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Discovery with the Light Microscope

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We owe our understanding of the modern world to a single instrument above all others – the light microscope. These days, it is often assumed that the electron microscope is at the pinnacle of microscopy, and it seems to have eclipsed the light microscope. Indeed, there are informal reports of academics claiming that light microscopes should now be consigned to the broom-closet, since electron microscopes are so superior. The monochrome images offered by an electron microscope may provide unmatched resolution, but high resolution is not what we need for the great majority of investigations. Only the light microscope can show us the color of our specimens, and color is often crucial. And it is the light microscope alone that can reveal life as it is lived. A variable-pressure scanning electron microscope can briefly show us gray images of the movements of moribund arthropods, though only a light microscope can reveal the voluptuous twisting and turning of living cells as they pursue their complex little lives, the captivating colors of crystals under polarized light, or the selectively stained microorganisms we need to identify.

The majesty of the living cell is our current focus of attention and electron microscopes have no part to play in that. Light microscopes are among the most crucially important instruments in the world of science, and one of the few you will find in every field of investigation. Not only can they solve otherwise intractable problems, but the insights they provide influence the way we interpret the world. A trained and experienced forensic analyst can identify a particle, some strange fiber, a pollen grain, or a fragment of mineral, and solve a crime in an instant. These days, authorities rarely resort to images from light microscopes; they like to have analyses and fancy graphs to illustrate their reports, whereas microscopists recognize reality.

Microscopists are curious; we look differently at the world and are insatiably inquisitive. Indeed, this is how microscopy was born. It was on March 15, 1663, that young Robert Hooke, the 27-year-old curator of experiments at the newly formed Royal Society of London, was presented with a microscope constructed by Christopher Cock, an instrument maker from Long Acre in London (Figure 1.1). Cristopher Cock flourished in the middle 17th century. He pioneered the production of compound microscopes, the details of which appear in Robert Hooke's great work Micrographia, published by the Royal Society in 1665. They were provided with brass fittings and covered in polished shagreen

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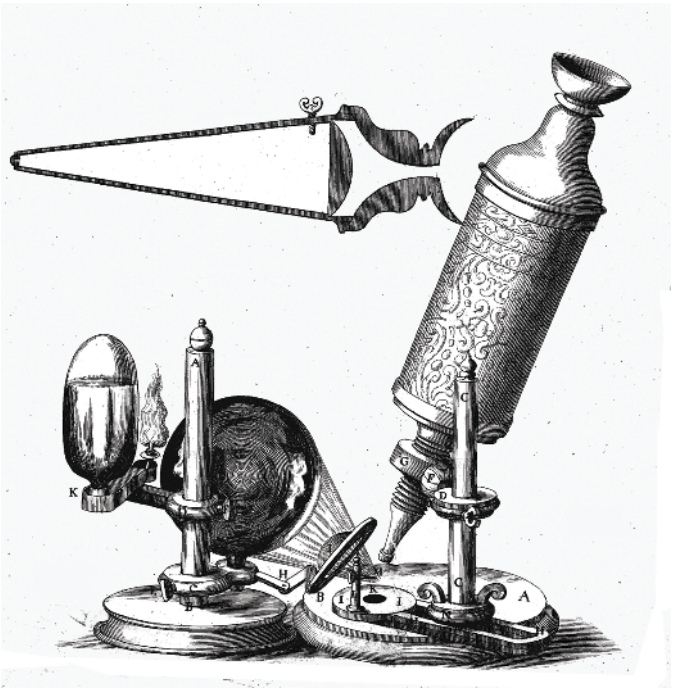

Figure 1.1 Robert Hooke's published engraving of his compound microscope.

or embossed leather. Although impressive as possessions, they gave optical results greatly inferior to those obtained with a single lens.

