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**Transformation of Scientific Knowledge in
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Changes in our Understanding of the Living Cell through Microscopic Imaging

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1. Introduction

The origin of knowledge in cell biology was from the first recognition of the existence of cells by Antoni van Leeuwenhoek (1632-1723) and Robert Hooke (1635-1703) almost solely dependent on microscopic images. Only since the middle of the 19th century chemistry (biochemistry), later molecular biology and recently systems biology have started to contribute to the accumulation of knowledge on the morphology and physiology of cells and tissues. Light microscopy lost its dominating role when around 1950 electron microscopy with its highly increased resolving power opened new windows into the cells' composition and architecture. It is, however, restricted to imaging of carefully dehydrated, water-free, dead remnants of cells. Beginning in 1981 a renaissance of light microscopy can be observed (Webb 1986, Shotton 1987 a,b) due to the addition of electronic cameras and computers to microscopes (Allen et al 1981a,b; Inoué 1981, see also Inoue 1986). Now image contrast could be increased enormously, and with the new video-microscopy attention concentrated again on live cell imaging. It is therefore, that we discuss the development of the different microscopy techniques and evaluate the clearly different quality of the images obtained and their contribution to our knowledge of cells' structure and functions.

The combination of electronic imaging and image processing techniques with classical methods of optical microscopy allowed to surpass the resolution limits of conventional microscopy and to reach useful magnifications up to 10 000x which were previously only accessible with electron microscopes. The dogma, that with visible light of wavelengths from 400 to 750 nm only objects of the dimension of one half of the applied wavelength could be observed, had to be abandoned. This electronic revolution of light microscopy led to a series of rapid and profound changes in cell biology.

The previously dominating static image of the cell, derived from electron microscopic images as the only high resolution technique available, was replaced, by a completely new understanding, of living cells and led to the discovery of the whole new class of cytoplasmic motors, to the quantification of the dynamics of macromolecules and to the quantitative visualization and tracking of single protein molecules in the living cell. Physical-chemical properties and their dynamics such as fluctuations of ion concentrations, pH and membrane potentials were visualized, and microscopic intracellular processes were reconstructed in 2, 3, 4, and 5 dimensions (space, time, wavelength or color and concentration).

In this study, a historical analysis of the different types of microscopic images, including those obtainable by electron microscopy and those obtainable after the renaissance of light microscopy will be attempted as well as an evaluation of the different qualities of the images obtained. Based on this we want to analyze the implications for the current perception of life at the cellular level and the continuation down to the molecular level, where "in silico" models and graphic representations provided by bio-informatics and systems. This leads us to study the transformation of

our understanding of the living cell. To this end we examine the impact of images and discuss their informative value. The revolutionary technological progress of the last one hundred years led to new kinds of images which are not simply magnified representations of the biological specimens but visualize specific aspects of cells' morphology and function, e.g. by delineating the distribution of a single class of molecules in one cell. For the different types of new images we will discuss their compatibility with existing classifications and suggest new microscopy-specific aspects and categories. A consideration of the value of different microscopic images and the epistemic virtues of the microscopist will demonstrate differences in the evaluation and responsibility between those dealing with macroscopic and those dealing with microscopic images.

In the course of this study on transformations of scientific knowledge of the cell the question about the way of scientific progress in this field will be discussed.in general. More specifically we will argue whether it is progressing steadily, in abrupt stages or even in the form of paradigm changes. To this end we present in more detail a case study which focuses on the concept of the cytoskeleton.

Taken together, we aim at contributing answers to the following questions:

- Which types of images are produced by different microscopy techniques and what is their information content?
- How can microscopic images be classified and what are the contributions of the different types of microscopic images to the process of transformation of our all biological knowledge?
- Are microscopic images and especially the electronically generated and digitally enhanced images giving an objective or a distorted view of the specimens under study?
- What is the attitude toward the epistemic virtue of objectivity of investigators working with microscopic images of different kinds of abstraction from the real object?
- How has the digital revolution of light microscopy paved the way from the classical view of cells to that of the micro- and nano-world and further to molecular analyses and simulations of cells?
- What is the attitude toward theories and predictions in cell biology and what if a theory successfully predicts a novel result, yet the result later turns out to be an artifact?
- Did the technological revolutions in microscope design and image generation lead to paradigm changes in cell biology?
- What can we learn from this analysis to better understand the process of transformation of scientific knowledge in the life sciences?

2. Development of microscopic techniques and their influence on the understanding of the cell

We present here a short historical overview about the development of the different techniques of microscopy. In order to evaluate microscopic images and to discuss their epistemic value it is essential to know their ways of origin and something on the physics involved.

2.1 The invention of the microscope and the discovery of microorganisms

The invention of microscopes can not clearly be ascribed to a certain person or a certain date. There are reports about compound microscopes (with two stages of magnifying lens systems) made around 1590 by the spectacle makers Hans and Zacharias Jansen and Hans Lippershey all at the lens-making centre Middelburg/Holland, but none of their instruments is preserved or documented. One of the early microscopists was Antoni van Leeuwenhoek (1632-1723). Although he had built himself only a one-lens high power magnifying instrument, he used it to study objects in his household and for the first time described living specimen. He discovered and described in 1675 bacteria (caries bacteria from his teeth), but also red blood cells and the blood circulation as well as moving sperm cells and protozoans (van Leeuwenhoek 1685). Since there is an extensive literature on early microscopy history (see Turner 1980, de Martin & de Martin, 1983, Gerlach 2009) we will not discuss it here in more detail.

2.2 Conventional light microscopy: Microscopes made by arts and crafts

After the simple one-lens systems mainly the compound microscope was further developed with a magnifying objective system and magnifying oculars. This principle is used until today. Up to the middle of the 19th century the optical parts were crafted by experience and tradition in families of lens makers as an art, much like violins made in the families of Stradivari and Amati. Famous microscope builder families were located first in Italy and France, later also England and Germany, including Carl Zeiss (1816-1888) in Jena (reviewed in Stolz and Wittig, 1993), Friedrich Adolph Nobert (1806-1881) in Barth, Edmund Hartnack (1826–1891) in Paris and others.

Optical elements were produced, tested and improved by trials with test specimens which contain regular repetitive patterns with a very narrow spacing from a few μm down to 100nm such as butterfly scales, diatoms and the renowned Nobert test plates (Nobert 1846; Pohl 1886). Contrary to the general public opinion that a specimen is put under the microscope and looking into its ocular yields a true and objective representation of the specimen - only at higher resolution - we have to deal with a large variety of microscopy techniques which yield completely different images from the same specimen. Images created with these contrasting techniques are each restricted to a different aspect, mainly physical material properties, of the same specimen.

The optical techniques used in the beginning were brightfield and darkfield microscopy (Figs. 1 and 2). They follow different physical laws and thus their appearance and information content is quite different.

