a specimen through a microscope, we must make sure that we are seeing what is there and find meaning in what is there, as opposed to seeing only that which is already meaningful to us.

In his *Optics* (Kheirandish, 1999), Euclid introduced mathematics into his extramission theory of vision. Euclid demands (or postulates) that we accept seven assumptions. If we accept these assumptions, we will be able to explain the geometry of vision, including the cause of optical illusions. Euclid's postulates can be summarized like so:

- Infinite straight lines, known as visual rays, proceed from the eye, forming a cone such that the vertex is at the eye and the base is at the surfaces of the objects being seen. Objects touched by the visual rays are visible; those untouched by the visual rays are not seen.

- Objects seen through a larger angle appear larger, those seen under a smaller angle appear smaller, and those seen under equal angles appear equal. Lastly, objects touched by visual rays coming from more angles, or a greater angle, are seen more clearly.

While Euclid's assumption of the reality of visual rays may seem reasonable to little children who cover their eyes in order to hide during a game of hide and seek, they seem absurd to us. Nevertheless, if we keep our historical perspective, we will see anon that it will be very helpful in understanding the formation of virtual images (see Chapter 2) and optical resolution (see Chapters 3 and 4).

Historically, most theories of vision were synthetic theories that combined the two theses, suggesting that the sun and the eye were kindred beings, and if like combined with like, then sunlight emitted from an object combined with the visual rays coming from the eye in order for vision to occur (Plato, 1965). Many writers, from Euclid to da Vinci, wavered back and forth between the two antithetical explanations of vision. In 1088, Al-Haytham, a supporter of the intromission theory, described vision as the transfer of a set of points from the external surface of an object to a surface in the eye, and suggested that images may be formed by eyes in a manner similar to the way that they are formed by pinholes. The similarity between the eye and a pinhole camera was also expressed by Giambattista della Porta, Leonardo da Vinci (1970), and Francesco Maurolico (1611). Nevertheless, they were unable to reasonably explain the logical consequence that, if an eye formed images just like a pinhole camera, then the world should appear upside down (Arago, 1857).

By 1604, Johannes Kepler developed, what is in essence, our current theory of vision (Kepler, 2000; Schickore, 2007). Kepler inserted an eyeball, whose back had been scraped away to expose the retina, in the pinhole of a camera obscura. Upon doing this, he discovered that the eye contains a series of hard and soft elements that act together as a convex lens that projects an inverted image of the object on the concave retina. The image formed on the retina is an inverted point-by-point replica that represents the brightness and color of the object. Kepler dismissed the problem of the "upside up world" encountered by Porta, da Vinci, and Maurolico by suggesting that the brain subsequently deals with the inverted image. The importance of the brain in vision was expanded by George Berkeley (1709).

Before I discuss the physical relationship between an object and an image, I will take a step backward and discuss the larger philosophical problem of recognizing which is the object and which is the image. Plato illustrates this point in the *Republic* (Jowett, 1908; also see

FIGURE 1.2 The troglodytes in a cave.

Cornford, 1945) where he tells the following parable known as *The Allegory of the Cave* (Figure 1.2):

And now I will describe in a figure the enlightenment or unenlightenment of our nature: Imagine human beings living in an underground den which is open towards the light; they have been there from childhood, having their necks and legs chained, and can only see into the den. At a distance there is a fire, and between the fire and the prisoners a raised way, and a low wall is built along the way, like the screen over which marionette players show their puppets. Behind the wall appear moving figures, who hold in their hands various works of art, and among them images of men and animals, wood and stone, and some of the passers-by are talking and others silent ... They are ourselves ... and they see only the shadows of the images which the fire throws on the wall of the den; to these they give names, and if we add an echo which returns from the wall, the voices of the passengers will seem to proceed from the shadows. Suppose now that you suddenly turn them round and make them look with pain and grief to themselves at the real images; will they believe them to be real? Will not their eyes be dazzled, and will they not try to get away from the light to something which they are able to behold without blinking? And suppose further, that they are dragged up a steep and rugged ascent into the presence of the sun himself, will not their sight be darkened with the excess of light? Some time will pass before they get the habit of perceiving at all; and at first they will be able to perceive only shadows and reflections in the water; then they will recognize the moon and the stars, and will at length behold the sun in his own proper place as he is. Last of all they will conclude: This is he who gives us the year and the seasons, and is the author of all that we see. How will they rejoice in passing from darkness to light! How worthless to them will seem the honours and glories of the den! But now imagine further, that they descend into their old habitations; in that underground dwelling they will not see as well as their fellows, and will not be able to *compete with them in the measurement of the shadows on the wall; there will be many jokes about the man who went on a visit to the sun and lost his eyes, and if they find anybody trying to set free and enlighten one of their number, they will put him to death, if they can catch him. Now the cave or den is the world of sight, the fire is the sun, the way upwards is the way to knowledge, and in the world of knowledge the idea of good is last seen and with difficulty, but when seen is inferred to be the author of good and right – parent of the lord of light in this world, and of truth and understanding in the other. He who attains to the beatific vision is always going upwards . . .*

