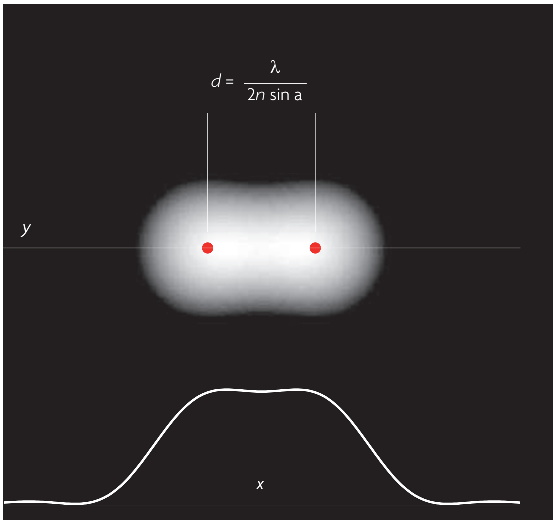
MILESTONE 3

Knowing the limit

Without light microscopy, our knowledge of 'little biology' would be severely impaired. The ability to observe migrating cells, examine the distribution of organelle populations or predict putative protein interactions (based on proximity) drive modern cell-biological research. However, these observations have physical limits, governed by the properties of light, which consequently restrict our view of this cellular world.

In light microscopy, when light passes through an opening it is diffracted, affecting the spatial resolution or, in other words, the smallest separation that two objects can have and still be discerned. When this opening is a lens, the diffraction pattern created by light passage through the illuminated circular aperture appears as an 'Airy disc', as described by George Airy in 1835. Later, the mathematical foundations for quantifying diffraction-limited microscopy were noticed by Emile Verdet but fully described and formalized by Ernst Abbe.



Blurred by diffraction, the image of two point objects (red) can just about be resolved at distance d. Line profile (bottom) quantifies the brightness along the direction of separation. Diagram courtesy of S. Hell, MPI Biophysical Chemistry, Göttingen, Germany.

In his landmark paper of 1873, Abbe reported that the smallest resolvable distance between two points using a conventional microscope may never be smaller than half the wavelength of the imaging light. Although no mathematical equations actually appear in the paper, Abbe stated in this and following papers that the resolution was limited by diffraction to half the wavelength modified by the refractive index of the medium and the angle of the cone of focused light. Based on the resulting equation, one can improve spatial resolution by using light with a shorter wavelength (for example, ultraviolet), but in biological samples, this is undesirable because of the greater potential for sample damage and increased light scattering within a tissue. Reciprocally, longer wavelengths improve tissue penetration at the expense of point separation.

Abbe's mathematical foundations of image formation and lens aberrations provided for the proper design of microscope lenses, accomplished in collaboration with Carl Zeiss and Otto Schott. Abbe's quantitative insights greatly enhanced the quality of microscope optics, contributing enormously to improved data collection and an enhanced user experience for the microscopist. Of course, the success of Abbe, Zeiss and Schott in designing lenses also had an enormous impact on the eventual success of the microscopy manufacturer Carl Zeiss itself. But besides these tangible legacies, another long-lasting legacy brought about by Abbe's work included the establishment of physical boundaries in imaging for quite some time. In fact, Abbe's study influenced the field so greatly that very few attempts were made to overcome the diffraction limit, despite the increasing necessity to enhance resolution and to improve the visualization of cellular structure. Rather, other techniques were developed to provide these data, such as replacing photons with electrons in the development of electron microscopy. Only more recently have scientists revisited Abbe's limit and successfully increased the resolution of light microscopy through a variety of innovative strategies (see MILESTONE 21).

> "… Abbe's study influenced the field so greatly that very few attempts were made to overcome the diffraction limit…"

Noah Gray, Senior Editor, Nature

PRIMARY REFERENCE Abbe, E. Beiträge zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. *Archiv für Mikroskopische Anatomie* **9**, 413–418 (1873)

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