Transformation of Scientific Knowledge in Biology:

Changes in our Understanding of the Living Cell through Microscopic Imaging

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4. Transformation of our knowledge of the cell and the cytoskeleton: From the static to a dynamic concept

Motility is one of the central criteria for life. Studies on movement of cells and cellular components are therefore a major field of study in biology. As discussed above, microscopic imaging techniques play a dominating role in studying cell motility, and with the rapid improvement of microscopy techniques dramatic transformations in our views have occurred.

Here we present a case study of cell science with a particular interest in the ways biological thought has changed over the decades and how these changes in thought may have affected scientific approaches. We have found that the history of research on the cytoskeleton and its role in intracellular motility provides a valuable example to examine the influence of technological innovations of the scientific toolkit on scientific reasoning. Since philosophy of science in the 20th century has focused mainly on physics, we want to analyze whether the specific biological episodes that we are giving an account of can also be made fruitful for philosophical reflections. One of our central questions is: How well do the common criteria of "scientific theories" or "predictions" work in cell biology? We will discuss whether there are such things as paradigms and scientific revolutions in cell biology and if this field functions by constant alternation of the two, as proposed by Kuhn for all of natural science.

4.1. A short history of cell biology

4.1.1 Early cell biology

It was the invention of light microscopy in the 17^{h} century that allowed the initial observations of the cell and channeled the interest of early naturalists into exploration of the new miniature world. Cell biology therefore started out as a science dealing mainly with structural and descriptive data, a status maintained perhaps until the end of the 19th century - as thorough observation and documentation of what the early optic apparatuses revealed to the previously naked eye. The function of the observed intracellular structures could be interpreted only in the light of the contemporary understanding of living systems until methods were invented to collect the necessary data by experimentation.

The wealth of observed structural detail grew rapidly with the establishment of selective staining procedures first introduced by Francois-Vincent Raspail (1794-1878), (reviewed in Schliwa 2002) and the development of microscopes based for the first time on optical knowledge by Joseph von Fraunhofer, Friedrich Adolph Nobert, Ernst Abbe and others, which provided the ability to resolve structures close to the diffraction limit (reviewed in Gerlach 2009, S399-462). Little however could be said of the function of the newly determined structures. As structures could be made visible only in chemically fixed cells, a debate on reality or artefact of the observed structures ensued (see for

example: Rumjantzew and Wermel 1925). The highly speculative character of functional interpretation posed a serious threat to objectivity before the advent of high resolution vital staining and high resolution microscopy of living cells. Scientists were well aware of this danger, as put by Henry Baker in 1866 (see above). Functional understanding of cellular substructures or mechanisms of cell motility remained a field of hypotheses and predictions but without empirical testing, since live observation with the necessary resolution was not possible and the technology for analytic experimental approaches not developed. Nevertheless, the pioneers of cell biology such as Matthias Jakob Schleiden, Theodor Schwann or Rudolph Virchow (for review see for example Marcello 1999) recognized the cell as living unit which possesses the ability to reproduce, to detect and to react to external stimuli, and with internal mechanisms of maintenance, distribution and translocation of molecules and organelles.

4.1.2 Discovery of the cytoskeleton

The cytoskeleton, as we know it today, describes a network made up of different types of filamentous protein polymers which are found in every living cell and represent part of the cytoplasm. The cytoskeletal fibers are highly dynamic, which is shown as constant elongation and shortening by polymerization and depolymerization. We know now that the fibers are important for maintaining the mechanical stability of the cell but also for cell motion, changes in cell shape and internal transport of organelles or smaller particles.

One of the first scientists to get a glimpse at the cytoskeleton was Robert Remak who observed cytoskeletal fibers in nervous tissue of the crayfish (1843, reviewed in Frixione 2000, Schliwa 2002). These observations were extended by Sigmund Freud (1856-1939) in his doctoral dissertation on vertebrate nervous tissue (Freud 1881). At the Institute of Physiology at the University of Vienna, Freud carried out an investigation on the internal structure of nerve fibers and cells. In pursuing the nature of the “neurofibrils” that formed the basis of the Golgi method, Freud was able to describe fine fibrils following straight courses in the nerve fibers, as well as loose loops surrounding the nuclei. He confirmed and extended the observations made by Remak almost 40 years earlier, which had remained controversial. Later, electron microscopy of the crustacean nervous system confirmed Freud's main points and in turn vindicated those of Remak. Freud was in this way probably the first to picture the intracellular framework that future cell biologists would call the cytoskeleton. However, the existence of these structures *in vivo* had to be defended against accusations of artifact caused by the chemical fixation procedure (see section 3.1).

This could be resolved with the first empirical support for the existence of an elastic intracellular scaffold. The support arose from experiments for which micromanipulation with fine dissection needles or centrifugation were used to actively displace organelles in the body of living cells. This work was carried out on single cells

2.5.3. Video-intensified fluorescence microscopy: Localizing molecules in the cell.

Video intensification is the procedure for making visible low light level objects and scenes generating too few photons to be seen by the naked eye (Fig. 10). Video-intensifier (VIM) or highly sensitive slow scan CCD cameras are needed which amplify low light signals so that extremely weak fluorescence and luminescence, not visible when looking down the microscope, can be visualized (see reviews by Weiss et al.1989, Lange et al. 1995). This is of utmost importance in biology because living specimens benefit from the sparing application of potentially hazardous vital dyes and phototoxic effects caused by excessive illumination. The localization in the living cell of a multitude of proteins under all kinds of different physiological or pathological conditions has led to the situation that we now know exactly which of thousands of proteins are located at which organelle, how they move to their target structures, which their neighbors or ligands are, and where the effectors and signalling molecules are located which cause changes under varying physiological conditions (Figs. 5 and 16).