Although this parable can be discussed at many levels, I will use it just to emphasize that we see images of the world, and not the world itself. Plato went on to suggest that the relationship between the image and its reality could be understood through study, particularly the progressive and habitual study of mathematics. In *Novum Organum*, Francis Bacon (in Commins and Linscott, 1947) described four classes of idols that plague one's mind in the scientific search for knowledge. One of these he called "the idols of the cave":

The Idols of the Cave are the idols of the individual man. For everyone (besides the errors common to human nature in general) has a cave or den of his own, which refracts and discolors the light of nature; owing either to his own proper and peculiar nature or to his education and conversation with others; or to the reading of books, and the authority of those whom he esteems and admires; or to the differences of impressions, accordingly as they take place in a mind preoccupied and predisposed or in a mind indifferent and settled; or the like. So that the spirit of man (according as it is meted out to different individuals) is in fact a thing variable and full of perturbation, and governed as it were by chance. Whence it was well observed by Heraclitus that men look for science in their own lesser worlds, and not in the greater or common world.

Charles Babbage (1830) wrote, in *Reflections on the Decline of Science*, about the importance of understanding the "irregularity of refraction" and the "imperfections of instruments" used to observe nature. In his book, entitled, *The Image*, Daniel Boorstin (1961) contends that many of the advances in optical technologies have contributed to a large degree in separating the real world from our image of it. Indeed, the physical reality of our body and our own image of it does not have a one-to-one correspondence. In *A Leg to Stand On*, Oliver Sacks (1984) describes the neurological relationship between our body and our own image of our body.

Thus it is incumbent on us to understand that when we look at something, we are not directly sensing the object, but an image of the object projected on our retinas, and processed by our brains. The image, then, depends not only on the intrinsic properties of the object, but on the properties of its environment, the light that illuminates it, and the physical, physiological, and psychological basis of vision. Before we even prepare our specimen for viewing in the microscope, we must prepare our mind and keep the following general questions in mind:

1. How do we receive information about the external world?
2. What is the nature and validity of the information?
3. What is the relationship of the perceiving organism to the world perceived?
4. What is the nature and validity of the information obtained by using an instrument to extend the senses; and what is the relationship of the information obtained by the perceiving organism with the aid of an instrument to the world perceived?

LIGHT TRAVELS IN STRAIGHT LINES

It has been known for a long time that light travels in straight lines. Mo Tzu (470–391 BC) inferred that the light rays from luminous sources travel in straight lines because:

- A shadow cast by an object is sharp, and it faithfully reproduces the shape of the object.
- A shadow never moves by itself, but only if the light source or the object moves.
- The size of the shadow depends on the distance between the object and the screen upon which it is projected.
- The number of shadows depends on the number of light sources: if there are two light sources, there are two shadows (Needham, 1962).

The ancient Greeks also came to the conclusion that light travels in straight lines. Aristotle (384–322 BC, Physics Book 5, in Barnes, 1984) concluded that light travels in straight lines as part of his philosophical outlook that nature works in the briefest possible manner. However, evidence for the rectilinear propagation of light came in part from observing shadows. Euclid observed that there is a geometric relationship between the height of an object illuminated by the sun and the length of the shadow cast (Figure 1.3). Theon of Alexandria (335–395) amplified Euclid's conclusion that light travels in straight lines by showing that the size of a shadow depended on whether an object was illuminated by parallel rays, converging rays, or diverging rays (Lindberg and Cantor, 1985). The Pilobolus Dance Company, founded in part by Jonathan Wolken, son of the photobiologist Jerome Wolken (1968) uses their knowledge of light and shadows to perform Shadowland. We can use the fact that light travels in straight lines to

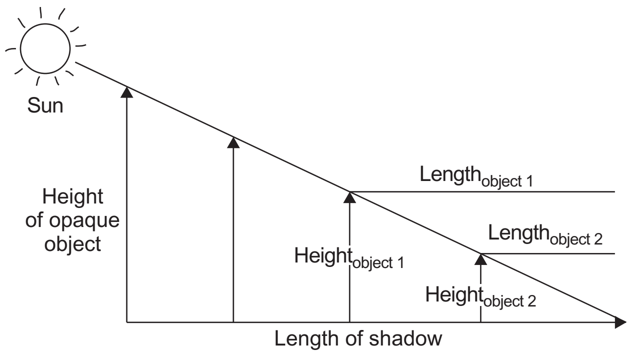

FIGURE 1.3 There is a geometrical relationship between the height of an object illuminated by the sun and the length of the shadow cast. Height$_{\text{object 1}}$/Length of Shadow$_{\text{object 1}}$ = Height$_{\text{object 2}}$/Length of Shadow$_{\text{object 2}}$ = constant.