In darkfield microscopy the image originates from light reflected and diffracted from different areas of the specimen and indirectly reaching the objective. Reflection can be wavelength-specific so that colors originate. No direct light from the light source reaches the eye. In reflected darkfield microscopy we have the same physical situation as in our every day macroscopic viewing of objects in sunlight. This is also the way of the "unarmed" eye sight and thus appears "natural" to us. Darkfield microscopy using transmitted illumination (Fig. 1) can be compared with looking at a small angle against dust particles in sunrays while not directly looking into the sun. This type of microscopy produces bright object images against a dark background, a situation very convenient for our brain which distinguishes bright objects better against a dark background than in the opposite situation (compare Figs. 1 and 2). In this technique extremely small particles and dirt in the light path become very conspicuous and can be sometimes disturbing (compare images of the alga in Fig. 1 and 2).
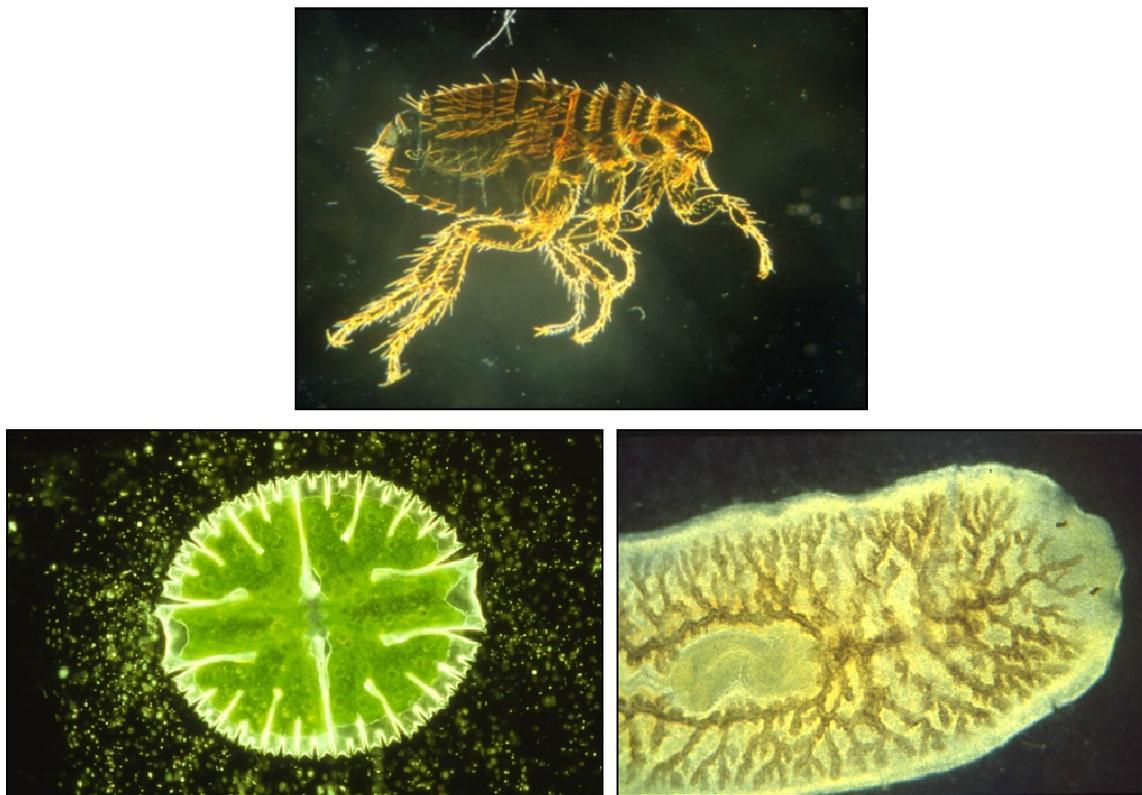
Figure 1. Darkfield microscopy images of a flea (top), the unicellular green alga Micrasterias (bottom left), and the flatworm Dicrocoelium sp.(bottom right).
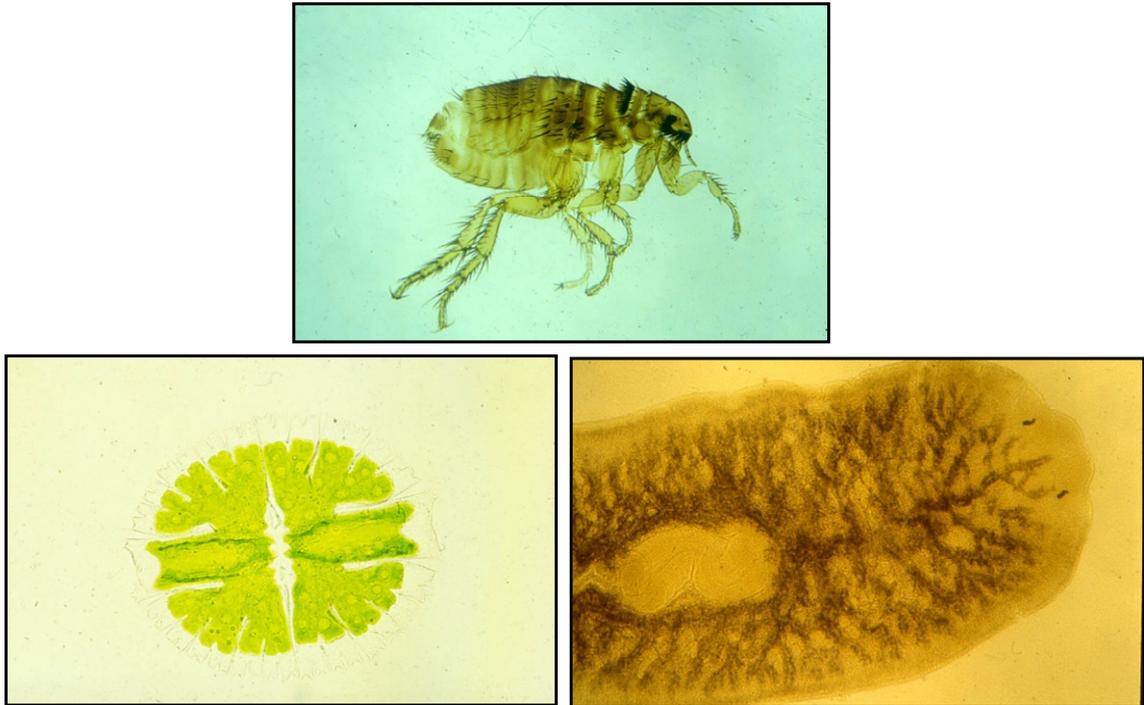
Figure 2. Brightfield microscopy images of the same specimens as in Figure 1.

It is noteworthy that this technique visualizes self luminous objects far beyond the limit of resolution which was defined by Ernst Abbe (1873, 1880) as approximately half the wavelength of the light used. Here, the size of visualizable objects depends mainly on the intensity of the illumination so that with extremely bright lamps objects down to a few nanometers can be visualized, but not optically resolved. This means that we can see the position and movement of colloidal and nanoparticles but not their true size and shape (Zsigsmondy, 1909).

Brightfield microscopy is the technique most used in biology. It derives its contrast from absorption, when different parts of the specimen absorb the transmitted light either totally or partially. With its help the fact that living organisms are built from cells was first described as a general principle for plants by Matthias Jakob Schleiden (1838) and for animals by Theodor Schwann (1839).

We must accept that beginning with brightfield and darkfield microscopy all techniques create contrast by virtue of different physical phenomena. In this sense, the question whether microscopic images contain artifacts can be simply answered by "all microscopic images are completely artifactual". This becomes especially obvious when looking at images of the flea created by a modified darkfield technique called Rheinberg contrast microscopy. Special color filters with a colored central stop and a differently colored peripheral ring allow the "optical staining" of any object in arbitrary colors. When placed in the focal plane of the condenser, optical image generation is influenced in such a way that the central stop defines the background color, while the specimen

itself appears in the color of the peripheral ring. If the top filter depicted in Fig. 3 is employed, horizontal object structures appear in the vertically oriented filter color and vertically oriented specimen detail appears in the horizontal filter color. In Fig. 3 a tri-color filter was used which let the horizontal hairs appear in red and the vertical hairs in yellow, while a central black stop causes the darkfield effect. This again underlines the fact that microscopic images are created by physical effects and that the result is not a one-to-one image of the real object. Colors do not exist in nature but are an interpretation of electromagnetic waves of different wavelengths perceived by our eyes and the brain. In addition it should be mentioned that nature has more wavelengths of electromagnetic waves than those which can be detected by eyes, such as infrared light that is detected by the skin and interpreted by the brain as heat, and yet other wavelengths (x-ray, radio waves, radar etc.) which cannot be seen or sensed by us. The question of objectivity of such images and of contrast and color must be raised and this will be discussed below.
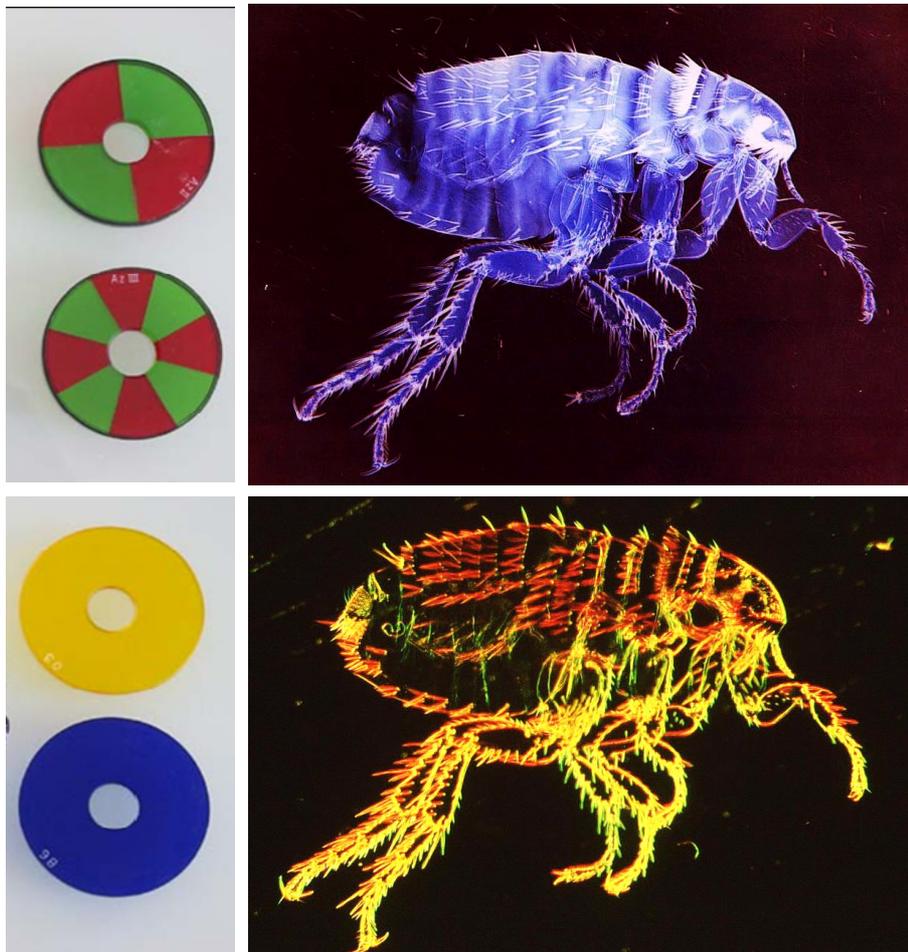
Figure 3. Microscopic image of a flea viewed with Rheinberg contrast in which arbitrary colors are generated by color filters in the aperture plane. Left: Selection of Rheinberg filters of which the peripheral ones define the specimen color and central filters the background color. Right: images obtained with a central black stop and a peripheral four zone filter simlar to the one on top but with different colors. Photo Live Cell Imaging Center Rostock, courtesy of Willi Maile.

2.3 Building microscopes based on scientific knowledge: Imaging physical aspects of the cell

The following era is chracterized by the development of microscopes that are no longer just the product of unique craftsmanship and manufacturing tradition, but their construction is based on knowledge of the physical laws of optics. One of the first physics-trained microscope builders was Joseph von Fraunhofer (1787-1826). He established own glass melting and lens making works at Benediktbeuern in Bavaria and was the first to produce in a reproducible manner faultless optical glass which allowed the design of error-free achromatic objectives. Others started this era later, e.g. Carl Zeiss (1816-1888) employed the physicist Ernst Abbe (1840-1905) only in 1868. The design of the optimal illumination system (Köhler 1893), the calculation of lens systems (Abbé 1904), the use of specially selected types of glass as introduced by Otto Schott (1875), together with the high resolution test gratings of Nobert (1846), allowed the assembly of optimized microscopes and the control of their quality.