The Society instructed Hooke to provide weekly demonstrations to the Society's fellows. A man given to enjoying the fine things in life, he sat there with his microscope, and toying with a cork from a bottle of wine. It's strange stuff, cork. It is incredibly light, buoyant, and compressible, yet it readily springs back to its original shape. For all its porous nature, it cannot absorb fluids, so it was unrivaled for stopping up bottles of liquid. Why – since it was so light and so porous – did it not leak? Hooke decided to solve the puzzle posed by cork and wrote: "I took a sharp pen-knife and cut a thin piece of it, placing it upon a black object Plate, because it was itself a white body, and casting the light on it with a deep *plano-convex Glass*, I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb …" He reported that his microscope would "presently inform me" how cork was so light, why it would never "suck and drink in water," and how was it possible to take compression more than any other substance, before it is "found to extend it self [*sic*] again into the same space." These were unique attributes, and Hooke's meticulous investigations provided the explanation. He observed that cork was composed of little boxes, or cells, "altogether fill'd with Air." Cork contained mostly empty space, and very little solid substance. It was the microscope that had revealed the truth.

1.1 Hooke, Leeuwenhoek and the Single Lens

He published his coinage of the word “cells” in his great book Micrographia, published two years later (Hooke, 1665) and the term has come down to us today. But it was wrong. To us, a cell is a living, succulent, microscopic organism and not the empty box that Hooke observed. He was identifying the empty walls inside which living cells had once existed. Far more momentous (though dismissed at the time and ignored until my revelations (Ford, 1989) more than three centuries later) was his observation of living cells in the moss *Funaria hygrometrica*. Although he wisely compared the complexity of a moss plant with that of familiar plants – like a *Sempervivum*, the houseleek – he did not mention the delicate tracery of its component cells, even though they were accurately portrayed in his fine engraving of the moss. Hooke was also the first to document a microbe, when he presented an exhibit of mildew fungi growing on old leather and recorded the details in diligent drawings. One of the paradoxes about Hooke, which scholars missed for centuries, is that you cannot observe with his microscope the fine details that he published in his engravings (Figure 1.2). I have shown that he must have used a simple – i.e. single-lensed – microscope to fill in the details, and confirmation of his methods lurks in the unnumbered pages of the Preface to Micrographia. Hooke explains how to grind and polish a tiny plano-convex lens and mount it in a metal plate. These lenses offer far higher magnification and much improved resolution though, he

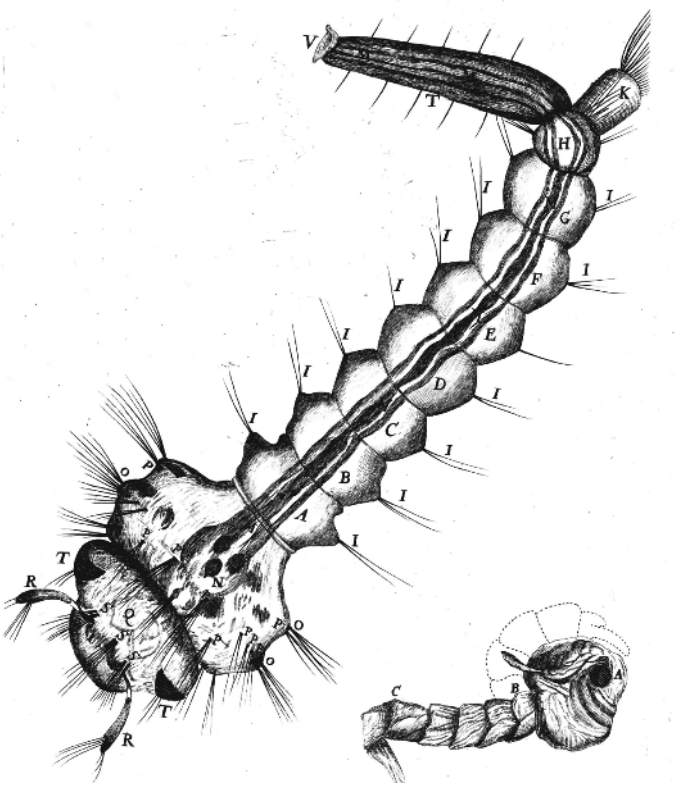

Figure 1.2 Robert Hooke’s detailed engraving of a mosquito larva.