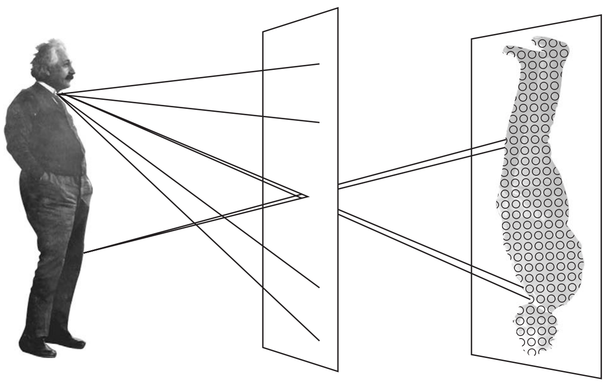

FIGURE 1.4 A pinhole forms an inverted image because light travels in straight lines. The pinhole blocks out the majority of rays that radiate from a single point on the object. The rays that do pass through the pinhole form the image. The smaller the pinhole, the smaller the circle of confusion that makes up each "point" of the image.

make amusing shadows of animals with our hands (Bursill, 1859,1860; Patterson, 1895; Trewey, 1920; Nikola, 1921). Raymond Crowe has elevated shadowgraphy to a performing art, especially in his production of Louis Armstrong's *What a Wonderful World*. Shigeo Fukuda (Seckel, 2004b) and Tim Noble and Sue Webster (Januszczak, 2012) cleverly arrange rubbish and illuminate it in such a manner that its shadow, as opposed to the three-dimensional object itself, reveals a surprising figure that represents the desired form.

Mirrors and lenses have been used for thousands of years as looking glasses and for starting fires. Aristophanes (423 BC) describes their use in *The Clouds*. Euclid, Diocles, and Ptolemy used the assumption that a light ray (or visual ray) travels in a straight line in order to build a theory of geometrical optics that was powerful enough to predict the position of images formed by mirrors and refracting surfaces (Smith, 1996; see Chapter 2). According to geometrical optics, an image is formed where all the rays emanating from a single point on the object combine to make a single point of the image. Bright points emit many rays and darker points emit fewer rays. The image is formed on the surface where the divergent rays from a given point converge and meet each other. The success that the geometrical theory of optics had in predicting the position of images provided support for the veracity of the assumption that light travels in straight lines, upon which the theory of geometrical optics is based. Building on the atomistic theories of Leucippus, Democritus, Epicurus, and Lucretius—and contrary to the continuous theories championed by Aristotle, Simplicus, and Descartes—Isaac Newton (1730) proposed that light traveled along straight lines as corpuscles.

Interestingly, the fact that light travels in straight lines allows us to "see what we want to see." The mathematician, William Rowan Hamilton (1833) began his paper on the principle of least action in the following way:

The law of seeing in straight lines was known from the infancy of optics, being in a manner forced upon men's notice by the most familiar and constant experience. It could not fail to be observed that when a man looked at any object, he had it in his power to interrupt his vision of the object, and hide it at pleasure from his view, by interposing his hand between his eyes and it; and that then, by withdrawing his hand, he could see the object as before: and thus the notion of straight lines or rays of communication, between a visible object and a seeing eye, must very easily and early have arisen.

In his song, "*Blowin' in the Wind*," Bob Dylan put it this way: "Yes, 'n' how many times can a man turn his head, Pretending he just doesn't see?"

IMAGES FORMED IN A *CAMERA OBSCURA*: GEOMETRIC CONSIDERATIONS

Mo Tzu, whose philosophy emphasized self-reflection, provided further evidence that rays emitted by each point of a visible object travel in a straight line by observing the formation of images (Needham, 1962; Hammond, 1981; Knowles, 1994). He noticed that although the light emitted by an object is capable of forming an image in our eyes, it is not able to form an image on a piece of paper or screen. However, Mo Tzu found that the object could form an image on a screen if he eliminated most of the rays issuing from each point by placing a pinhole between the object and the screen (Figure 1.4). The image that appears, however, is inverted. Mo Tzu (in Needham, 1962) wrote,

An illuminated person shines as if he was shooting forth rays. The bottom part of the man becomes the top part of the image and the top part of the man becomes the bottom part of the image. The foot of the man sends out, as it were light rays, some of which are hidden below (i.e. strike below the pinhole) but others of which form an image at the top. The head of the man sends out, as it were light rays, some of which are hidden above (i.e. strike above the pinhole) but others of which form its image at the bottom. At a position farther or nearer from the source of light, reflecting body, or image there is a point (the pinhole) which collects the rays of light, so that the image is formed only from what is permitted to come through the collecting-place.

The fact that the image can be reconstructed by drawing a straight line from every point of the outline of the object, through the pinhole, and to the screen, confirms that light does travel in straight lines. According to John Tyndall (1887), "This could not be the case if the straight lines and the light rays were not coincident." Shen Kua, who lived from 1031 to 1095, extended Mo Tzu's work by showing the analogy between pinhole images and reflected images. However, Shen Kua's work could not go too far since it lacked a geometric foundation (Needham, 1962).