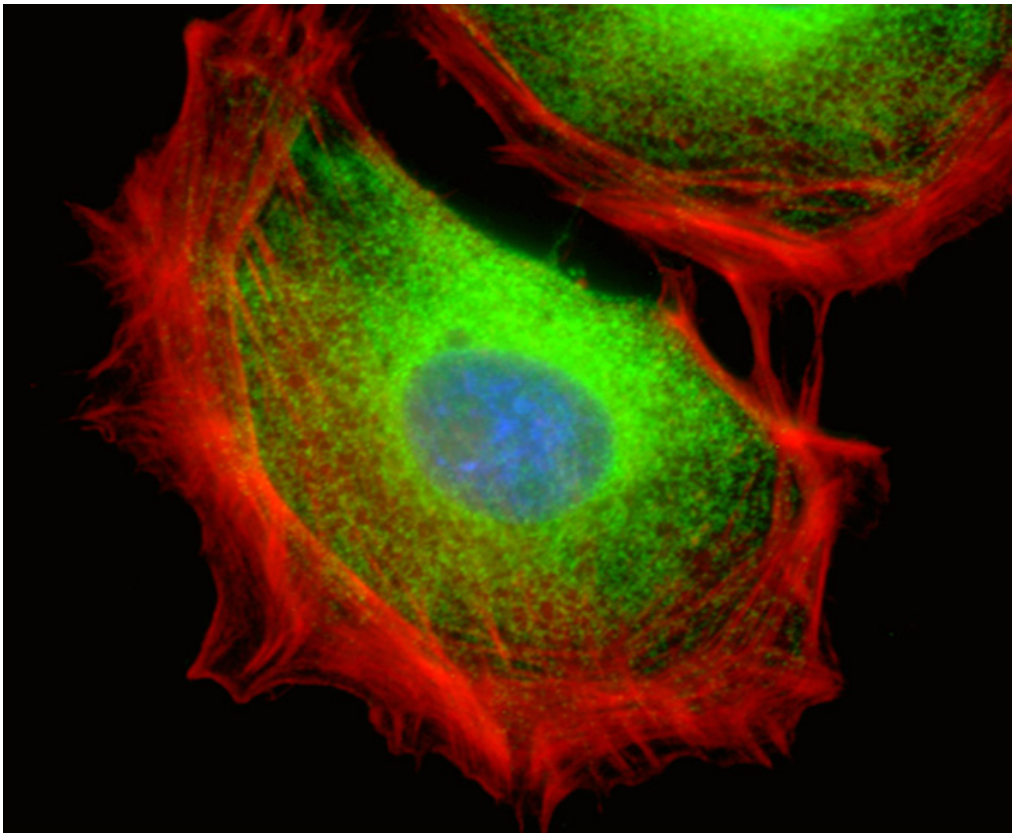

Figure 16. Video intensified fluorescence microscopy (VIM). Simultaneous staining of three cell components in fibroblast cells in culture: actin (red by immunofluorescence), a marker enzyme for the endoplasmic reticulum (green fluorescence caused by GFP-labeling) and DNA (stained with the dye DAPI, blue). Photo Live Cell Imaging Center Rostock, courtesy of Eik Hoffmann.

Table 1: Resolution limits of different viewing techniques shown in comparison to the size of cytoskeletal elements.

Limit of resolution and the size of cell components	
Naked eye	0,3mm
Light microscope	250nm (2,000-fold magnification)
Electron microscope	0.2nm (in biology: 400,000-fold magnification)
Cell components	
Actin filaments	7nm
Intermediate filaments	10nm
Microtubules	25nm
Organelles	40 - 2000nm

While in brightfield and darkfield microscopy objects such as the flea or a transparent, stained tissue section are seen in a similar way as macroscopic objects by visual experience, this changed with knowledge-driven microscopy design. Additional physical and material properties of the specimen were now used to create contrast by inserting specific optical elements in the light path. The object is, therefore, often not seen as a whole object, but only some of its optical properties such as birefringent or fluorescent regions are selectively depicted. An overview of contrasting methods and their underlying physical principle is shown in Table 2. (Table 2).

Frits Zernike discovered that differences in the velocity of a traveling light wave passing through materials of different refractive index can be used to generate contrast by inserting a phase retarding ring in a modified light path (Zernike 1935). When a light wave passes through a cell and a closely adjacent wave passes just outside the cell, they will exhibit a relative shift of phases. These, when interfering with each other, lead to constructive and destructive intererence which causes a bright halo and dark ring around all objects. This means that the image contains information on the different velocities of travelling light waves passing materials of different refractive index. Zernikes phase contrast microscope creates images of cells without staining (Fig. 4). It is an elegant method to visualize completely transparent, non absorbing objects such as living cells. Phase contrast microscopes are today used in all cell culture laboratories around the world to check the growth of living cells.