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Microscopy, Optical

The discovery of how to grind glass lenses to magnify tiny objects was one of the critical first steps in the Scientific Revolution. Seventeenth-century natural philosophers hoped that the microscope might reveal the "corpuscles" thought to be the elementary constituents of matter. When this proved not to be the case, interest in microscopy ebbed for almost two centuries. In the late 19th century, optical microscopy rebounded as mass-produced instruments with fewer distortions became available. In the 20th century, other techniques of magnification—such as electron microscopy—began to compete with optical microscopes. Because of its first-mover advantage and continuing improvements, however, optical microscopy has remained crucial to industry and research even in the era of nanotechnology.

Hooke and Leeuwenhoek

People have peered through quartz or glass to magnify objects since antiquity. Like alchemy, astronomy, algebra, and much of medicine, classical knowledge of lenses was preserved in the Arab and Muslim world. It was only in the 14th century that Western Europeans figured out how to grind lenses for spectacles. Two centuries later, Dutch lensmakers had learned to combine multiple lenses, thereby inventing the telescope and the microscope.

Italian natural philosophers, including Galileo, took up microscopy in the 1620s, but elsewhere it was not an important tool of the Scientific Revolution until the 1660s. At that point, alchemists and philosophers of matter such as Robert Boyle came to believe that the microscope could provide direct evidence for "corpuscles," the particulate constituents of matter that—perhaps through differences in shape—accounted for observed qualities such as the taste and hardness of materials. Boyle's assistant, Robert Hooke, published a beauti-

fully illustrated compendium of microscope images, the *Micrographia* of 1665. Boyle also encouraged an extraordinarily gifted Dutch lensmaker, Antoni van Leeuwenhoek, whose keen eyesight and exquisite simple microscopes offered resolution of the microscale that was unsurpassed until the 19th century.

Abbe and Aberrations

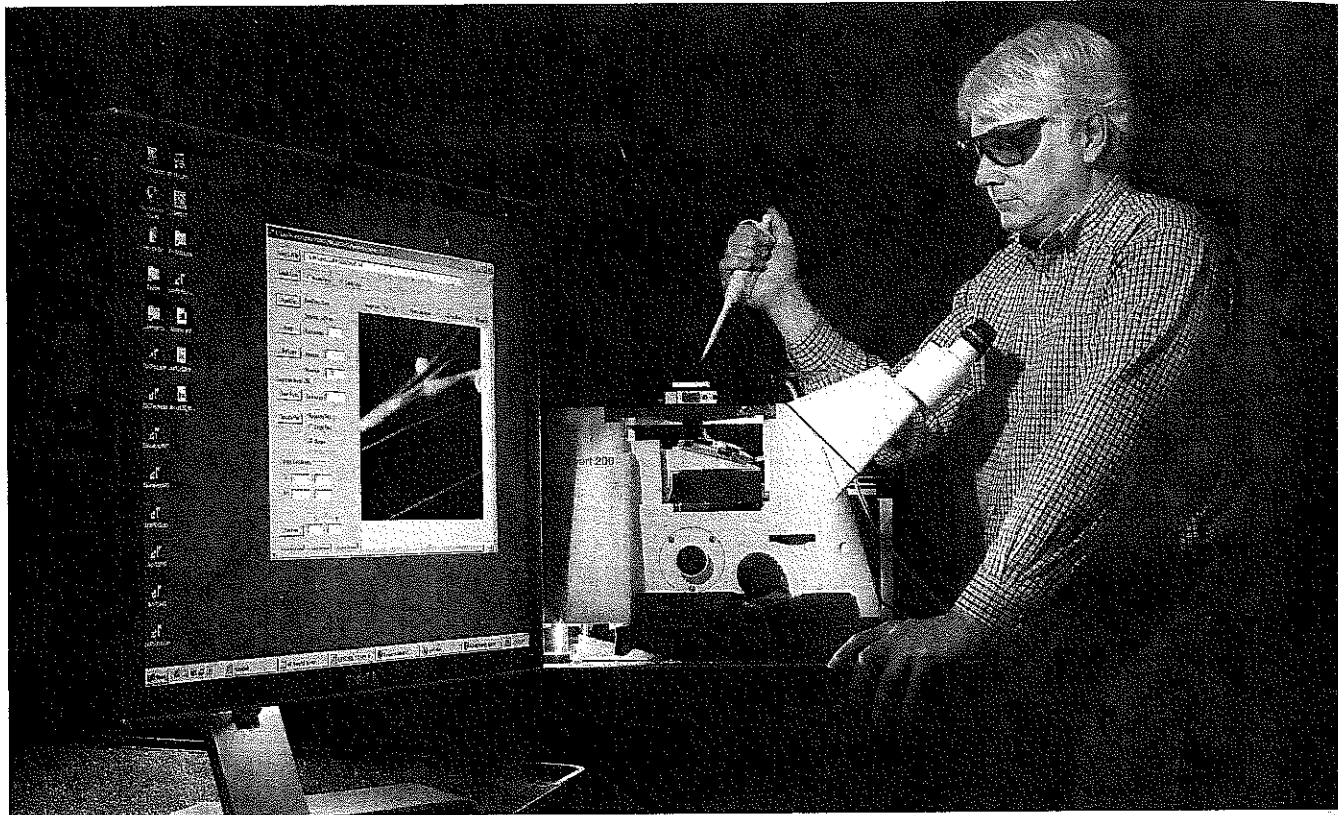
By 1700, though, it was obvious that microscopes would be unable to resolve the elementary constituents of matter, and natural philosophers rapidly lost interest. Microscopes contributed little to science for the next century. However, technical improvements starting in the 1820s—particularly new lenses to correct for spherical and chromatic aberration, and new dyes to stain biological samples—reignited interest, particularly from botanists and zoologists.