The Greeks also discovered that images could be formed by a pinhole. Aristotle noticed that the light of the sun during an eclipse coming through a small hole made between leaves casts an inverted image of the eclipse on the ground (Aristotle; Problems XV:11 in Barnes, 1984).

During the Middle Ages, the description of image formation based on geometric optics by Euclid and Ptolemy was extended by scholars in the Arab World. Al-Kindi in *De Aspectibus* showed that light entering a dark room through windows travels in straight lines. Likewise, the light of a candle is transmitted through a pinhole in straight lines (Lindberg and Cantor, 1985). Al-Kindi's work was extended by Al-Haytham, or Alhazen as he is often known (in Lindberg, 1968), who wrote in his *Perspectiva*,

The evidence that lights and colors are not intermingled in air or in transparent bodies is that when a number of candles are in one place, [although] in various and distinct positions, and all are opposite an aperture that passes through to a dark place and in the dark place opposite the aperture is a wall or an opaque body, the lights of those candles appear on the [opaque] body or the wall distinctly according to the number of candles; and each of them appears opposite one candle along a [straight] line passing through the aperture. If one candle is covered, only the light opposite [that] one candle is extinguished; and if the cover is removed, the light returns . . . Therefore, lights are not intermingled in air, but each of them is extended along straight lines.

Each and every point on a luminous object forms a cone of light that passes through the pinhole (Figure 1.4). The tip of the cone is at the luminous point and the base of the cone forms the image. The size of the pinhole determines the effective cross-sectional area of the luminous cone that forms the image of a point. Al-Haytham and his fourteenth century commentator Al-Farisi realized that the image formed by a pinhole was actually a composite of numerous overlapping images of the pinhole, each one originating from an individual luminous point on the object (Omar, 1977; Lindberg, 1983; Sabra, 1989). The fact that light originating from a point on an object forms a circle of light known as the "circle of confusion" at the image results in a blurring of the image (Derr, 1922; Time-Life, 1970). The features of an image will be distinct only when the bases of cones originating from adjacent points of that feature do not overlap. Given this hypothesis, the sharpness of the image would increase as the size of the aperture decreases. However, the brightness of the image also decreases as the size of the aperture decreases. Using geometry, Al-Haytham found the optimal diameter of an aperture when viewing an object of a given diameter (y_o) and distance (s_o) from the aperture. He showed that when the object is circular, and the object, aperture, and plane of the screen are parallel, two light patches originating from two points on the object will touch when the ratio of the diameter of the aperture (a_o) to that of the object (y_o) is equal to the ratio of the distance between the image and the aperture (s_i), and the distance between the image and the object ($s_i + s_o$). That is:

$$a_o/y_o = s_i/(s_i + s_o)$$

The position of the optimal image plane (s_i) and the optimal size of the aperture (a_o) are given by the following analysis (Figure 1.5).

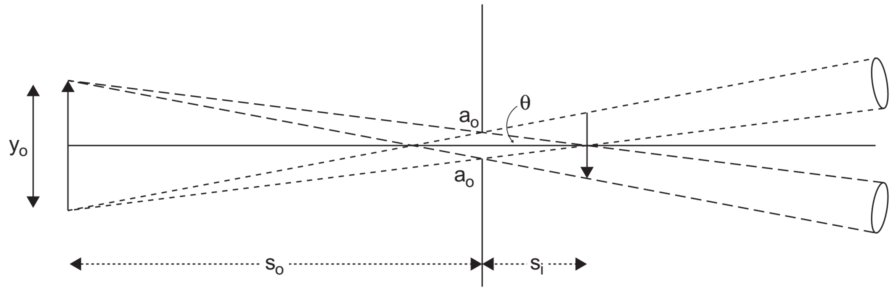

FIGURE 1.5 The position of the optimal image plane (s_i) and the optimal size of the aperture (a_o) for an object of height (y_o) placed at the object plane (s_o).

Since $\tan\ \theta = (\frac{1}{2}\ a_o)/s_i = (\frac{1}{2}\ y_o)/(s_i + s_o)$, then $a_o/y_o = s_i/(s_i + s_o)$ and $y_o/a_o = 1 + s_o/s_i$. For large distances between the object and the pinhole, $y_o/a_o \approx s_o/s_i$, and for a given s_o, the greater the aperture size, the greater is the distance from the aperture to a clear image.