admits, they are "very troublesome to be us'd, because of their smallness, and the nearness of the Object." (Lawson, 2016)

Robert Hooke's superbly detailed engravings were plagiarized over the centuries, and they reveal that he was an extraordinary draftsman. Here we see an aquatic larva of the mosquito Culex. Crucially, I have shown that the precise detail visible in this image cannot be seen with Hooke's microscope. Clearly, he used a single-lensed (simple) microscope – a design for which he published in his Preface – to observe fine structures which he incorporated into the final engraving.

It was this method of making a magnifier that caught the attention of the Dutch draper Thonis Leeuwenhoek, whom we know as Antony (and who added a "van" to his name in 1686, to give himself greater respectability). Although these simple microscopes were problematic, they were cheap and easy to make at home if you were a dedicated enthusiast, and Leeuwenhoek became single-minded in his quest for optical perfection. He had visited London in 1666, when he came across Hooke's great book. It inspired him to take up microscopy, and he was soon making his own little microscopes at home, based on Hooke's design (Figure 1.3).

Figure 1.3 Optical and SEM images of a newly discovered Leeuwenhoek microscope.

*Details of the handmade original Leeuwenhoek microscope can be seen with this uniquely detailed study compiled from over 130 separate images obtained with a light microscope (*Figure 1.3*, left). For the first time, I have imaged the same instrument through a Hitachi S-3400N scanning electron microscope at the Cavendish laboratory, Cambridge University, and we can compare the lifelike image obtained with light microscopes (*Figure 1.3*, left) with the higher resolution of the scanning instrument (*Figure 1.3*, right).*

When Robert Hooke wrote of Pulex irritans, the flea, he did so from personal knowledge (Figure 1.4). The large engraving published in Micrographia, measuring some 43 cm (17 in) long, was often removed from the book and framed for public view. It is extraordinarily detailed – yet, as this micrograph taken with a Hooke-type microscope demonstrates, the features that Hooke depicts are not visible with his microscope. For the fine details he resorted to the use of a single-lens, simple microscope.

To fully appreciate the detailed structure of a head-louse (Figure 1.5), Pediculus capitis, we need to use a simple, single-lensed microscope. In this micrograph, a lens ground by the late Horace Dall – a British optical specialist who ground lenses of the type that

Figure 1.4 Flea through Hooke-type microscope.

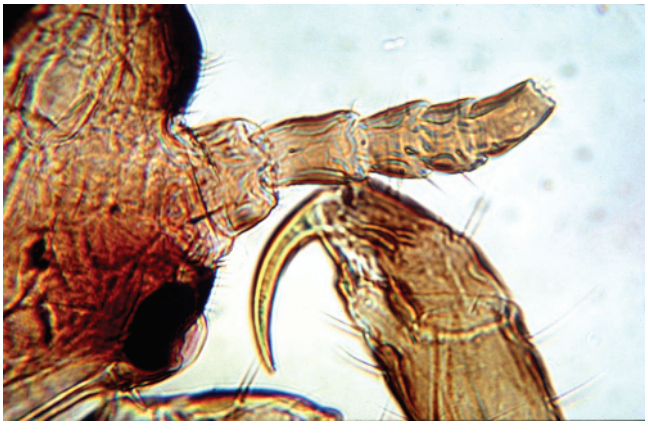

Figure 1.5 Head-Louse through single lens.

Leeuwenhoek used in his research – has been employed to show the greatly improved view that a single lens can provide. The resolution of these lenses is within a factor of four of the achromatic lenses that were to supersede them.

