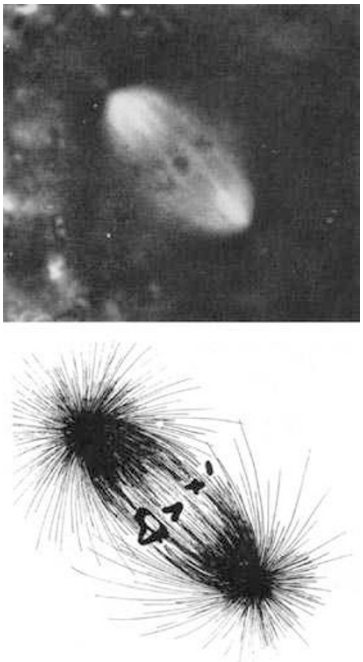
MILESTONE 6

Seeing is believing: subcellular structures brought to life



First maturation division spindle in a living oocyte of the marine annelid *Chaetopterus pergamentaceous* imaged with a polarization microscope (top) and traced the projected image negative (bottom). Image is reproduced, with permission, from S. Inoué © 1953.

The development of fluorescence microscopy technologies has made live-cell imaging routine for the current generation of cell biologists. However, the ability to visualize the cell's architecture was not taken for granted until the 1950s. Although images of cells and subcellular organelles had been produced as early as the mid-nineteenth century, they originated from fixed material. As a result, the existence of three-dimensional macromolecular structures within cells had remained a source of controversy.

The view of the cell's internal organization was revolutionized in the 1940s and 1950s with techniques that were initially developed to image the mitotic spindle. Until then, the observed assembly of polymers into fibres was believed to be an artefact originating from fixation and staining treatments that induced protein coagulation and gave the spindle a fibrous appearance. The first evidence of fibrous organization in the living spindle came from W. J. Schmidt's observations of developing sea-urchin eggs made in 1937, and reinterpreted in 1939, with a polarizing microscope. This apparatus exploits the optical anisotropy of transparent materials to reveal detailed information about the internal structure of samples — information that was not available with any other optical microscopy technique at the time. Schmidt reported that spindles have positive birefringence — a measure of optical anisotropy — a feature that he concluded to be caused by aligned protein units. However, although football-shaped spindles were visible in Schmidt's pictures, his microscope did not provide adequate resolution to see discrete fibres.

The definitive visual demonstration of the existence of spindle fibres in untreated living cells was given by Shinya Inoué more than a decade later. Inoué built his first polarizing microscope by hand in post-war Japan and managed to repeat Schmidt's observations. However, it was only later at Princeton University in New Jersey, USA, that he built an improved polarizing microscope that could image weakly birefringent structures. In 1953 this led to the publication of the structure of the metaphase and anaphase spindles in oocytes of the marine annelid *Chaetopterus pergamentaceous* and in *Lilium longiflorum* pollen mother cells: "With the increased resolution and sensitivity achieved by the new instrument, I have been able to observe the detailed structure of the spindle in living cells and to follow its change during mitosis," he wrote. Further observations led Inoué to suggest how microtubule dynamics generate the force for chromosome movement and also to deduce the arrangement of DNA and chromatin folding in living sperm.

> "Schmidt's and Inoué's findings were the first demonstrations of the structural complexity of the cell interior …"

Schmidt's and Inoué's findings were the first demonstrations of the structural complexity of the cell interior and showed the great potential of live-cell imaging for understanding complex biological processes. Polarization microscopy offers certain advantages over fluorescence microscopy in that it is label-free and therefore a non-invasive mode of imaging that can be used, for example, to monitor the viability of *in vitro* fertilized oocytes. Furthermore, the development of the 'pol-scope' in 1995 overcomes the limitation of previous microscopes in that the polarization contrast is independent of the orientation of the sample.

Kim Baumann, Associate Editor, Cell Migration Gateway

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