The better knowledge of optics also led to Abbe´s prediction of the limit of resolution (diffraction limit), which was found to be approximately one half of the wavelength of the light used, given that one uses optimal optics (Abbe 1873, 1880). For visible light this is approximately 250nm, for UV-light about 150nm. This limit is referred to as the "beneficial" or "useful" magnification. Additional magnification can be obtained by optical means or projection of the image onto large screens or photographic paper but no additional detail will come about ("empty" magnification). We will see later that this limit is not unsurmountable, but that it depends on the limited ability of the human eye to distinguish between differences in gray shades. When using electronic eyes (cameras), the resolution limit can be overcome, as well as by various optical tricks, including darkfield microscopy (see above), by the so called ultramicroscopy of Richard Zsigmondy (1909), by Henry Friedrich Wilhelm Siedentopfs Schlieren microscopy and by the most recent, advanced laser optical techniques such as the STED-, PALM- or SIM-technology which are together given the popular name "super-resolution microscopy" and presented in a recent review by Lothar Schermelleh (2010).

In the second half of the 19th century physics-trained manufacturers built micro-scopes which soon reached the diffraction limit. This enabled cell biologists to describe the cell nucleus and the larger organelles, mitochondria (Lewis and Lewis, 1914), chloroplasts, storage granules and lysosomes. All smaller organelles, such as vesicles and small granules ranging from 40nm to 250nm as well as all cytoplasmic protein filaments remained invisible (Table 1). Intracellular movements of large organelles had been observed very early, such as the cytoplasmic streaming in plant cells (Corti 1774) or mitochondria movements in unicellular organisms described for example by Felix Dujardin (1835) (see Bereiter-Hahn 1990 for review). However, even though the importance of cytoskeletal filaments in the cytoplasm was assumed, the common belief that these objects would never be accessible to light microscopy persisted.

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.
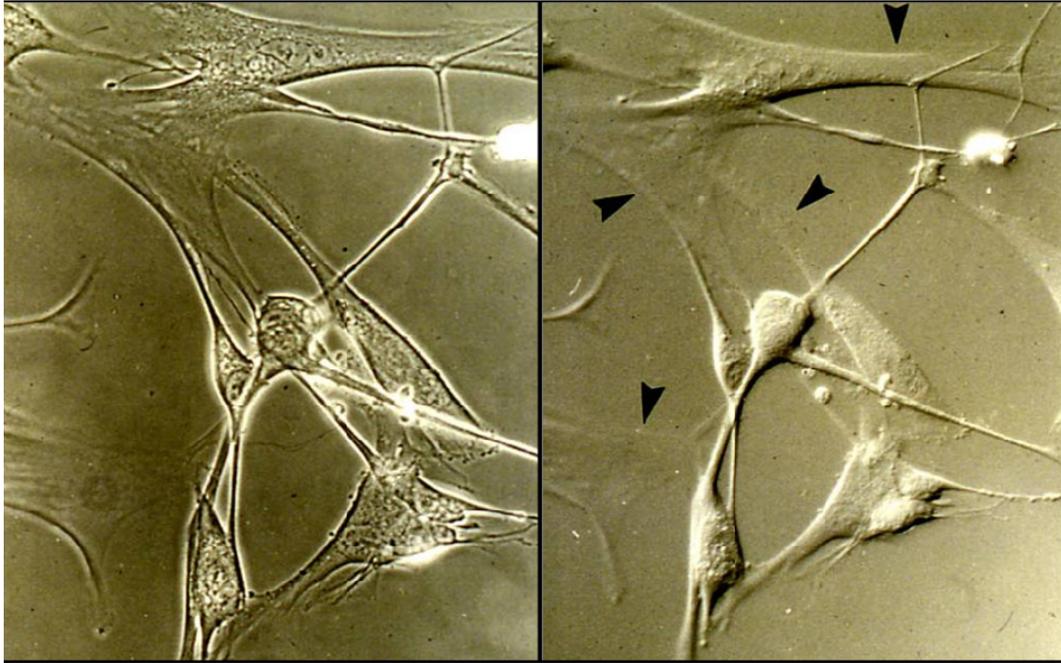
Figure 4. Differences in the retardation of those light waves that pass through the object and those passing by the object are used for constructive or destructive interference at the object borders. Left: Phase contrast according to Zernike, right: Differential interference contrast (DIC) according to Nomarski. No dyes are used. The bright halos around the cells are complete optical artifacts but render the cells visible. The 3D impression of the Nomarski image is another optical artifact and does not reflect the real hight of the objects. The arrows point to fine structures resolved by Nomarski contrast, but would be covered by the artificial halo in phase contrast. Photo courtesy of Elisabeth Möncke.

A second likewise elegant technique was invented by Georges Nomarski (Nomarski 1957, Allen et al. 1969), which is also based on the phenomenon that rays passing through materials with different refractive index exhibit phase retardation on which contrast formation can be based. Nomarski´s differential interference contrast (DIC) microscope uses polarized light, part of which is shifted laterally before passing though the object and recombined afterwards. The resulting contrast is a lateral shadowing at the specimen margins which are interpreted by our brain as threedimensional objects (Fig. 4). Microscopists need to know that optical sections through axially extended or through rod-like structures in DIC microscopy will create an optical illusion and artifactually appear as 3D convex bodies or beads (Fig. 4 right).

DIC is characterized by the fact that only light from a very thin layer or optical section contributes to image formation, while all out-of-focus information does not contribute. The optical parts needed are expensive, but it pays off because DIC has a transfer function which prefers the highest contrast frequencies, i.e. the finest object details, so that it is the method of choice for unstained phase objects such as living cells. The proper interpretation of these images requires considerable previous knowledge of the physical processes employed, but on the other side one gains valuable scientific insight.

Retardation of the phase of light waves passing through objects and the subsequent interference at object margins is a phenomenon in optics that we cannot observe with the naked, unarmed eye. Its use in microscopy makes phase objects visible in a specific manner and creates images (Fig. 4) which were unaccessible and unknown to our experience. In addition, non-absorbing, i.e. transparent phase objects such as unstained cells would be invisible in bright field, but contrast is generated by Nomarski or Zernike microscopy. Both types of phase object microscopy create artificial images by virtue of optical effects. Therefore the images may be considered as artifactual, but phase objects can only be visualized in this manner, so that we may speak here of a physical trick rather than a physical artifact.

The phenomenon of fluorescence (Table 2) led to one of the most powerful and widely applied microscopy techniques in biology. Certain molecules, endogenous metabolites or externally applied dyes can be excited into an energy-rich electron configuration, which equilibrates after about 10^{-9} sec by emitting a photon of somewhat lower energy (red shift). Excitation and emission wavelengths are material constants of fluorescent dyes. With the proper monochromatic light and a set of filters an image can be created from the light emitted from only one species of molecule. Some dyes are non-toxic (vital dyes) so that concentration changes, transport and distribution of cellular components can be followed and quantified (Fig. 5). By coupling fluorescent dyes to antibodies and utilizing the immune reaction the antigenic protein molecules can be selectively localized in fixed or live cells (Fig. 5) (Coons 1960, Lange 1995). Vital dyes are also used to report the intracellular position and movement of the dye or dye-coupled molecules. These images are topological 2D-measurements of the amount of substances.

Table 2. The most commonly used contrasting techniques for light microscopy.