Leonardo da Vinci (1970) also came to the conclusion that light travels through a pinhole in straight lines to form an image. He wrote "All bodies together, and each by itself, give off to the surrounding air an infinite number of images which are all-pervading and each complete, each conveying the nature, color and form of the body which produces it." Da Vinci proved this hypothesis by observing that when one makes "a small round hole, all the illuminated objects will project their images through that hole and be visible inside the dwelling on the opposite wall which may be made white; and there in fact, they will be upside down, and if you make similar openings in several places on the same wall you will have the same result from each. Hence the images of the illuminated objects are all everywhere on this wall and all in each minutest part of it." Da Vinci (1970) also realized that the images formed by the pinhole were analogous to the images formed by the eye. He wrote,

An experiment, showing how objects transmit their images or pictures, intersecting within the eye in the crystalline humour, is seen when by some small round hole penetrate the images of illuminated objects into a very dark chamber. Then, receive these images on a white paper placed within this dark room and rather near to the hole and you will see all the objects on the paper in their proper forms and colours, but much smaller; and they will be upside down by reason of that very intersection. These images being transmitted from a place illuminated by the sun will seem actually painted on this paper which must be extremely thin and looked at from behind.

The pinhole sets a limit on the size of the cone that is used to form a distinct image of any given point. When

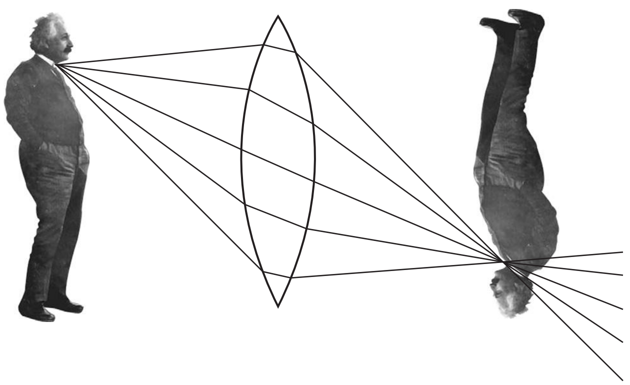

FIGURE 1.6 A converging lens can collect more of the rays that emanate from a point on an object than a pinhole can, thus producing a brighter image.

the aperture is too large, the cone of light emanating from each point of the object illuminates every part of the screen and there is no image. As the aperture decreases, a clearer image is formed but less light passes through the aperture and the image becomes dimmer. The dimness problem can be overcome without compromising image clarity by putting a converging lens over a pinhole (Wright, 1907; Figure 1.6).

In 1550, Girolamo Cardano suggested that a biconvex lens placed in front of the aperture would increase the brightness of the image, and this suggestion was repeated by Daniel Barbaro in 1568 (Gernsheim, 1982). Such a lens can capture a larger cone of light emitted from a given point on the object than an aperture can. A biconvex lens also focuses all the rays emanating from each point of an object on the corresponding conjugate point on the image. In contrast to an image formed by a pinhole, an image formed by a lens is restricted to only one plane, known as the image plane. In front of or behind the image plane, the rays are converging to a spot or diverging from a spot, respectively. Since the "out-of-focus" image is formed from cones originating from many points on the object, there is no clear relationship between the brightness of an out-of-focus image point and the brightness of a single object point.

The brightness of the image increases as the diameter of lenses with a given focal length increases. This is because the larger a lens, the more rays it can collect from each point on the object. The sharpness of the image produced by a lens is related to the number of rays emanating from each point that is collected by that lens.

The *camera obscura* was popularized by Giambattista della Porta in his book *Natural Magick* (1589). When a *camera obscura* was open to the public, the crowded dark room was used both as a venue to present shows of natural magic and as a convenient place to pick the pockets of the unsuspecting audience. By the seventeenth century, portable versions of the *camera obscura* were used by Johann Kepler (who coined the term *camera obscura*, which literally means dark room) for drawing the land he was surveying and for observing the sun. Kepler also suggested that the *camera obscura* could be improved by adding a second biconvex lens to right the inverted image and a concave lens to increase the focal length. The distance of the image plane from the lens, as well as the magnification of the image, depends on the focal length of the lens. For an object at a set distance in front of the lens, the image distance and magnification increases with an increase in the focal length of the lens (Figure 1.7). Johann Zahn and Athanasius Kircher used *camera obscuras* in order to facilitate drawing scenes far away from the studio, and Johann Hevelius (1647) may have connected a *camera obscura* to a microscope to facilitate drawing enlarged images of microscopic specimens (Hammond, 1981).

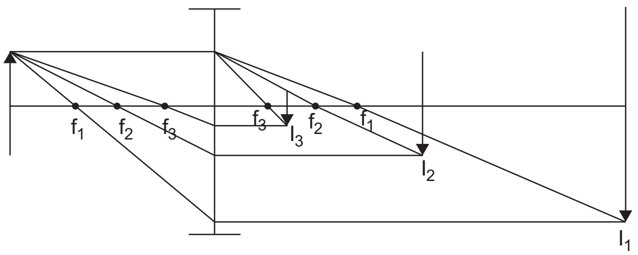

FIGURE 1.7 As the focal length of a lens increases ($f_1 > f_2 > f_3$), the image plane moves farther from the lens and the image becomes more magnified.

