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Celebrating 50 years of Live Cell Imaging

Carl Zeiss UK and The Royal Microscopical Society, London, 15 October 2003

by Mark Burgess
(Executive Editor)

In 1930, Frits Zernike developed a way of making the invisible visible: he had perfected a method for the examination of living, unstained cells. The human eye and brain are good at distinguishing the amount of light (contrast) or its wavelength (colour), but are unable to distinguish differences in phase (there is no common name for it). Zernike had invented a technique that would make the invisible phase difference of a living cell a visible difference in light and shade. He took his invention, which he called phase contrast, to the greatest microscope manufacturer, Carl Zeiss, in 1932. Zeiss told him to get lost.

"If this had any practical value, we would ourselves have invented it long ago," Zernike was told by one of Zeiss' experts, and so he returned home to Holland. Zeiss, of course, believed that all revelation had ended with the death of Ernst Abbe. Yet Abbe's theory of image formation by microscopes has some serious omissions that begin to create difficulties as soon as the microscopist strays from the object of regular structure which, with the point source of light, underpins the hypothesis.

Portrait of Zernike, taken to accompany the award of the Nobel Prize

Generally, the living cell is not a regular object. In fact, most look structureless. But it affects the phase of the light that passes through it; although it might not absorb the light, it can slow it down. By combining light that had been affected by the object with light that had not passed through it, Zernike produced interference artefacts that made the phase difference visible.

Zeiss relented and invited Zernike back. Köhler and Siedentopf built Zeiss' first working prototype in 1936. In this, the back focal plane of the objective was situated far into the body tube so that they could test different phase plates. Lines and crosses were tried before Zeiss settled on the annulus that has becomes standard ever since.

Having proved that it could work, Zeiss forgot all about phase contrast and concentrated on war work. It was not until 1941, with the Nazi regime looking for any invention that might help the war effort that phase contrast was re-discovered and the first phase-contrast microscope, the Lumiplan, was manufactured. Meanwhile, of course, Zernike's country was occupied and its citizens subjugated.

In 1953, Frits Zernike received the Nobel Prize in Physics for his invention and, in October of this year, Carl Zeiss decided to make amends and celebrate the 50th anniversary of this belated recognition.

The meeting, held jointly with The Royal Microscopical Society, was titled 'Celebrating 50 years of Live Cell Imaging' and brought together scientists who investigate the mysteries of the cell using the optical microscope working at its limits.

The introduction given was by Tim Hunt (Cancer Research UK), who, like Zernike, is a Nobel Laureate and is someone who has known rejection. His famous paper on cyclins provoked one referee to comment: "wild speculation based on faulty logic". Tim cheerfully confessed that he was mainly a biochemist and had not even known about Köhler illumination until 1974, but he gave an outsider's view of the difficulty of fundamental optical phenomena. He remarked "physics has simple questions, but hard answers, while biology has complex questions with simple answers".

The next speaker, Victor Small (Austrian Academy of Sciences,

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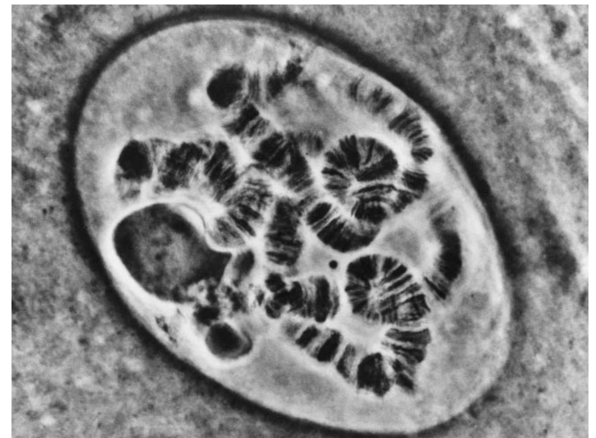
Salzburg, Austria), uses phase contrast to probe living cells for the secrets of how they move and die. He is particularly interested in the motility of cells. They expand at the front and retract at the rear: how are they attached? How do they move? In a series of stunning moving images, he showed cells moving under phase-contrast microscopy. Microtubules snaked towards adhesion sites, as if the cell was dragging itself along by its fingertips. How are the microtubules guided? Tensile stress in the actin cytoskeleton has an important role, but more work is needed.

The cell edge was the object of the next presentation. Alexander Verkhovsky (Swiss Federal Institute of Technology, Lausanne, Switzerland) uses digital techniques to draw more information from the phase contrast image than can the eye. A 16-bit collector, contrast enhancement, background subtraction, pseudo-DIC (differential interference contrast) with pixel shifting: the full armoury produced another set of extraordinary images.