Contrasting technique	Contrast is generated by:
Brightfield microscopy	Absorption
Darkfield microscopy	Diffraction, Tyndall effect
Phase-contrast and differential interference contrast (DIC) microscopy	Delay in the velocity of light propagation (phase shift)
Polarization microscopy	Birefringent properties of objects
Interference microscopy	Interference-effects at thin layers, refractive index
Fluorescence microscopy	Excitation followed by fluorescence light emission
Low light fluorescence microscopy	Very weak autonomous light emission such as in bioluminescence or chemiluminescence
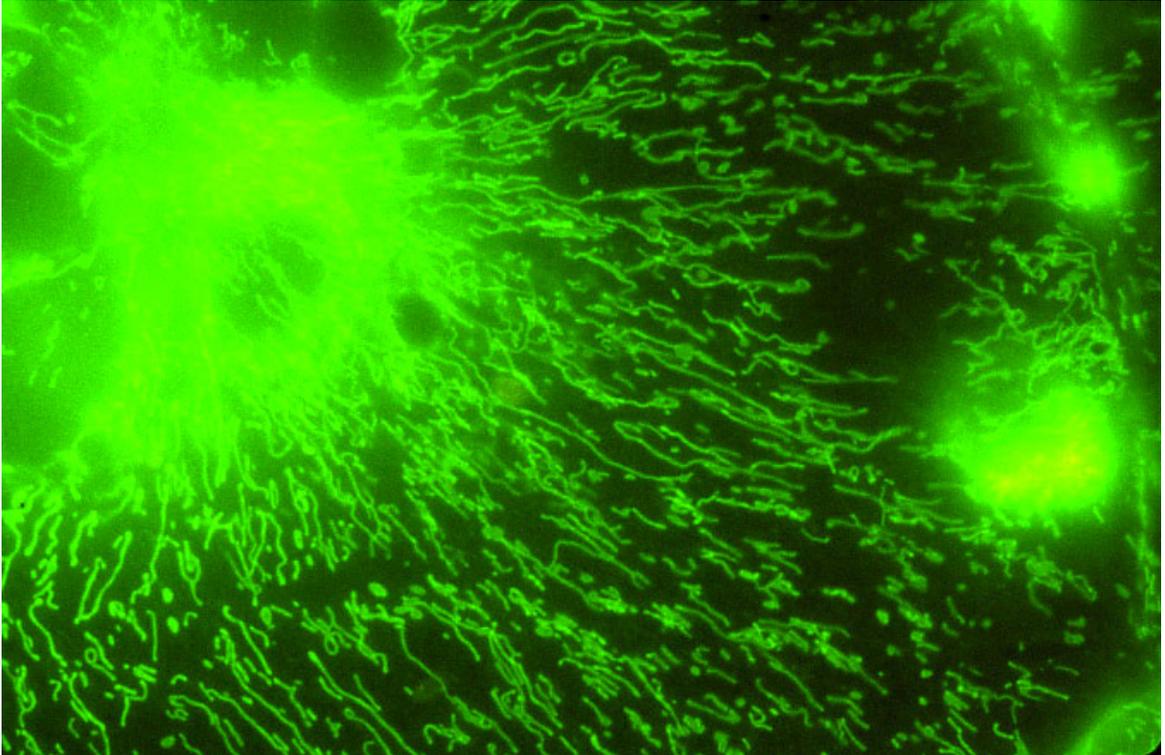
Figure 5. Selective imaging by fluorescence microscopy. Vital staining of mitochondria in a live neuroblastoma cell in culture. Fluorescence is caused by the mitochondria-specific vital dye rhodamine123. Photo Live Cell Imaging Center Rostock, courtesy of Simone Stüwe.
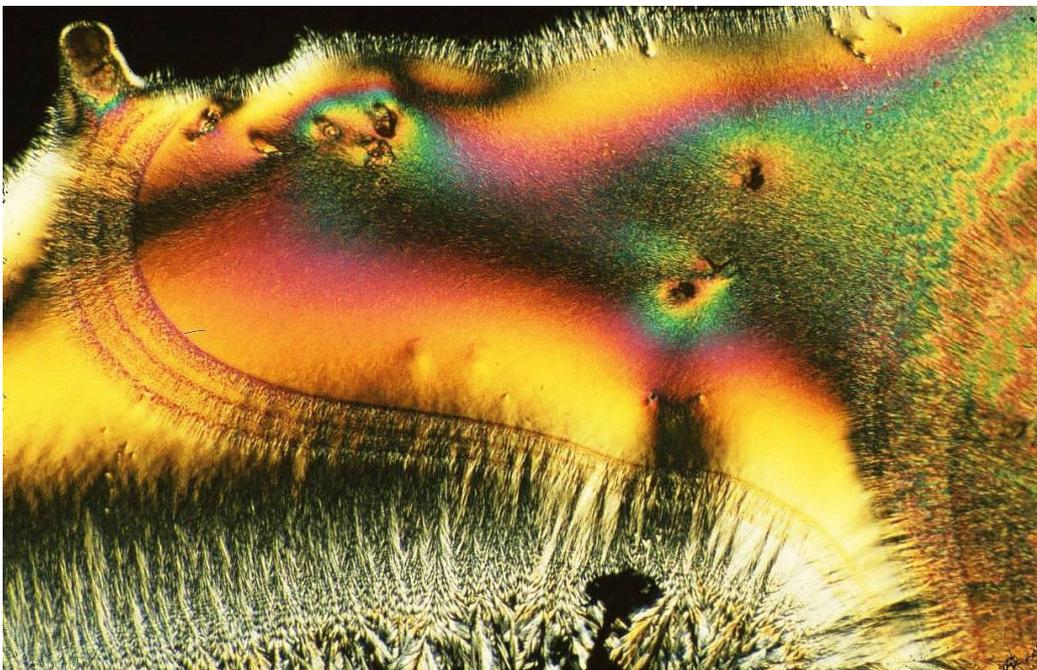
Figure 6. Interference at micro-crystals from a drop of ascorbic acid solution dried on a microscopic slide and viewed between crossed polarization filters. Photo Live Cell Imaging Center Rostock, courtesy of Willi Maile.

The most recent improvement in fluorescent imaging is the Green Fluorescent Protein (GFP) technique. The gene coding for a green fluorescent protein molecule of a jellyfisch (Shimomura 1969, 2005) is coupled by molecular genetics technology to the gene of the protein to be visualized and this construct is introduced with a vector into a target cell. This cell then produces not only its own proteins but an additional one in a green fluorescent variant (Fig. 5). In many cases the GFP-construct becomes stably inserted into the genome so that all descendent cells are fluorescent. Often whole fluorescent plants or animals originate. The cells stain themselves by gene expression. Several fluorescent dyes can be combined and now, since the GFP-technology has developed additional fluorescent proteins of different colors, the technique was considered worth the Nobel Prize, awarded to the three inventors of functional vital cell markers Osamu Shimomura, Martin Chalfie and Roger Tsien in 2008.

With the latter two techniques thousands of proteins could be individually localized in a multitude of cells and tissues and many cellular processes have been clarified during the last decades. The fluorescence microscopy methods are the most powerful and therefore probably most applied imaging techniques in present day molecular and cell biology, as well as in genetics and medicine.

Another type of physical imaging technique is *polarization micros*copy, which uses the optical rotation of polarized light by birefringent objects to selectively make them visible. In cell biology this technique is used when ordered supramolecular structures need to be detected that are birefringent such as crystalline deposits, fibrils in muscle cells, microtubule bundles, or the myelin sheath of nerve fibers (Schmidt 1937). *Interference microscopy* creates colorful images of specimens of a thickness of a wavelength or below. In this technique light is reflected from the upper and lower surfaces creating interference colors which are also known from Newton's rings and contain the thickness information (Fig. 6).

Most of the techniques in Table 2 can be used with both live cells or fixed and sectioned material. Hundreds of dyes were developed for staining different cell types and components in fixed histological sections in brightfield microscopy (see the standard work on staining recipes "Mikroskopische Technik" edited by Benno Romeis in 18 editions from 1919 until today) and their application led to our profound knowledge of cell and tissue morphology in healthy and pathological situations.

Cell biologists also concentrated their efforts on selecting fluorescent and absorbing dyes that are non-toxic, so that dynamic events could be studied. Application of such vital dyes and brightfield and fluorescence microscopy together with phase contrast and DIC microscopy, which are live cell techniques by themselves, led to a good understanding of the basic dynamic events in cell biology such as cell division, protoplasmic streaming in plants, amoeboid movements, egg fertilization, phagocytosis, egg cleavage, and the early stages embryo development. This field is connected with many names including Rudolf Virchow (1821-1902, cellular pathology), Oscar (1849-

1922) and Richard Hertwig (1850-1937) (lower invertebrate development, sea urchin fertilization), Theodor Boveri (1862-1915, chromosomes and cell division), Hans Spemann (1869-1941, embryonic organogenesis), Earnest Everett Just (1883-1941, egg cell fate and stem cells), Victor Hamburger (1900-2001, neuroembryology, nerve growth factor), Johannes Holtfreter (1901-1992, cell adhesion, recognition and migration) and Josef Spek (1895-1964,vital microscopy of the cytoplasm, pH imaging) to name just a few.

2.4 Electron microscopy: The ultrastructure of dehydrated cells

While conventional light microscopy is limited in its useful magnification to about 2000fold, electron microscopes magnify up to several hundred thousand fold. The specimens are "illuminated" by an electron beam in a vacuum chamber and must therefore be free of water, extremely thin (100 to 200 nanometer) and coated with heavy metals such as gold, osmium or uranium salts in order to create contrast by absorption of electrons. Comparable to light microscopes, electron microscopes (EM) work either in a transmitted configuration (TEM) invented by Ernst Ruska (1932) or in a reflected mode (scanning electron microscope, SEM) developed by Manfred von Ardenne in 1939. Starting around 1950 the stream of new information on viruses, cells and isolated cellular components down to single proteins (Fig. 7) was so overwhelming that light microscopy seemed to become almost reduced to an ancillary technology in many laboratories only needed to pre-screen the EM samples to find the positions to be studied in greater detail later in the electron microscope.
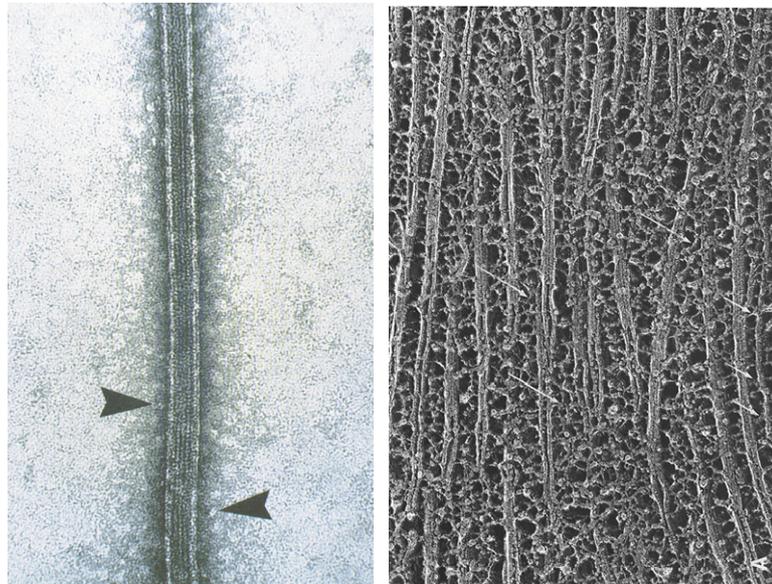
Figure 7. Electron-microscopical images. Left: A single microtubule is seen to consist of rows of globular tubulin molecules after processing for the negative contrasting EM technique. Courtesy of George M. Langford. Right: Neurofilament lattice (8) from an IDPN-intoxicated myelinated axon that was extracted with saponin. Cross-bridges are seen between microtubules and tend to form a branching and anastomosing network. Scale bar, 0.1 ,μm. Reproduced from N. Hirokawa (1985).