Leeuwenhoek began by faithfully reprising observations described by Hooke in Micrographia. Leeuwenhoek is widely disregarded by much present-day science teaching, though his personal inquisitiveness gave us the first glimpse of cell nuclei and spermatozoa, the discovery of bacteria and protozoa, and he single-handedly launched the science of microbiology. Whereas Hooke concentrated on the study of familiar objects – a magnified razor, a piece of moldy leather, fossilized wood, moss, seeds, and small insects from gnats to fleas – Leeuwenhoek was preoccupied by what the naked eye could not perceive. Hooke, you might say, was a macroscopist [*sic*]; it was Leeuwenhoek who was the first true microscopist.

Between them, these two irascible, independent, single-minded, and diligent investigators laid down the science of investigation using light microscopes to solve problems. Hooke's perplexity over the lightness and porosity of cork – combined with its extraordinary ability to resist penetration by fluids, while springing back to shape after being compressed – made him realize that only a microscope could offer the answer. Leeuwenhoek's curiosity about mucilaginous matter floating in lake-water led him to peer at it under a lens and bequeathed to us the first description of microorganisms in history.

1.2 Single-lens Microscopes come of Age

When Hooke and Leeuwenhoek died, their science died with them. There were no students to follow, and no devotees who would carry on their endeavors. When Carl Linnaeus set out to name and categorize every known form of life from 1735, he used a microscope (I have been to examine it in Sweden) yet he largely ignored the microscopical world and grouped microbes as "Microcosmus" (Ford, 2009). He didn't mention bacteria. A few philosophers recorded magnified images and made useful discoveries, including Marcello Malpighi whose observations of kidney anatomy (the Malpighian corpuscle and the Malpighian tubules) are familiar to present-day students. Jan Swammerdam used careful microdissection to elucidate the structure of insects, and in 1686 a microscope by Joseph Campani of Bologna shows in a woodcut portraying the first recorded use of a microscope in medical investigation (Ford, 2009). A handheld microscope is in use, with an assistant directing candlelight upon the area under investigation.

Giuseppe Campani was a Roman philosopher of the 17th century whose screw-barrel microscope (Figure 1.6, left, enlarged) was the first to be portrayed in medical diagnosis. This engraving of his microscope in use was published in the Acta Eruditorum published in Leipzig in 1686 by Christoph Günther (Mencke, 1686). The investigator is shown using the microscope to scrutinize the details of a wound in a patient's leg, while an assistant holds a concave mirror that reflects candlelight onto the site.

Other writers mentioned microscopy, but their claimed originality was disproved by a comparison between Hooke's and Leeuwenhoek's published illustrations and those in later books (Ford, 2010). Indeed, when Hooke published pictures of snowflakes, he was actually plagiarizing them from an earlier work by Bartholin, and when Leeuwenhoek sent to the Royal Society his reprise of Hooke's demonstrations of cork, elder-pith, bovine optic nerve and the quill of a feather, he did not acknowledge his predecessor's example. Persistent plagiarism is still prominent in today's Royal Society yet has its roots in that body's earliest days (Ford, 2020).

Figure 1.6 First use of microscope in medical investgation (Pictorial Press Ltd/Alamy Images).

When the Swiss naturalist Abraham Trembley came across *Hydra* in the 1730s, he launched into a series of diligent investigations showing how this tiny multicellular animal could be cut, spliced and grafted. Trembley was the first to carry out such extraordinary experiments yet did so without knowing of Leeuwenhoek's descriptions of *Hydra* published in 1677 (Dobell, 1932). His skill at micromanipulation using a microscope, and his remarkable prescience in undertaking far-sighted investigations, have made Trembley the father of experimental biology (Lenhoff *et al.*, 1986).