Heinz Gundlach (Carl Zeiss, Germany) then picked up the story of phase contrast. In 1941, the first phase-contrast micrograph was published in *Die Naturwissenschaften* by Kurt Michel[1]. "The chromosomes appear with unusual clarity," he said, "Also, the mitochondria are excellently visible." Michel used phase contrast for a time-lapse film of spermatogenesis in the grasshopper *Psophus stridulus*, which was released on 15 November 1941. The quality of this early film has to be seen to be believed. It is as good as anything done today.
As the audience watched the process of meiosis, the intervening 60 years of technical achievement in microscopy and imaging seemed to count for nothing.

(Left) The first phase-contrast microscope prototype, Jena 1936. Note the modified body-tube to take experimental stops.



(Above right) One of the first phase-contrast images showing the salivary gland chromosomes of the larvae of the non-biting midge species *Chironomus*. K. Michel, 1941, Jena.

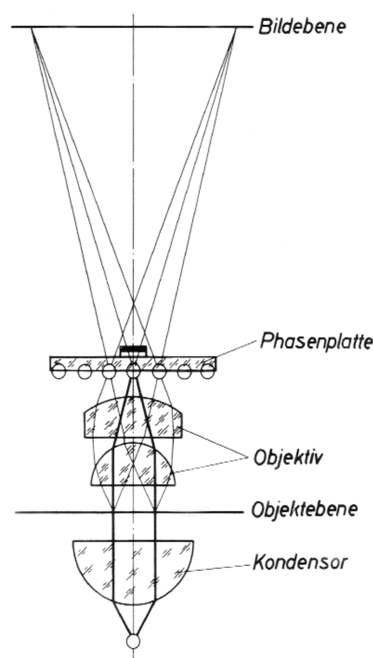
Heinz Gundlach then introduced Alan Boyde (University College London, UK) for the Abbe Lecture. The subject was 'Oblique views in the microscopy of hard tissues'. Oblique illumination has persisted as a valuable microscopical technique, in spite of sneers from optical purists. The modelling effect is simply too useful in interpreting the image. Abbe produced an oblique condenser (a version is still made in Russia) and there have been a number of takes on oblique illumination.

Edge microscopes use multiple sources to create a three-dimensional image, but Alan Boyde noted that parallax depth cues are best appreciated when the light source is in continuous motion, rather like the sweep of a spotlight. "You can't see three-dimensional information by focusing up and down," said Alan, "but you can by rotating the light source."

With this in mind, he had built a condenser with a rotating, but otherwise traditional (quarter sector), oblique patch stop. The images of bone matrix were remarkable, very close to the results from a scanning electron microscope.

The next speaker was Hort Wolff (National Research Centre for Environment and Health, Munich, Germany), who was using some very elegant technology in his research on Rev function in HIV-1. He pointed out that many journals publish fluorescent images only, although they need to be married to a phase-contrast image to see what else is in the cell and what else is happening there.

One of the things that emerged from this meeting was the way in which fluorescent and phase-contrast techniques are being used in tandem, and producing more



(Left) An original diagram showing the position of Zernike's phase plates

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Alan Boyde (left) and Heinz Gundlach

information than the sum of their parts.

Graham Dunn (King's College, London, UK) spoke on 'Life at the cell's leading edge: actin dynamics revealed by FLAP [fluorescent localization after photobleaching]'. The technique is similar to speckle fluorescence, which it is used mainly to vizualize motion. FLAP does this too, but you can also see where the molecules go. He also used interference reflection microscopy to show where a cell is in contact with a substrate. This research has provided some surprising insights into the diffusion of actin, especially near the leading edge of the cell, and has led to some speculation on the involvement of myosin-2.

The final speaker was Justin Molloy (National Institute for Medical Research, London, UK) who gave a presentation on 'Visualizing single molecules in cells using TIRF [total internal reflectance fluorescence] microscopy'. This is a technique that exploits total internal reflectance to generate an evanescent field that is about half the wavelength of the light. This provides the great surface-selectivity of TIRF: only fluorophores adsorbed, adhered or bound to the surface will be excited and fluoresce. The point of all this is that several cell events occur with single molecules. Although there are formidable technical challenges in catching the photons from a fluorophore before photobleaching, there is the advantage that they are behaving as a point source of light, with a diffraction-limited point spread function. We were treated to another show-stopping film as single molecules of green fluorescent protein winked out against a dark background.

It was a remarkable meeting that gave a historical background to live cell imaging and some reports from the frontiers of science. It is often said that fluorescence was the saviour of the traditional compound microscope and that without it we should be using some CCD (charged couple device). The meeting presented a strong argument for the continuing usefulness of phase contrast, because it does things that nothing else can. It has made its main application, the observation of the living cell, its own.

Reference

1. Michel, K. (1941) Die Naturwissenschaften **29**, 61–62

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