In the beginning electron microscopy was limited to the study of very thin sections while viewing of whole cells or the arrangement of cells in tissues was precluded. Biologists rather studied cell organelles and supramolecular protein assemblies such as ribosomes and the cytoplasmic filaments which were considered to strengthen the cell shape and increase cytoplasmic viscosity. Since microtubules, intermediate filaments, and actin filaments (microfilaments) appear as stiff rods and are often found to bundle into thicker and longer strands traversing the cytoplasm they were called in conjunction "cytoskeleton". Indeed, as the electron microscope images are unable to show any movements the understanding of the cell and especially the cytoplasm slowly changed. The impression developed that the cellular constituents can be described as an assembly of protein molecules, which are associated in a certain order for which the term cytoarchitecture was coined. But the terms cyto"-skeleton" and cyto-"architecture" reflect the then prevailing static view of the cell. (Figs. 7 and 8).

Since electron microscopy requires rather rigorous steps of sample preparation and visualization such as removal of water, impregnation with heavy metal salts, embedding the sections in polymer resins, irradiation with a close-to-damaging electron beam, warnings that biologists may fall victim to actual modifications of the real structure were numerous. But different methods of dehydration and variants of electron microscopy led to comparable images which were in addition validated by results from physical chemistry, biochemistry, molecule spectroscopy and other techniques. Therefore, consensus was reached that electron microscopic images represent true images of the cell (Bechtel 1990), only with the exception of a few extreme views (Hillman 1980, Hillman and Sartory 1980) who continuously warned that electron microscopy may create a plethora of distorting artifacts.

In the late 1970s High Voltage Electron Microscopes were developed. With more than 1 million Volt, they allowed for the first time to study the ultrastructure of whole dehydrated cells (whole mounts), which could not be penetrated by the lower voltage electron beams of conventional electron microscopes (50-100 kV). Again a new world opened which seemed to confirm the concept of a rigid cytoarchitecture consisting of the cytoskeleton and microtrabecular structures connecting all cellular components (Fig. 8).

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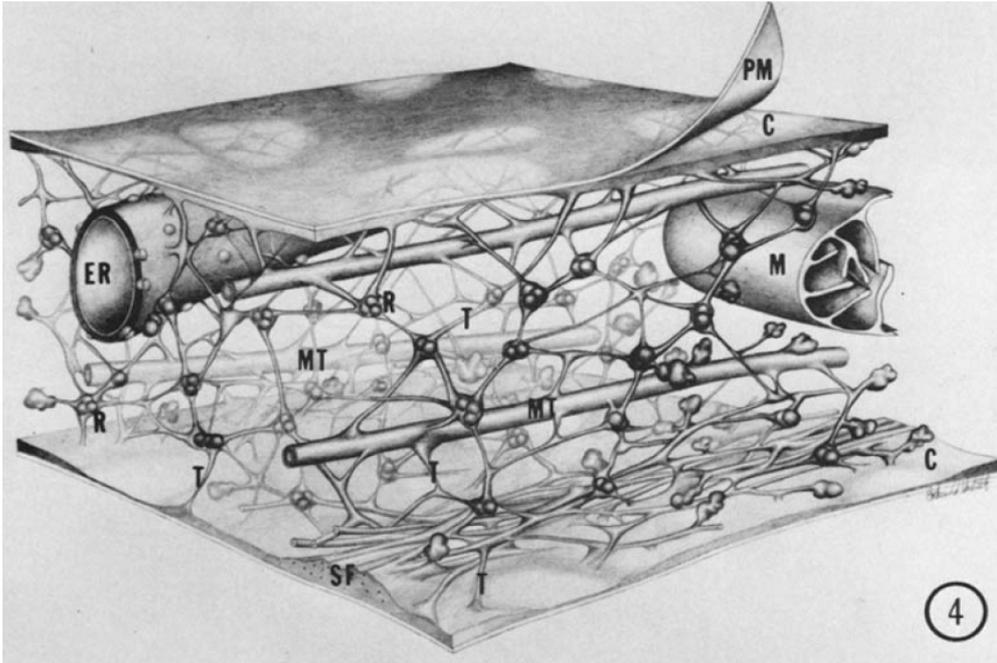
Figure 8. Idealistic drawing of the microtrabecular structure of the cytoplasm, as proposed by Wolosewick and Porter since 1979. The microtrabeculae were later shown to be artifactually created by critical point drying with liquid nitrogen. Reproduced from Pawley und Ris 1987.

In general it can be stated that the wealth if electron microscopic images obtained in the 1960s and 1970s led slowly to a more or less static image of the cell. Although movements of cells (locomotion) as well as in cells (organelle motility) were not forgotten, the enthusiasm to uncover the rules according to which the proteins as "cellular building blocks" are arranged, dominated the minds of most cell biologists. This situation will be discussed in more detail in a case study on the view of and the roles ascribed to the cytoskeleton at around 1980 (see section 4.).

2.5. Light microscopy supported by electronic cameras and computers: The living cell revisited

2.5.1. An overview of Electronic Light Microscopy

In 1981 two laboratories published about remarkable and unexpected improvements in light microscopy by the use of a video camera and a real-time image processor (Allen et al 1981 a.b, Inoué 1981). The new method of video microscopy allowed for the first time a direct observation of a wealth of very small previously unseen and highly dynamic cellular constituents including microtubules and vesicles down to a size of 50 nm.

Robert D. Allen (Fig. 9) had fortuitously discovered video microscopy while teaching a microscopy class and projecting with the aid of a video camera and a

monitor the effects of opening and closing the aperture diaphragm in DIC microscopy to the students. While the image in the microscope worsened with opening the aperture due to overwhelming light intensity, it improved on the monitor for the students. His camera was accidentally wrongly set to remove a large amount of excess (stray) light so that the maximal resolution of the fully open aperture diaphragm could be utilized.

This almost immediately shifted the biologists view of cell away from the static electron microscopy-dominated view to the new dynamic one. It was exciting to see objects of a size range that had previously required the use of electron microscopes at magnifications up to 15,000fold (Table 1, Fig. 10) in the living state, albeit everybody knew and believed that this was absolutely impossible since Abbe had reported a principle limit of light microscopy at about 200 – 250 nm (Bradbury 1989).

In the beginning video microscopy used analog electronic equipment such as tube cameras, analog contrast enhancement and analog video monitors (Allen and Allen 1983). Later digital frame memory operations were added. The details of the method and the early discoveries made are summarised in Allen and Allen (1985), Inoué (1986), and Weiss and Maile (1993).
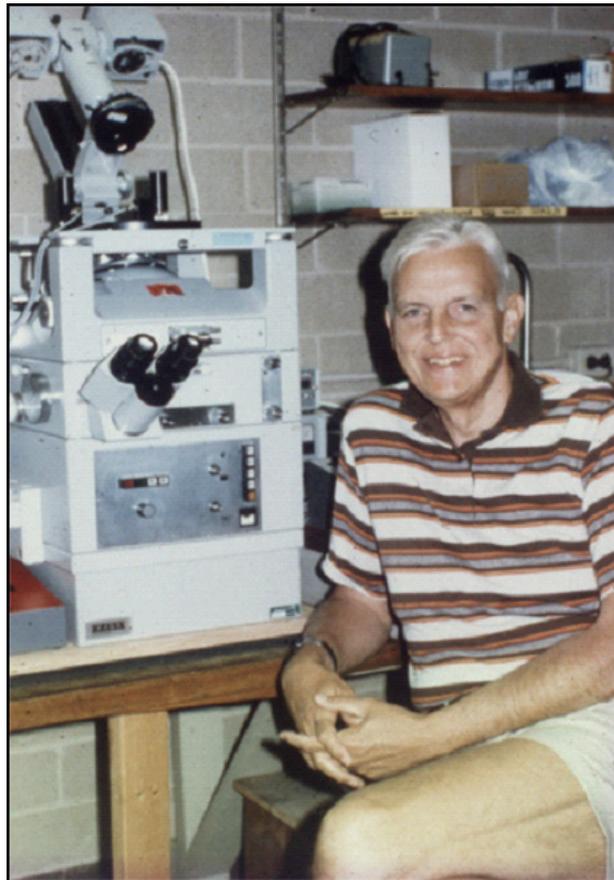
Figure 9. Robert D. (Bob) Allen and his Zeiss Axiomat microscope at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer of 1984. Photo courtesy Bob Allen.

This video microscopy is much more then just adding a camera and a monitor to the microscope to share the images with a larger audience. A new quality of microscopy emerges, if one observes the specimen instead with the human eye with a video camera connected to video processing equipment which works in real time. More recently, electronic devices other than video cameras, such as high sensitivity charge-coupled device (CCD) cameras and scanning light detector systems for confocal microscopy have been added to microscopes.

It is important to note that in electronic video and light microscopy image information may be coded either in analog or in digital form:

- **analog:** the brightness at each point of the optical microscope image is converted into a voltage signal by the camera. The analog signal is a continuous signal where 0.4 V represents black and 1 V white. It is interrupted by synchronization signals defining the end of lines and fields. Normally, one frame consists of 576 visible lines (European standard, CCIR).