Simple microscopes of the kind Hooke designed (and which Leeuwenhoek used all his life) were handheld, though Trembley's investigations of the 1740s necessitated having the lens mounted on a stand, leaving both hands free to manipulate the specimen. An articulated arm was one answer to the problem; a fixed lens with a stage and mirror was even better. This idea was taken up in England by John Ellis, who had an instrument-maker named John Cuff produce a portable microscope that could be packed in a small box. It was popular for examining small plant specimens (and so gained the name "botanical microscope") and equally useful for observing *Hydra* and other pond organisms (instruments known as "aquatic microscopes"). The terms are interchangeable. By the early 1820s, in Regency England, these became popular possessions of a growing scientific class and their lenses provided remarkably clear images. One of the investigators who realized their potential was the Scottish surgeon Robert Brown, who named the cell nucleus in 1831 and painstakingly

Figure 1.7 Single-lensed microscope from the 1820s by Bancks and Son.

dissected the ovules of plants to study the process of fertilization. He also documented the ceaseless movement of microscopic particles, due to molecular bombardment, that we still refer to as Brownian motion. His battered and bruised microscope survived the centuries, dismissed by all as crude and incapable of serious microscopical use. Its restoration proved to be a revelation, and the images it generates compare favorably with those from present-day instruments. Single lenses are universally described as producing chromatic, rainbow-hued images, though the limitations have proved to be less pronounced in practice.

Single-lensed microscopes for research were manufactured from brass (Figure 1.7), and supplied with a range of lenses, by Robert Bancks and his son of London for luminaries including Robert Brown, the originator of Brownian motion, who named the cell nucleus in 1828; Charles Darwin, who took his on his voyage aboard the Beagle in 1835; Sir William Hooker, the first director of the Royal Botanical gardens at Kew, England; and George Bentham, the premier systematic botanist of the 19th century.

During his research on plant tissues in 1831, botanist and surgeon Robert Brown examined the upper surface of an orchid leaf under a simple microscope (Figure 1.8). He clearly discerned a small ovoid body within each cell, and named it "an areola, or nucleus." His term "nucleus" has come down to modern science. Others had observed the cell nucleus before (it was first recorded by Leeuwenhoek in the erythrocytes of fish in 1682) though no previous observer had commented upon it, or offered a name.

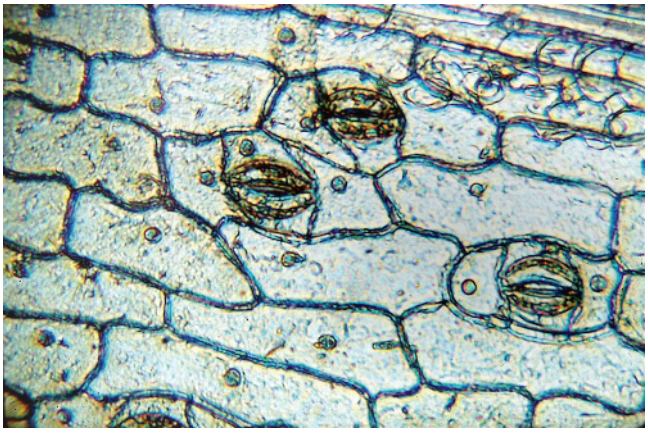

Figure 1.8 Orchid epidermis showing nuclei, under Brown's microscope of 1831.

1.3 Light Microscopes in the Modern Age

The smallest object a Leeuwenhoek lens can resolve measures 0.7 μm, whereas the resolution limit of a corrected microscope lens is >0.2 μm, and it was the introduction of achromatic lenses in 1758, and apochromats in 1763, which first allowed microscopists to approach this limit. It took decades before the advantage was accepted by microscopists (Darwin took a simple microscope on his voyage aboard the Beagle in 1831) but those improved lenses would open a new world to investigators. In 1858, a German anatomist, Joseph von Gerlach, experimentally infused brain tissue in a solution of the red dye carmine, and noted that the tissue when sectioned, showed clearly stained nuclei and he became the first microscopist to recognize the value of differential staining. The biological and medical sciences were not the only disciplines to benefit; in 1863 Henry Clifton Sorby explored metallurgical microscopy and realized the significance of carbon in creating steel from iron. His microscopical investigations led directly to the invention of the Bessemer Converter which underpinned the development of modern steelmaking.