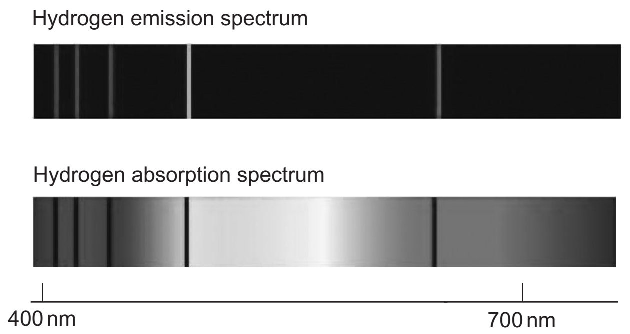

FIGURE 1.8 A diffraction grating resolves the light emitted from an incandescent gas into bright lines. When a sample of the same gas is placed between a white light source and a diffraction grating, black lines appear at the same places as the emission lines occurred, indicating that gases absorb the same wavelengths as they emit.

Some Renaissance painters, including Vermeer, may have used the *camera obscura* as a drawing aid (Huerta, 2003). Perhaps *A View of Delft* was painted with the aid of the *camera obscura*, given that the edges of the painting are out of focus. In 1681, Robert Hooke suggested that the screen of the *camera obscura* should be concave, as a retina is, since the image formed by either a pinhole or a simple lens does not form a flat field at sharp focus, but a curved field. The *camera obscura* evolved into the camera of today (see Chapter 5), and Abelardo Morell (1995, 1999, 2004) uses the *camera obscura* to create beautiful photographs.

WHERE DOES LIGHT COME FROM?

To understand where light comes form, we have to turn to quantum mechanics. The realist interpretation championed by Albert Einstein states that particles such as electrons really exist and have definite positions independent of human consciousness. Accordingly, quantum mechanics is an incomplete theory since it cannot predict the positions of real electrons independent of measurement. By contrast, the Copenhagen interpretation, which is the most widely accepted interpretation (d'Espagnat, 1979; Griffiths, 2005), states that since only measured things are known to exist, quantum mechanics is a complete theory, but elementary particles such as electrons are really not anywhere until we compel them into existence through measurement. I adhere to the realist interpretation and will use it throughout this book.

Light comes from matter composed of atoms, which are in excited states that have more energy than the most stable or ground state (Clayton, 1970). An atom becomes excited when one of its electrons, makes a transition from an orbital close to the nucleus to an orbital farther from the nucleus (Bohr, 1913; Kramers and Holst, 1923). Atoms can become excited by various forms of energy, including heat, pressure, an electric discharge, and by light itself (Wedgewood, 1792; Nichols and Wilber, 1921a, 1921b). Heating limestone ($CaCO_3$), for example, gives off a very bright light. Thomas Drummond (1826) took advantage of the incandescence of limestone to design a spotlight for theatrical productions so that actors could be "in the limelight."

Although the ancient Chinese invented fireworks, the stunning colors were not added until the discovery of the characteristic optical properties of the elements. Thomas Melville (1756) discovered that when he added salts to the flame of a spirit lamp, the color constitution of the light was altered, and he wondered if sunlight might be composed of "such colours and in such proportions as were seen in the lights of salts and burning spirits." John Herschel (1827) and William Henry Fox Talbot (1834c) followed up on this observation and suggested that chemicals might be identified optically by their spectra.

Joseph von Fraunhöfer (1817) found that chemicals burning in an alcohol lamp produced purer colors than colored glasses and used the more homogenous light to characterize the optical properties of glass. He then realized that the lines produced by burning chemicals were related to the dark lines that he and William Wollaston (1802) observed in the solar spectrum (Figure 1.8). Fraunhöfer quantified the positions of the dark lines so that he could make use of the multiplicity of lines to better characterize the optical properties of glass (Anon., 1829,1919; Jackson, 2000). Fraunhöfer identified the major lines, beginning with the red and ending with the violet, with uppercase letters (A, B, C, D, E, F . . .). These lines are still used to characterize optical glass and lenses in terms of dispersion and chromatic aberration (see Chapter 4).

Robert Bunsen and Gustav Kirchhoff used the coal gas powered flame burner Bunsen invented to heat chemicals to incandescence and characterize their spectrum (Kirchhoff and Bunsen, 1860; Roscoe, 1869,1873; Schellen and others, 1872; Schellen, 1885; Sommerfeld, 1923; Pauling and Goudsmit, 1930; Herzberg, 1944; Gamow, 1988). Fraunhöfer's A (759.370 nm) and B (686.719 nm) lines turned out to be due to oxygen, the C (656.281) line was due