- **digital:** by the use of an analog-to-digital converter (ADC) or a digital camera the continuous light intensity signal is converted into discrete numbers which are assigned to an array of picture elements (pixels). A common format is 768 x 576, i.e., 768 pixels per line and 576 lines. If an 8bit conversion is used, one obtains images with 256 gray levels where 0 represents black and 255 white.
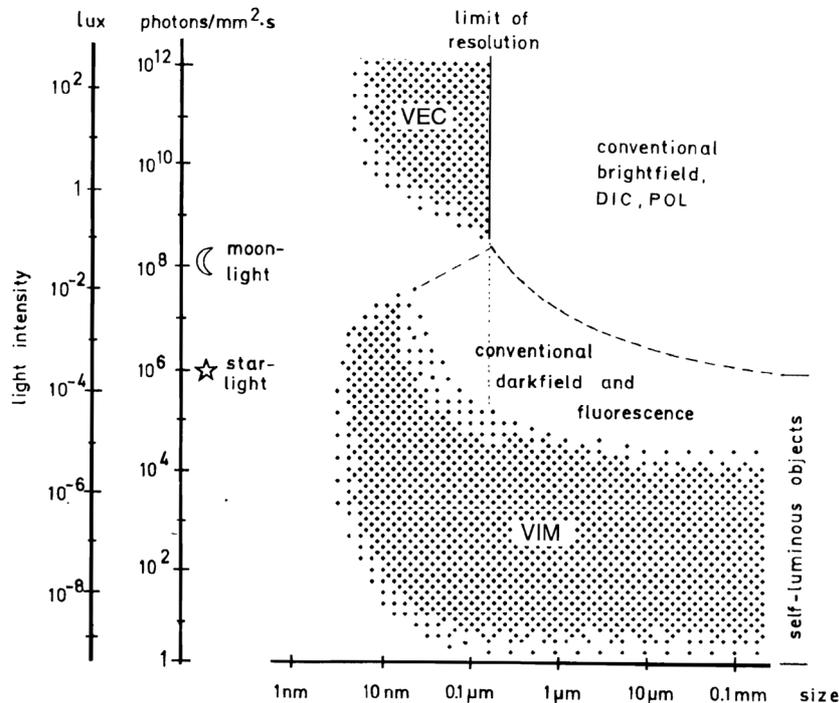
Figure 10. Video microscopy works beyond the limits of conventional light microscopy. When plotting light intensity against object size, the new application areas for video microscopy are seen (dotted areas) both in low light situations (VIM) and when working with very small objects (VEC microscopy). Modified from Weiss et al. 1998.

The human eye can distinguish somewhat less than 100 gray levels. In analog image processing contrast can be amplified electronically up to several thousand-fold. In digital image processing contrast is enhanced numerically and the upper limit of useful digital contrast enhancement is only about three to five-fold; however, image quality can be improved considerably by a large number of additional digital filtering algorithms. This means that maximal image improvement is only gained, if first analog and then digital contrast enhancement and, if needed, further image processing procedures are applied (Fig. 11).
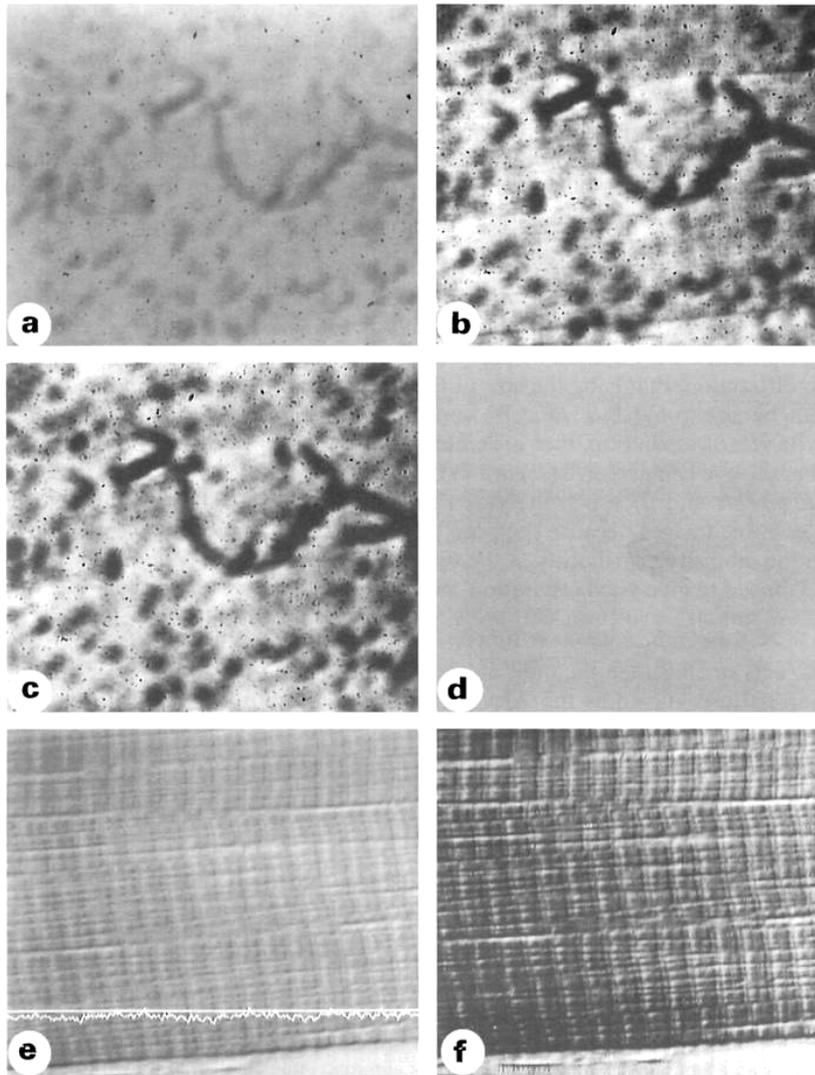
Figure 11. Steps of improving image quality of a poor contrast specimen by AVEC-DIC microscopy. The specimen, an unstained thin section of a striated muscle prepared for electron microscopy was viewed by differential interference contrast (DIC) microscopy. This kind of specimen exhibits no contrast in conventional light nor in electron microscopy, but it is visible with video microscopy. Microscopy was intentionally carried out **prior** to proper cleaning of the optics after arriving at a marine station in order to demonstrate value of the procedure in the presence of unusually heavy dust in the microscope and on the camera and other imperfections (mottle). (a) In-focus, not enhanced image. (b) In-focus, analog-enhanced image. (c) Out-of-focus image, with mottle. (d) Out-of-focus image, background (mottle) subtracted. (e) In-focus image, mottle subtracted. (f) Digitally enhanced image. (Zeiss IM 405, Plan Neofluar, 63x, N.A. 1.4, 16x eyepiece, processor ARGUS Hamamatsu Photonics. Frame width 42 μm. From Weiss et al 1989.

The three fields (i) *video-enhanced contrast microscopy* for highest resolution work, (ii) *video-intensified microscopy* for low light applications, and (iii) *electronic scanning microscopy* for confocal fluorescence microscopy and 3-D imaging use different camera technologies but images are processed in a common way by basically the same types of analog and digital image processing steps. These techniques are combined under the term *electronic light microscopy* (Fig. 10). Although the latter technique uses also digital cameras and detectors, we speak here generally of *video microscopy*.

Video microscopy made the traditional light microscope a new powerful tool especially for those who are working on dynamic aspects of biological systems. It has given further resolving power to the light microscope enabling the observation of particles of a size range between those which were already well known from light microscopy and those normally studied only by electron microscopy, with the added advantage that specimens can be examined in the living state. The technique has also the capacity to "clean up" the image by digital steps (background subtraction, digital filtering) which leads - in many cases for the first time - to the visibility of the object under study (Fig. 11).

The improvement of images obtainable by video-microscopy is due to the following reasons:

1. Electronic cameras are able to detect differences in intensity which are much smaller than those detectable with a conventional microscope by the human eye (Fig. 10). Hence, two closely adjacent objects can be better resolved which increases the resolving power by a factor of about 2; this is due to the fact that the range of gray levels can be stretched and stray light, that does not contribute to image formation, can be subtracted (Fig. 12);

2. If objects are even smaller they may not be truly resolved but become visible in their position and live movements (Fig. 12); this leads to an improvement in visualization by a factor of 10 for organic and up to 50 for inorganic material such as colloidal gold (Fig. 13);

3. Weakly self-luminous objects can be detected by extremely sensitive low light cameras (Fig. 10) thus allowing images to be created at illumination situations with 1 million times less light than required for night vision with the well-adapted naked eye under starlight conditions. Video-intensified microscopy (VIM) and photon counting imaging are applied for studying live cells loaded with small amounts of dye and illuminated at low intensities so that photo- or dye toxicity is avoided, or for visualizing very weakly fluorescent and self-luminous samples such as bioluminescent bacteria.