The notion of an insatiably curious investigator, who sees the significance of traces that others cannot perceive, brings to mind Sherlock Holmes. The capacity of this fictitious detective for deriving complex truths from simple evidence is well-known, though he was never depicted using a microscope. The closest we come is in *A Study in Scarlet* (Doyle, 1888) where Arthur Conan Doyle writes of Holmes taking out "a large round magnifying glass" to search for evidence. This image has become associated with Holmes ever since, though a "large round" magnifier has low magnification. The capacity of a lens to magnify is inversely proportional to its surface curvature, and hence its radius, so a small lens can magnify more than a large glass.

Although Holmes's diagnostic skills are fanciful, they are mirrored in the real world of microscopy. The pioneer of forensic microscopy was Edward O. Heinrich of the University of California at Berkeley. In 1923 he was brought in to investigate evidence after a train robbery. He considered statements from witnesses, and examined a pair of dirty overalls recovered from the scene. Grease reported from the fabric proved to be resin from fir trees found in the Northwest, confirmed by wood fragments and pine needles in the right-hand pocket. He also found traces of mustache wax, nail clippings, and cigarette butts ... by the time he'd finished, Heinrich led the police to their suspect after informing them that he was a fastidious, left-handed Caucasian lumberjack some 5 ft 10 in tall with light brown hair and a new coat. A receipt hidden in a pencil pocket even gave a family address. This was the dawn of what we now know as CSI, a subject which has since gained an enormous public following. The annual survey by the Eurodata TV Worldwide organization in 2012 said that "CSI: Crime Scene Investigation" was the most-watched show in the world (Ford, 2015).

Today there is unremitting pressure to ignore light microscopy in a move toward digital diagnosis. It cannot work as well. Nobody can diagnose asbestos fibers in a building – or in a patient – as rapidly and reliably as a microscopist with a wise eye. In an era where tuberculosis is rapidly spreading, there are pressures to use nucleic acid amplification (NAA) tests to spot the causative organism. The commercially available Xpert MTB/RIF tests disposable cartridges of sputum that are analyzed in the GeneXpert Instrument System and can give a result within two hours. These machines cost tens of thousands of dollars and

the cheapest cartridges cost over $10. The light microscope allows a smear to be examined at minimal cost with results in a matter of minutes. *Aspergillus* is regularly identified using an enzyme-linked immunosorbent assay (ELISA) test though most laboratories around the world lack the equipment. Polymerase chain reaction (PCR) tests are also available, though are not reliable. Yet an experienced microscopist can recognize *Aspergillus* spores in seconds, without even needing to stain the specimen.

Microscopists identify pollen grains and can use the characteristic surface profile to diagnose the origins of a specimen (Figure 1.9). They variously possess spikes, protuberances, and surface vesicles. These are from the conifer *Pinus*, and the pollen from those conifers have a unique structure, because attached to each haploid cell are two air-sacs in the manner of water-wings. These serve to provide buoyancy which allows each pollen grain to catch the drafts of air which facilitate their air distribution.

High magnification is not always what we need. Living pond protozoa are among the most enticing organisms to study under a light microscope, as Leeuwenhoek discovered in 1767. The use of low power – typically, less than 10× – can frequently provide a sense of structure that otherwise eludes us.

The sagittal section of a full-term rat embryo has been stained with hematoxylin and eosin (Figure 1.10). It is 15 mm long and is viewed here under low power in a study comprising four separate micrographs. Details, from the anterior cerebellum to the genitals and posterior caudal structures, yet including the kidney and suprarenal, allow us to gain a revealing view of the internal anatomy of the entire embryo. Low-power microscopy is often ignored by researchers yet can provide unique insights.