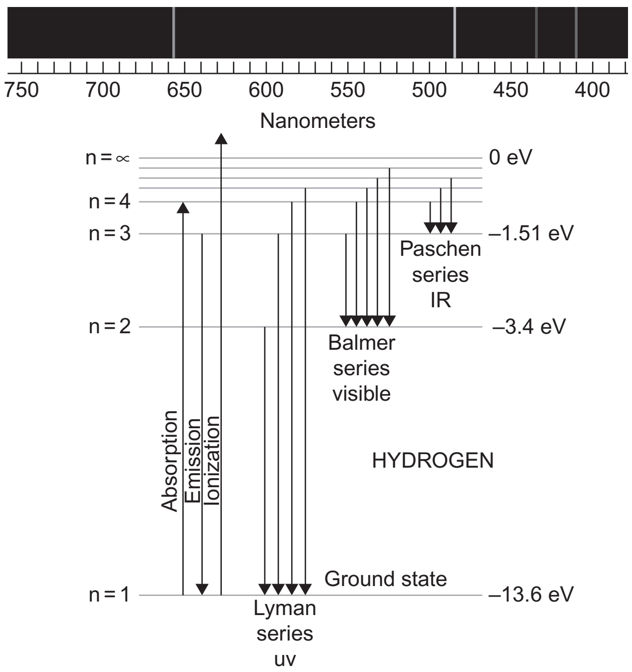

FIGURE 1.9 The bright spectral lines represent light emitted by electrons jumping from a higher energy level to a lower energy level. The dark absorption lines (shown in Figure 1.8) represent light absorbed by electrons jumping from a lower energy level to a higher energy level. The energy levels are designated by principal quantum numbers (n) and by binding energies in electron volts (1 eV = 1.6×10^{-19} J). Transitions in the ultraviolet range give rise to the Lyman series, transitions in the visible range give rise to the Balmer series, and transitions in the infrared range give rise to the Paschen series.

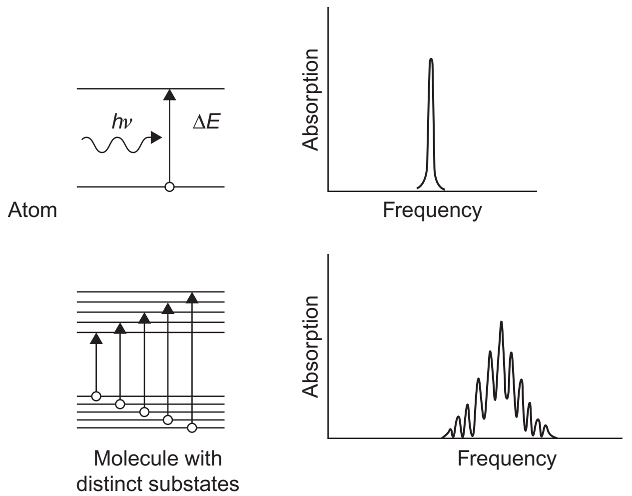

FIGURE 1.10 The absorption (and emission) spectra broaden and the peaks become less resolved as a chemical gets more and more complex. This occurs because a complex molecule can utilize absorbed energy in more ways that a simple molecule by vibrating, rotating, and distributing the energy to other parts of the molecule. Likewise, the various vibrational, rotational, or conformational states of a molecule give rise to more complex spectra. The absorption and emission spectra of molecules are used to determine their chemical structure.

to hydrogen, the D1 (589.592 nm) and D2 (588.995 nm) lines were due to sodium, the D3 (587.5618 nm) line was due to hydrogen, the e (546.073 nm) line was due to mercury, the E2 (527.039 nm) line was due to iron, and the F (486.134 nm) line was due to hydrogen. In the process, Bunsen and Kirchhoff discovered cesium and rubidium using flame emission spectroscopy.

The dark lines of the solar spectrum could then be explained if the elements found on earth were also present on the sun. In order to cause dark lines, these elements must exist in a cool gaseous state in the atmosphere of the sun where they absorb and re-emit individual wavelengths of the continuous blackbody spectrum produced by the sun's incandescence. The lines are dark because the gaseous atoms absorb these wavelengths from one direction but re-emit them in all directions (Kirchhoff, 1860; Shu, 1982; Phillips, 1999). Interestingly, the chemical compositions and velocities of stars and nebulae, as well as the structure of atoms, have been determined to a large degree by analyzing the characteristic spectral lines that are emitted from them (Brode, 1943; Serway et al., 2005).

The spectral lines represent the energy levels of the atom (Figure 1.9). When an excited electron returns to the ground state, the energy that originally was used to excite the atom is released in the form of radiant energy or light. The wavelength of the emitted light can be determined using Planck's Law:

$$\lambda = hc/\Delta E$$

where λ is the wavelength (in m), h is Planck's Constant (6.626×10^{-34} J s), c is the speed of light (3×10^{8} m s^{-1}), and ΔE is the transitional energy difference between electrons in the excited and the ground states (in J). Niels Bohr (1913) introduced the total quantum number (n) to describe the distance between the electron and its nucleus. The multiplicity or fine structure of the spectral lines is caused by the spin of the electrons, while the hyperfine structure is caused by the spin of the nucleus (Tolansky, 1948).