Taking these advantages together, video microscopy allows one to overcome the three basic limitations of conventional microscopy, i.e. too low illumination of the scene, too little contrast and too low resolving power, i.e. the resolution limit.
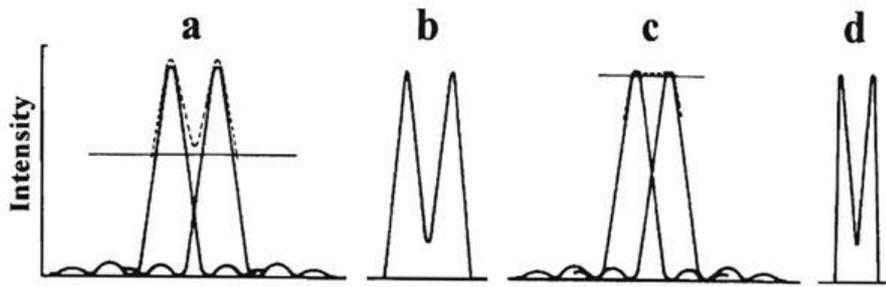
Figure 12. Improved resolution by video microscopy. The diffraction pattern (Airy pattern) of a very small object is characterized by a central zero order maximum and smaller maxima of first, second, and higher orders. (a) The overlapping images of two closely adjacent objects (pin-holes) with their summed intensity distribution (dashed) are shown. The two objects are resolved according to Rayleigh's criterion since the central depression is sufficiently deep to be perceivable. (b) A much improved image is obtained by redefining the low intensity (black) end at the position indicated by the horizontal line by applying offset, and subsequently amplifying the signal by applying gain. (c) The same objects are somewhat closer so that they are not resolved according to Rayleigh's criterion. (d) However, if contrast is enhanced as for (b), even in this situation an image can be obtained which shows the two objects separated. Sparrow's limit of resolution is reached when there is no trough between the two peaks. From Weiss 1999.
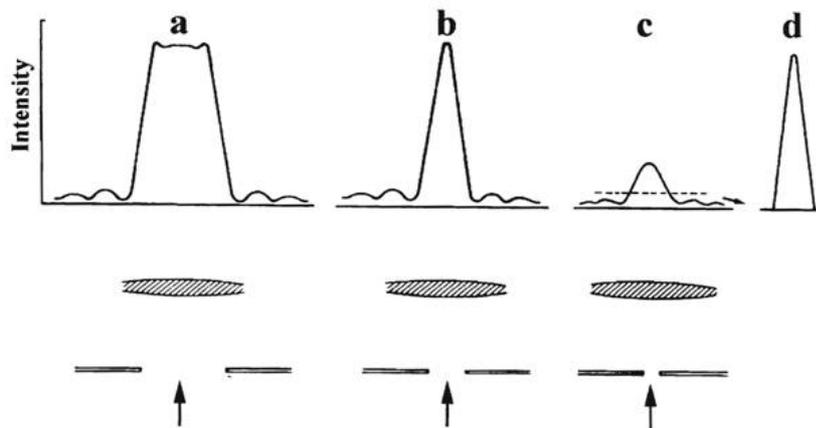
Figure 13. Improved visualization of sub-resolution size objects by VEC microscopy. Positive objects (slits) that are larger than (a), equal in size (b), and much smaller (c) than the limit of resolution are imaged by transmitted light (arrows) using an ideal diffraction-limited optical system represented schematically by a single lens. The top panels show the resulting intensity distributions across the images (diffraction disks, Airy disks). Before digitization, this corresponds to the voltage of the analog video signal, i.e. brightness, along a video scan line. The sub-resolution size object (c) yields a very low contrast "image" which cannot normally be distinguished from surrounding noise and therefore remains indiscernible by eye. However, its contrast can be enhanced by applying offset and gain, i.e. applying a negative DC voltage of a magnitude indicated by the dashed line and subsequent electronic amplification. This results in the definition of a new black level (intensity zero) and a higher signal, as seen in (d). As a result of such analog contrast enhancement, objects much smaller than the limit of resolution (c) can be clearly visualized (d). However, their real size and shape cannot necessarily be inferred from the size or shape of their "images", such as image (d) of object (c), which is inflated by diffraction to be equal in size to image (b). From Weiss 1999.

Digital image processing. Microscopic images that have been picked up as or are converted into a digital signal allow digital image processing to be performed. This is used to reduce image noise by digital filtering or averaging, to subtract undesired background patterns, to further enhance contrast digitally, or to perform measurements in the image (e.g. intensity, size, speed, or form of objects). It is since the development of procedures for noise reduction and contrast enhancement in real time, that is at video frequency (40msec intervals from one frame to the next), that the microscopist has been able to generate electronically optimized pictures while working at the microscope.

2.5.2. Allen video contrast enhancement: The cytoskeleton is alive

Video enhancement increases contrast electronically in low contrast or 'flat' images. This process not only clarifies images containing details visible to the eye, but renders visible structures 5–20 times smaller than can be detected in microscopes by looking down the eye-pieces or by taking photomicrographs (Fig. 14). A special variation developed by Allen proved to be especially powerful. Allen Video-enhanced contrast (AVEC) microscopy uses polarized light for DIC or polarization microscopy and requires the introduction of additional retardation with the compensator, after offset adjustment and analog enhancement (Figs. 11 and 14) (Allen et al. 1981 a,b, Weiss 1999).

Specimens which are extremely weak in contrast or even invisible by conventional microscopy are best suited for AVEC-DIC microscopy. Examples are micelles, liposomes and single or double-layer membraneous material, colloids (see for example Kachar et al., 1984), live, actively transcribing rDNA genes (Trendelenburg et al. 1988), synaptic and other small cytoplasmic vesicles (Allen and Weiss 1984, Allen et a. 1985, Euteneuer et al. 1985, Weiss, 1986 a,b), artificial latex particles of 50 nm and smaller, and cytoskeletal elements such as microtubules or actin bundles (Kuznetsov and Weiss 1998, Weiss 1999). The process of microtubule gliding (Fig. 14, see also section 4) was discovered by AVEC-DIC microscopy (Allen et al. 1985a), its ATP-dependence and the completely new class of motor enzymes were discovered (Vale et al. 1985a, Euteneuer et al 1985, Paschal and Vallee 1987) using video microscopic motility assays (Allen, 1985). Even molecular events such as microtubule subunit assembly and disassembly could be directly observed for the first time in cytoplasm (Weiss et al., 1988). Video contrast-enhanced polarization microscopy (AVEC-POL) visualized even extremely weakly birefringent objects such as single microtubules (Allen 1985). When applied to bright-field or epi-polarization microscopy the VEC technique visualized 5-20nm diameter colloidal gold particles (DeBrabander et al. 1986).

Figure 14. AVEC-DIC microscopy shows organelles move on free microtubules and a careful comparison of the two images that are 40 sec apart shows that microtubules glide independently over the glass surface. Assay for motor enzymes adhering to organelles and to the glass surface. Modified from Allen and Weiss 1984.

Video microscopy developed to the method of choice for all studies of cell or organelle motility. Techniques to improve images of *moving objects* can be generated with the aid of digital processors. Tracing operations add frames at predetermined intervals to a frame memory thereby generating images showing multiple positions of moving objects. Averaging can be used as a filter to remove velocities greater than a certain pre-selected velocity from the images. Conversely, subtraction of sequential in-focus images can be used to image moving objects selectively, while stationary ones are absent from the image. In conclusion, the dynamics of bending, snaking and gliding movements of microtubules, formerly only known as a stiff cellular skeleton, the transport of minute cell organelles along individual microtubules and the discovery of cytoplasmic motors not only increased our knowledge but also converted our view of the cell and its components from the previously static view dominated by a rigid cytoskeleton into a highly dynamic image with active movements of practically all cell constituents.

Need for Interpretation. Intracellular objects in the size range of 1nm or smaller are truly resolved only in EM images. But the natural configuration of intracellular components may well be distorted during the procedures of fixation and dehydration for EM. In AVEC-DIC microscopy all objects smaller than the diffraction limit of about 200nm are visualized but not resolved, so that images may not necessarily reflect their

real size. Objects smaller than the limit of resolution are inflated by diffraction to the size of the resolution limit, i.e. the Airy disk diameter of about half the wavelength of the visible light used. (Hecht and Zajac 1974) Although the size of the image does not enable a decision on whether one or several objects of a size smaller than the limit of resolution are present, the degree of contrast sometimes permits this judgement to be made (Fig. 15). A pair of adjacent microtubules would, for example, appear to have the same thickness as a single one, but the contrast would be about twice as high. If large numbers of subresolution objects are crowded together and separated by distances less than 200 nm from one another (e.g. vesicles in a synaptic nerve ending), they will remain invisible, because contrast from lights and shadows of the particle images will cancel out when their DIC-shadowed Airy disk images overlap. However, they will be clearly visualized, if they are separated by a distance greater than the resolution limit (Figs. 13-15). Also one has to remember that, if averaging over several frames is applied to reduce noise, all moving parts in the field of view may be blurred or completely missing in the images. If in-focus background subtraction is used, solely moving parts are visible, while stationary ones are subtracted out. It is therefore evident that similar to the awareness of the sample preparation methods in electron microscopy one must be aware of the effects of the applied image processing steps in order to appropriately interpret the meaning of the video-microscopic images.
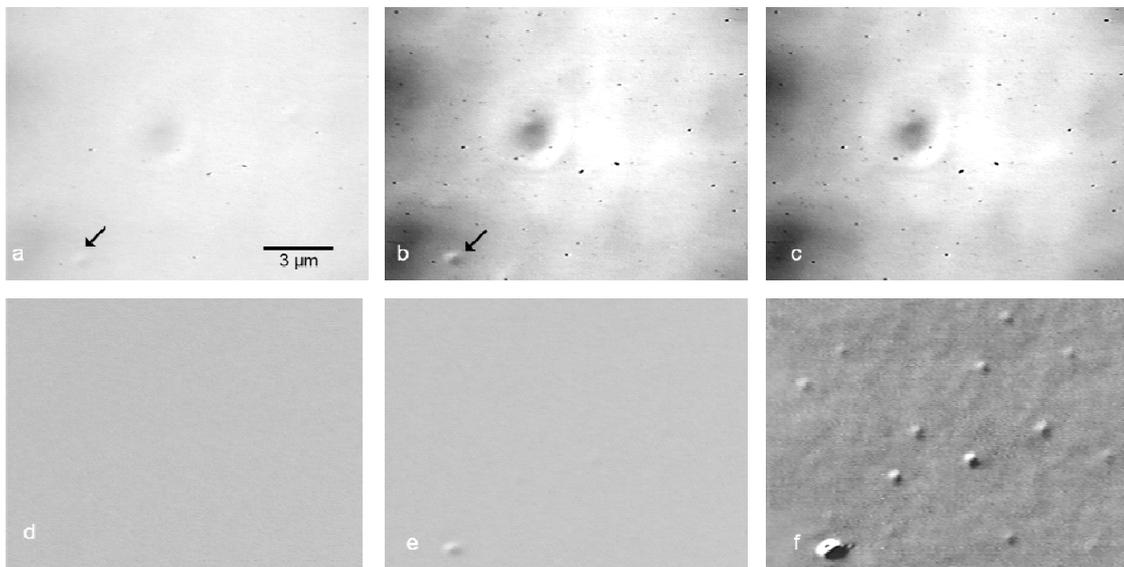
Figure 15. Visualizing single and clusters of 50 nm polystyrene nanoparticles. Video-enhanced-contrast-microscopy (AVEC-DIC) of a specimen with very weak contrast demonstrating the steps of image generation and contrast enhancement. Seeing by video camera and computer. (a, b and c) Analog contrast enhancement. (d, e and f) Digital background subtraction. (a) In focus, not enhanced; only the large aggregate of a size above the limit of resolution is visible (arrow). (b) In focus, analog enhanced; the background becomes annoying. (c) Out of focus background, analog enhanced. (d) Digital out of focus background image, subtracted from itself. (e) in focus, background subtracted. (f) The same digitally enhanced. The weakest particles are single, others are small or larger aggregates as shown by comparison with EM; only the cluster below left is larger than the limit of resolution. Photo Live Cell Imaging Center Rostock courtesy of D.G. Weiss (1998).