Pollen grains? We know them: there is no need for some costly analysis. Diatom frustules? They can be characteristic of a given environment and we know how to handle those. Mineral dust, or particulates? They can be easily and quickly characterized. Leaf fragments? Leave them to us. Animal hairs? No problem whatever. Paper fibers? Scraps of soil? Sand? Sawdust? For so many of these, there are costly, tedious, and time-consuming analytical techniques using sophisticated equipment. Yet there is also the knowing eye of a microscopist. Nobody needs to collect genetic data and analyze the genes to know your

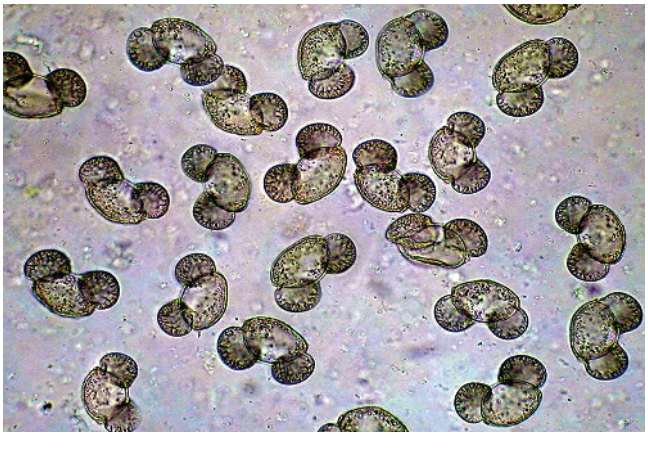

Figure 1.9 Characteristic pollen grains of *Pinus* under a modern light microscope.

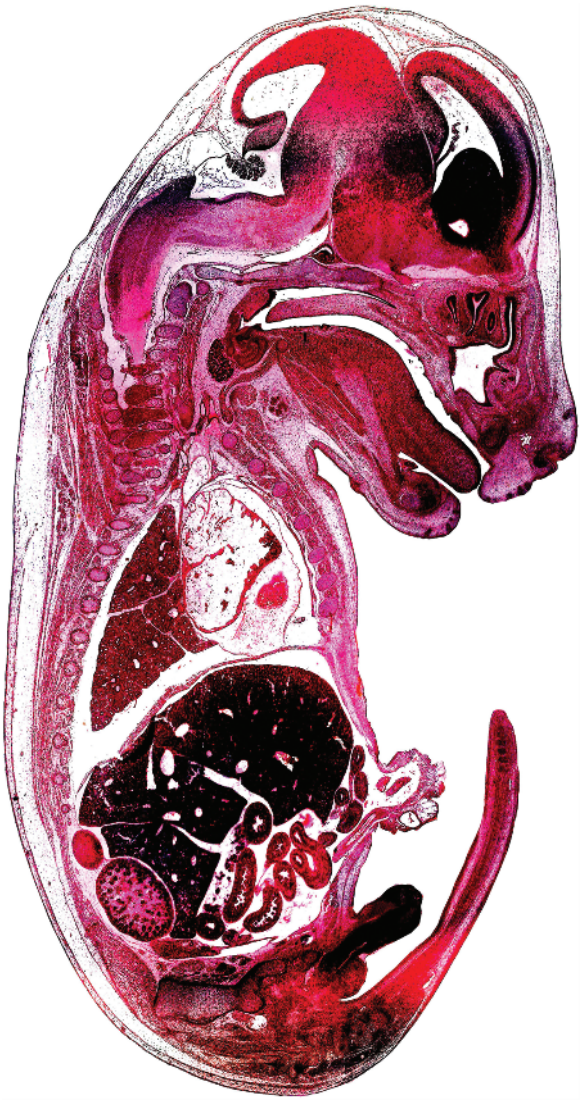

Figure 1.10 The anatomy of a rat embryo under the low-power light microscope.

grandmother when you can recognize her in a crowd. You don't need to order PCR printouts to distinguish an apple from an orange – you know by looking.

