MILESTONE 5

Finding phase

In 1930, physicist Frits Zernike was studying the optics of diffraction gratings when his lab obtained a large concave grating. Owing to its size, Zernike had to position a small telescope 6 metres from its surface to visualize the light patterns created by the gratings. Normally, this resulted in a striped pattern, the effect of seeing a principal spectral line of direct light and weaker spurious lines of diffracted light to the left and right caused by slight imperfections associated with the gratings. But when Zernike focused his telescope on the gratings themselves, something surprising happened — the striped patterns disappeared. Determining why this occurred would change how microscopists look at cells and earn Zernike the 1953 Nobel Prize in Physics.

Through a series of experiments, Zernike came to understand that the direct light and the diffracted light were actually in different phases. When the telescope was focused on the gratings, the resulting image, created through interference, was rendered invisible because, unlike changes in amplitude or brightness, the eye cannot pick up phase differences. But Zernike knew that the phase differences contained information about the object of interest, and realized that he would need an adequate reference surface if he were to make use of it.

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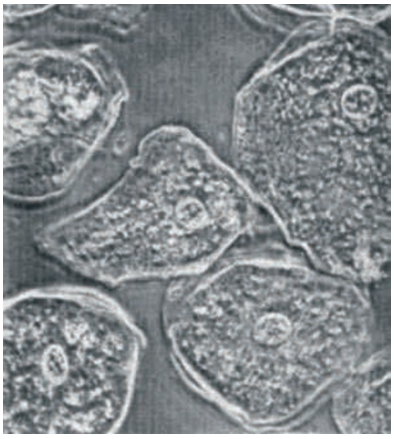
Zernike then recalled the 1900 work of Lord Rayleigh, who described an operation to make shallow etchings in glass with very dilute hydrofluoric acid. Using this approach, Zernike created 'phase strips', in which the direct light hit a thin etching on a glass surface while the diffracted light passed through the glass. The result was to shift the direct light by 90°, making it darker than a uniform background of coherent light. When Zernike added a phase strip to his telescope, the striped pattern on the grating reappeared.

Zernike immediately saw the potential of translating his discovery to the world of microscopy, where transparent objects found in cells had previously been compared to gratings. By placing his phase strip in the focal plane of a microscope, similarly to the way in which he had used it with the telescope, Zernike was able to visualize otherwise transparent objects without the need for a stain. Zernike published a description of his method, which he coined 'phase contrast', in 1935.



Negative phase-contrast micrograph of epithelial cells from the mouth. Image is reproduced, with permission, from L. C. Martin *Nature* **159**, 827–830 © (1947) Macmillan Publishers Ltd. All rights reserved

Shortly after his discovery, Zernike excitedly demonstrated the method to microscope maker Carl Zeiss, who were less than enthusiastic about its potential at first glance. It took another 10 years before companies started manufacturing microscopes with the ability to perform phase contrast, but today most high-end microscope systems have this capability, enabling researchers to see cellular structures in real time without the need for specialized stains or dyes.

Nathan Blow, Technology Editor,
Nature and Nature Methods

PRIMARY REFERENCES Zernike, F. Das Phasenkontrastverfahren bei der mikroskopischen Beobachtung. *Z. technische Physik* **16**, 454–457 (1935) | Zernike, F. Phase contrast, a new method for the microscopic observation of transparent objects. *Physica* **9**, 974–986 (1942)