When gaseous atoms are combined into complex gaseous molecules, there is an increase in the number of spectral lines because of the formation of molecular orbitals, which exist in many vibrational and rotational states. Consequently, a gaseous molecule gives a banded spectrum instead of a line spectrum (Figure 1.10). The spectra of liquids or solids are broadened further because a range of transition energies becomes possible as a consequence of the interactions between molecules. As a result, the various bands overlap and the spectrum appears to be continuous with colors that change smoothly from violet to red (Newton, 1730). The intensity of the various colors in a continuous spectrum depends on the temperature of the source (Planck, 1949), the gravitational energy of the source (Turner, 1923; Adams, 1925; St. John, 1928; Hentschel, 1993; Wayne, 2012b), and the relative velocity of the light source and the observer (Doppler, 1842; Stark, 1920). The sun and other stars can be considered to be black body radiators (Thiel, 1957).

Each point of an object emits light when an electron in an atom or molecule at that point undergoes a transition from a high energy level to a lower energy level. The light emitted by the excited electron travels along rays emanating from that point. If the rays converge on our retina or any other imaging device, an image is formed. In order to gain as much information as possible about the molecules that make up each point of the object, we have to understand the interaction of light with the atoms and molecules that make up that point; how the environment surrounding a molecule (e.g. pH, pressure, electrical potential, and viscosity) affects the emission of light from that molecule, how neighboring molecules influence each other, and finally how the light travels from the object in order to form an image.

Knowledge of the physical and chemical basis of light emission has allowed the design of fluorescent proteins and quantum dots made of semiconducting materials that can emit any given wavelength of light (see Chapter 11). By using a number of probes, each of which reports on the localization of a given DNA sequence, peptide sequence, protein, or environmental variable by emitting a different color of light, and computer-assisted spectroscopic detection techniques, one can do multispectral imaging (see Chapter 12) to determine the content and character of each point in a specimen and the spatial and temporal relations between various molecules (Conrad et al., 1989; Waggoner et al., 1989; Haraguchi et al., 1999; Bates et al., 2012).

HOW CAN THE AMOUNT OF LIGHT BE MEASURED?

The measurement of light, known as photometry, calorimetry, and radiometry, involves the absorption of light energy by a detector, and its subsequent conversion to another form of energy (Thompson, 1794; Talbot, 1834a; Johnston, 2001; Johnsen, 2012). A thermal detector, which converts light energy into thermal energy, is a type of thermometer whose detecting surface has been blackened so that it absorbs light from all regions of the spectrum. The temperature increase is related to the light energy, and it is equal to the product of the number of photons absorbed and the energy of those photons (hc/λ).

A thermocouple can be used as a thermal detector that converts radiant energy into voltage. When used this way, it consists of a junction of two metals coated with a black surface whose temperature increases in proportion to the light intensity. When light strikes the blackened junction, a voltage is generated relative to an identical reference junction kept in the dark. Several thermocouples arranged in series to increase the response of the system forms a thermopile. The effect of temperature on voltage was discovered by Thomas Seebeck (1821), and is known as the Seebeck effect.

The bolometer is a thermal detector developed by Samuel Pierpont Langley (1881), the founder of the National Zoological Park in Washington, DC, to measure solar radiation. Bolometers consist of a thin strip of blackened platinum foil or ceramic thermistors whose electrical resistance increases or decreases, respectively, with temperature relative to a reference foil or thermistor kept in the dark.

The amount of light can also be measured using photochemical reactions whose rate is proportional to the amount of light that strikes the chemical substrates. This technique, known as chemical actinometry, can be done by using photographic paper, where the degree of darkening of the silver bromide impregnated paper is related to the amount of incident light (Draper, 1837a,b,c, 1841). I will discuss the use of electrical detectors, including photodiodes, photomultiplier tubes, video cameras, and charge-coupled devices in Chapter 13.

WEB RESOURCES

Anamorphosis in Art

http://www.anamorphosis.com/what-is.html

Optical Illusions

http://www.illusions.org/
http://www.eyetricks.com/illusions.htm
http://www.michaelbach.de/ot/
http://www.echalk.co.uk/amusements/opticalillusions/illusions.aspx
http://www.moillusions.com/
http://www.ritsumei.ac.jp/~akitaoka/index-e.html
http://www.123opticalillusions.com/
http://educ.jmu.edu/~johns2ja/illusion/illusion.htm
http://www.colorcube.com/illusions/illusion.htm
http://www.illusionworks.com/optical-illusion.html
http://www.ted.com/talks/al_seckel_says_our_brains_are_mis_wired.html

Camouflage and Mimicry in Nature

http://somethinbeautiful.blogspot.com/2010/03/animals-camouflage-leaf-ordinary-leaf.html
http://dpughphoto.com/mimicry.htm