Not a week goes by without my using some aspect of microscopy to throw light on a problem, or to elucidate some mechanism that would otherwise elude us. Yet our modern world is hell-bent on a race to digitization and that curious concept of "artificial intelligence" which confers wondrous propensities upon costly gadgets while denying us the freedom to know what we know. I daresay there are apps that can probe our cooking and flash up on a screen when that egg is cooked – but a trained chef just knows. You can go to the laboratory and use a machine to amplify the genes in a plant and plot out its genetic profile, which could eventually be automatically linked to a botanical database that gave you the species.

Or a gardener will just tell you.

And so it is with the light microscope. Learn to use it. Study how to recognize what you see. Cultivate inquisitiveness and objectivity and be prepared to shock other people. Those blebs on the fine finish of your car? Microbes caused them. Why did that engine fail? Bacteria in the fuel. What caused those fish to act strangely? Flickering protozoa in the blood. Why did those rodents lose their fear of predators? Protozoa taking control of their brains. Have I been conned by paying a huge some for this jar of Manuka honey? Certainly, if it isn't rich with Manuka pollen grains. Can you be sure this banknote is a forgery? The printing is perfect! But its paper is made with Asian fibers, which we never use. What's this powder? Are these fibers genuine? Do I make sperm? Is this asbestos? Are these faked? Which is the right fabric? Is that genuine starch? What's in my urine? Have I got leukemia?

Ask a microscopist. They'll tell you. It won't be complicated, and it won't take long.

Could apps replace this skill? If you cannot find a light microscopist, and need some indication of what you've found, then apps will one day find the way. They do not yet exist, but within a few years they certainly will. We are told that they work through "artificial intelligence" but that's the wrong word. They aren't intelligent. What they offer is digitized automation so, if you wish to perform a routine assessment – a regular blood count, say, or a routine check for particulate contaminants – then an app will save time and tedium. But they won't eliminate the need for experienced microscopists. They alone will know where to search for what you need, how to collect the sample, how to prepare and mount the specimen, and will tell you what's there.

Apps are destined to lessen the drudgery of routine, but they aren't people. BloomOptix is the first for microscopy and identifies algae using data from over 200 microbial blooms. It is said to be 94% accurate. I have a state-of-the-art plant identifier on my phone which assures me that a pomegranate tree was a myrtle plant. I took a picture of a lei from Hawaii, an artificial garland of silk flowers, which the app assured me was the Christmas cactus *Schlumbergera*. Some years ago, a team of geneticists were puzzled by what seemed to be a miniature crossword puzzle appearing in a chromosome preparation. I recognized it as a fragment of a diatom (Ford, 1986). Diagnostic microscopy is always a challenge, and the results often pose the most unexpected juxtaposition.

The next decade will inevitably bring more automation, and an increased demand for graphs and digital data. Yet behind it all lies the microscopist. The light microscope will always entertain, illuminate, confound, and exasperate; yet – no matter how much the apps assist us – it will still be the keen eye, and that wise mind, which alone will solve the greatest diagnostic problems we face in the future.

About the Author

Brian J Ford is world-renowned as a leading microscopist, and the author of hundreds of research papers (and many books) on microscopes and microscopic life. He is also a popular television broadcaster and an international lecturer. His research has covered topics ranging from blood coagulation to paleontology, and from food science to the study of intelligence in living cells. Professor Ford has extensively researched early microscopy.

He discovered that Leeuwenhoek's original specimens still existed, was the first person to examine them through an original lens, and has recently identified two previously unknown Leeuwenhoek microscopes. He also restored Robert Brown's microscope to use and took the first photographs through it. Professor Ford has connections with several universities and is based in Cambridgeshire, England.

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