
Introduction: The Optical Microscope in Cell Biology

The simplest microscope is a single lens. The word “lens” is Latin, and lenses seem to have been known to the ancient Greeks and Romans, as well as to the Chinese, for thousands of years. Lenses were used as *burning glasses* to start fires by focusing the light of the sun on kindling, and in medieval Europe (and much earlier in China) they were used to correct vision. Yet the idea of using them to look at small objects does not seem to have occurred to the ancients. The problem was philosophical, not technical—they simply had no concept that there was anything significant to see beyond the limits of the human eye. The world, to them, was what they could see.

It was not until 1656 (according to the *Oxford English Dictionary*) that the word *microscope* entered the English language, and its equivalent is recorded in Italian at around the same time. This is far more significant than the coining of a new word; it reflects the new way of looking at the world that emerged in the Renaissance and grew into the Age of Enlightenment. There was a whole cosmos to study with telescopes—a universe hugely larger than that imagined by the ancients. And then there was the microcosm—the world below the scale of human sight, studied with the microscope.

A book was published in 1665 that changed the way people looked at the world. It was the first, and most famous, book on microscopy ever written: Robert Hooke’s *Micrographia*, published by the Royal Society. Hooke was not confident enough to use the new word *microscope* on the title page, referring instead to “physiological descriptions of minute bodies made by magnifying glasses.” But what he used was a compound microscope, with a focusing system, illuminator, and condenser, all recognizable albeit primitive. It was not very good, but one of his observations had great significance—he called the compartments he could see in cork “cells.” If fact, cells are also clearly shown in his drawing of a nettle leaf, though he did not comment on them in the text.

Then, and for at least two hundred years afterward, the *simple microscope* consisting of a single lens was also the best microscope, able to give better resolution than *compound microscopes* using multiple lenses such as Hooke’s. With the aid of very carefully made single lens microscopes, Hooke’s contemporary, the Dutch naturalist Antonie van Leeuwenhoek, was able to take his microscopic studies to a level of resolution an order of magnitude better than Hooke’s. van Leeuwenhoek saw and described spermatozoa, red blood corpuscles, and bacteria more than 300 years ago. (He took pains to explain that the sperm “were obtained from the overplus of marital intercourse and without committing any sin”.) It is impossible to overestimate the significance of these discoveries. Imagine a world in which nobody knew what caused disease, or how conception worked, or what blood did. Van Leeuwenhoek did not answer these questions, but he made them answerable.

Compound microscopes were used for greater convenience and versatility, but not until the late 19th century could they surpass simple microscopes in performance. They were inferior because of the aberrations (defects) of the lenses. The more lenses that were added to a microscope the worse it got because each one magnified the level of aberration. This topic is dealt with in detail in Chapter 7.

The last person to make major scientific discoveries with the simple single-lens microscope was the legendary botanist Robert Brown in the early 19th century. His name is perpetuated in Brownian motion, which he discovered (and understood), and he also studied fertilization in plants, describing the pollen tube and its function. In the course of this study—almost in passing—he described the cell nucleus. It was a tiny part of a very long paper, but his contemporaries spotted it and took note.

Between van Leeuwenhoek and Brown lay a timespan of over 100 years, and during that time compound microscopes were mostly the playthings of wealthy amateurs who used them mostly as expensive toys. Their quality simply was not good enough for science. This does not mean that the problems were being ignored.

In the 1730s Chester More Hall, an English barrister, set out to construct an achromatic lens, free from the color fringing that ruined the performance of current lenses. He realized that what was needed were two media of different dispersion, and fortunately for him a new glass ("flint glass," with a high lead content) had just been produced with a higher refractive index and dispersion than traditional "crown" glass. He would need to combine two lenses: a strong positive one of crown glass and a weak negative one of flint glass. To test his idea he needed to get these lenses made, but he was afraid that whoever made them for him would pinch the idea. So he commissioned two different opticians to make the two lenses. Unfortunately, both opticians subcontracted the job to the same lens maker, George Bass. Bass and Hall made several achromatic telescopes for Hall's use, the first in 1733, but neither commercialized the invention. The telescopes remained in existence, though, so Hall's priority was clearly proven.

Bass kept quiet about Hall's achromatic telescope lenses for 20 years, but then he discovered that the microscope and telescope maker John Dollond was experimenting along similar lines. Bass told Dollond about the lenses he had built for Hall, and Dollond promptly patented the idea and started production. Other optical manufacturers naturally disputed the patent. The court ruled that although Hall's priority was clear, Dollond deserved patent protection for his work in bringing the lenses to production and making them known to the world, so the patent was upheld. The company, now called Dollond & Aitchison, is still in business. From about 1758 achromatic objectives for telescopes, newly designed by Dollond but based on Hall's principles, were generally available. However, attempts to make such lenses for microscopes proved too technically difficult at the time, and it was not until the 19th century that achromatic microscope objectives became available.

The other major optical problem was spherical aberration. This can be minimized in a single lens by making it a meniscus shape. Van Leeuwenhoek discovered this empirically and Hall's first achromats also deliberately used this design to minimize spherical aberration. However, although this partial correction is not too bad in a telescope, it is not adequate in a microscope working at high magnification.