In the 1870s, Ernst Abbe, a physicist working for the Zeiss workshop of instrument makers, developed a theoretical understanding of optical microscopy that has defined the technique ever since. At the time, Abbe's work was probably meant simply to advertise Zeiss' microscopes as having a scientific underpinning. Over the medium term, Abbe guided Zeiss to more powerful instruments. In the long run, though, Abbe's work established a limit to improvements in optical microscopy. No matter how many aberrations microscope designers overcame, the smallest object a light microscope could resolve would be directly proportional to the wavelength of the light shining on the object, and inversely proportional to the sine of the index of refraction of the material from which the microscope's lenses were made.

Abbe's limit implies that even the best optical microscope will be unable to distinguish features less than about 200 nanometers apart. This would seem to bar optical microscopy from use in nanoscience. It also implies a limit to the use of optical lithography (the inverse of microscopy) in microelectronics manufacturing. Yet ingenious design gradually overcame Abbe's limit in both microscopy and lithography.

Competition and Continuing Improvement

In the 20th century, once quantum mechanics demonstrated the wave-particle duality, some researchers saw that a way around the Abbe limit was to "shine" electrons on a sample rather than optical photons—since the electron's wavelength is more than three orders of magnitude smaller than that of visible light.



A researcher positions a sample on the spectrally resolved, confocal imaging microscope. Confocal microscopy has allowed light microscopists to achieve somewhat better resolution than that defined by the Abbe limit.

Yet, despite the tremendous gains in resolution achieved by electron microscopy and other techniques, optical microscopy is still a ubiquitous and important part of research, particularly in the life sciences. This is partly because light microscopes are cheap and easy to use, and partly because many biological samples are insulating and therefore difficult to adapt for electron microscopy (often they must be coated with a layer of gold).

Optical microscopy is still relevant to nanoscience, however, because it is *still* improving, four centuries after scientists started using it. Perhaps the most important improvement in the past three decades has been confocal microscopy, in which a pair of pinhole apertures is used to collect only in-focus light in the focal plane—thereby allowing both a higher-resolution image, and greater three-dimensional imaging capability. Because confocal microscopy usually relies on fluorescent samples, it is a powerful technique in the life sciences, where particularly biological structures (such as expressed genes) can be tagged with a fluorescent marker. Confocal microscopy and other variants have allowed light microscopists

to “cheat” and achieve resolution somewhat better than that defined by the Abbe limit.

See Also: Microscopy, Electron (Including TEM and SEM); Microscopy, Exotic; Microscopy, Scanning Probe.

Further Readings

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