50 Years Ago: Frits Zernike (1888-1966) Got the Nobel Price in Physics for the Development of the Phase Contrast Method

Starting from diffraction experiments, Ernst Abbe in 1873 developed the theory of image formation in the microscope, which provided a scientific basis to the designing and manufacture of microscopes. With the introduction of apochromatic lenses and oil immersion, the light microscope reached the theoretical limit of resolution of 200 nm. Another milestone in the history of microscopy was the optimized illumination system devised by August Köhler in 1893, which allowed microscopic specimens to be imaged with absolute homogeneity and best possible contrast.

Whereas Abbe's studies were mainly focused on absorbing objects, the Dutch physicist Frits Zernike looked into the formation of images of phase objects, i.e. objects that were non-absorbing but influenced the phase of the light transmitted by them. Zernike realized that the diffraction pattern formed of such objects in the back focal plane of the objective differs characteristically from the pattern formed by absorbing objects. This discovery led to his development of the phase contrast method. In 1936, he collaborated with the Carl Zeiss corporation in Jena to develop the first prototype of a phase contrast microscope. The phase plates were arranged in a special tube, in a plane that is conjugate to the back focal plane of the objective so as to be easily interchangeable and centerable in experiments. In any standard batch-produced phase contrast microscope of today, the phase annuli are permanently mounted inside the objectives.
In the late thirties and early forties of the 20th century, A. Köhler, W. Loos and K. Michel in Jena made the first application studies. Michel, a biologist, first applied the method to the presentation of chromosomes in live cells. The stages of meiotic cell division in the testicles of grasshoppers proved particularly suitable objects: „The chromosomes appear with unusual clarity. Also, the mitochondria are excellently visible.“ (Michel, 1941). Another object studied were the salivary gland chromosomes of the larvae of the non-biting midge species *Chironomus*. Here again, the result exceeded what was anticipated.
As early as 1941, Kurt Michel began documenting the mitotic processes involved in cell division by time-lapse cinematography. He developed a microcinematographic system with a 35mm Debrie camera. The film entitled „Meiosis in the spermatogenesis of *Psophus stridulus L*.“ (a locust) was published by the Institute of Scientific Cinematography in Göttingen in 1958; it was used in academic teaching for many years.
The phase contrast method increasingly gained ground in medical research and routine. It allowed progress in the study of unstained, live specimens, especially in cytology, hematology, bacteriology and parasitology. In gynecology, phase contrast microscopy is employed for fast diagnoses on outpatients (Runge, Vöge, Haselmann, Zinser, Stoll et al.). The advent of inverted microscopes, micromanipulation and microinjection of cells and tissues, and microdissection of chromosomes further added to the importance of the method. Differential interference contrast (DIC), a method introduced by Nomarski in the late nineteen-sixties, complements phase contrast, especially with specimens of greater thickness. The advancements achieved with the phase contrast method in biology and medicine were of such far-reaching significance as to earn Frits Zernike a Nobel Price in physics in 1953. Phase contrast microscopy still is well established in routine and research. It is used for optical contrasting especially in „life cell imaging“ in conjunction with multiple fluorescence techniques, and in confocal microscopy.

Dr. Heinz Gundlach

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