Joseph Jackson Lister, father of the surgeon who introduced antiseptic surgery, set out to solve the problem in the early 19th century. He was a wine merchant and a Quaker (an odd combination) and this was only a hobby, but his science was impeccable, and his design principles are still the starting point for all microscope objectives. His first corrected microscope was built for him in 1826, and his paper was published in the *Philosophical Transactions of the Royal Society* in 1830. Optical manufacturers did not immediately beat a path to his door, but with some persuasion Lister got the large optical firm of Andrew Ross to take over the manufacture of his corrected lenses. Ross also designed a much more rigid microscope so that the high resolution these lenses produced could be used in practice.

Lister and Ross took the design one stage further by introducing a correction collar to adjust the spherical aberration correction for use with or without a cover slip, or with different thicknesses of coverslip, and this was published in 1837. It became a must-have feature so some less reputable manufacturers provided a collar that did not actually do anything, or even just engraved markings without anything that turned. The compound microscope was now a fit tool for scientists, and Lister himself was one of the first to put it to work. He showed that red blood cells (first seen by van Leeuwenhoek) were biconcave disks and discovered that all muscle was made up of fibers.

Two German scientists, Matthias Schleiden and Theodor Schwann, also eagerly adopted the new microscopes. In 1838 Schleiden (then professor of botany at the University of Jena) wrote *Contributions to Phytogenesis*, in which he stated that the different parts of the plant organism are composed of cells or derivatives of cells. He thereby became the first to formulate what was then an informal belief as a principle of biology equal in importance to the atomic theory of chemistry. He also recognized the importance of the cell nucleus, discovered in 1831 by the Scottish botanist Robert Brown, and sensed its connection with cell division.

Schwann studied medicine at the University of Berlin, and while there at the age of 26, discovered the digestive enzyme pepsin, the first enzyme ever isolated from animal tissue. Schwann moved to Belgium, becoming professor first at Louvain (1838) then at Liège (1848). He identified striated muscle and discovered the myelin sheath of nerve cells (Schwann cells are named after him). He also showed that fermentation was the product of a living organism. Schwann's *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstume der Tiere und Pflanzen* (1839; Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants) extended his friend Schleiden's work into a general theory applying to plants, animals, and protists.

The framework for cell biology was now in place, but the optics of the microscope were still not fully understood, and many microscopists were frustrated at not being able to obtain the results they thought they should get. Physicists were working on the problem. George Airy was an astronomer (in due course Astronomer Royal) who showed that the image of a point source formed by a lens of finite diameter was a disk with halos around it whose properties depended entirely on the size of the lens. This was a key point in understanding the performance of a microscope, but Airy was perhaps not the man to take it further. He was a vain and highly opinionated

man who refused to accept such major breakthroughs as the discovery of Neptune and Faraday's electrical theory.

John Strutt, Lord Rayleigh, was the man who saw the use of Airy's discovery. He was a brilliant scientist who first made his name in acoustics but then moved into optics and explained how the wave nature of light determined how it was scattered (Rayleigh scattering). He also discovered the element argon. In microscopy he gave the first mathematical analysis of resolution, defining a resolution criterion based on the Airy disk and showing how it was determined by the numerical aperture of the objective.

The man who more than any other completed the evolution of the microscope was Ernst Abbe, a junior professor of physics at the University of Jena, who joined the optical company founded by the university's instrument maker Carl Zeiss in 1866, became a partner in 1876, and took over the firm after Zeiss' death in 1888. Abbe realized that Rayleigh's treatment was not correct for the common case of a specimen illuminated by transmitted or reflected light, and developed his diffraction theory of image formation, which for the first time made the significance of the illumination system clear. He also designed a more perfectly corrected lens than any before, allowing microscopes for the first time to actually reach the theoretical limit imposed by the wavelength of light.

At this time, the end of the 19th century, the microscope had reached its limit. To some, this was the end of the line but with hindsight it was a boon, for it ended the quest for more resolution and set scientists on the road to expanding the capabilities of the microscope. This was the golden age of the histologist; microtomes, too, had been perfected, a wide range of fixatives and stains was in use, and superb images were obtainable of fixed, stained sections. However, cell physiologists wanted to understand the workings of the cell, and being restricted to dead, sliced material was a serious limitation.

The 20th century brought a revolution in what could be studied in the microscope. Fluorescence came early, introduced by August Köhler, the same man who gave us Köhler illumination, though it was another 60 years before the improvements introduced by the Dutchman J.S. Ploem made it widely popular. Zernike's phase contrast (yet another Dutch invention), followed by Nomarski's differential interference contrast, gave us for the first time convenient, effective ways to study living cells. Polarized light could reveal structural information about cell components that lay below the resolution limit. Midway through the century, the optical microscope had become a serious tool for the cell biologist. Then came confocal microscopy, and multiphoton, and a huge expansion in the realm of optical imaging tools in biology.

But they do not belong in the introduction; these techniques are what this book is about.