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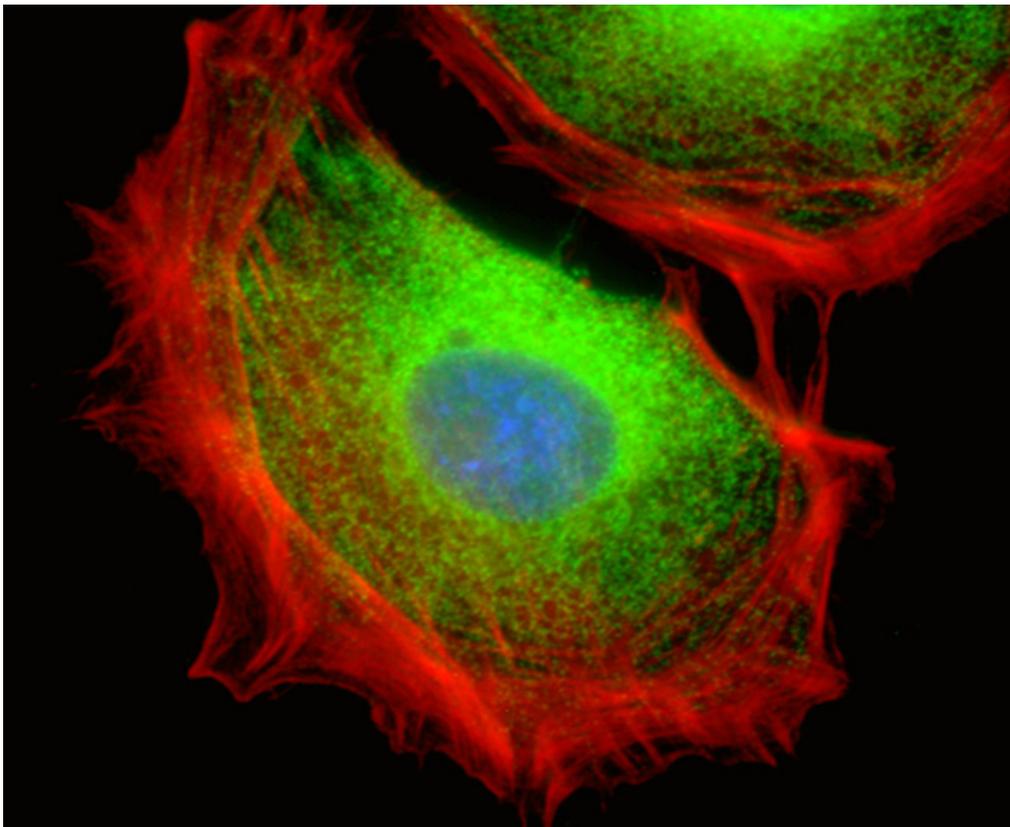
Figure 16. Video intensified fluorescence microscopy (VIM). Simultaneous staining of three cell components in fibroblast cells in culture: actin (red by immunofluorescence), a marker enzyme for the endoplasmic reticulum (green fluorescence caused by GFP-labeling) and DNA (stained with the dye DAPI, blue).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

Vital fluorescent dyes are also used to quantitatively report intracellular pH, Ca^{2+} concentration or membrane potential through changes of fluorescence intensity or wavelength. These images are topological 2D-measurements of substance concentrations or physical properties of cells. Together with the above mentioned green fluorescent protein technology (Fig. 16) and the possibility of pH and intracellular ion concentration imaging the VIM technology has dramatically contributed to our understanding of many intracellular events at the molecular level such as intracellular signalling, protein rearrangements, receptor-ligand interactions and many more dynamic phenomena. Many of the measurements can be performed in a quantitative manner because the light intensity of each pixel represents a measurement. In this way even the number of metabolite molecules can be measured when their enzymatic degradation is coupled to a luciferase enzyme reaction. Each metabolized molecule of glucose or ATP leads to the cleavage of one molecule of a luciferin derivative which causes the emission of one photon. By photon accumulation on the camera target for one minute one obtains an image representing a map of ATP or glucose concentration (Fig. 16). Thus the cell can be viewed as a measuring cuvette for quantitatively determining local substance concentrations and parameters of specific molecules in both time and space (Fig. 17).
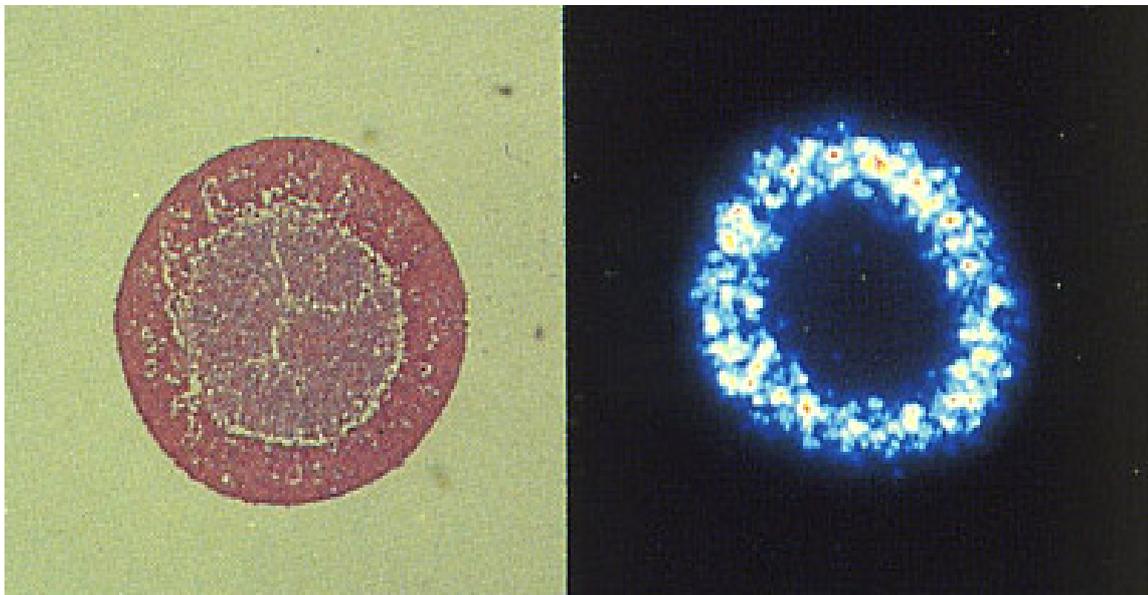
Figure 17. ATP-measurement by luciferin luminscence in a section through a frozen tumor speroid in tissue culture. ATP-concentration in false colors, blue and white are indicating concentrations lower than 1 mM, while the red tips mark cells with more than 1 mM ATP. The cells in the center are lacking ATP indicating necrosis. Left: Bright field image. Right: Visualization of ATP-activated luciferase reaction which generates photons by hydrolyzing ATP molecules. Size 1mm. Photo courtesy W. Müller-Klieser 1990, from Hamatsu Photonics 1995.

2.5.4. Confocal microscopy: Three-dimensional fluorescence images

In conventional fluorescence microscopy the light from out-of-focus planes is very prominent and precludes high resolution imaging in the axial direction (z-axis) of the object. Starting at around 1985 confocal microscopes came into use which allow in fluorescence microscopy to obtain optical section images, i.e. images that contain only the fluorescence signal from a thin plane of the object. Confocal microscopes generate stacks of images with a z-axis resolution of 0,6 μm rather than several μm as previously. Therefore, it is possible to merge the image stack into a three-dimensional image that can be looked at from all sides and cut in the computer in all planes to show internal structures (for reviews see Shotton 1987b, 1989) (Figs. 18 and 19). This technique changed our view of cell ramifications and multicellular arrangements, although the slow recording time of typically 1 sec per plane leads to blurred 3D images with living cells due to their locomotion or organelle movements and prevents the study of fast dynamic events in 3D. Only recently faster detectors became available which will allow the recording of true 3D movies. Confocal fluorescence microscopy also permits the determination of cellular components in defined columns such as a nucleus or in a whole cell or parts of it (Fig. 19).
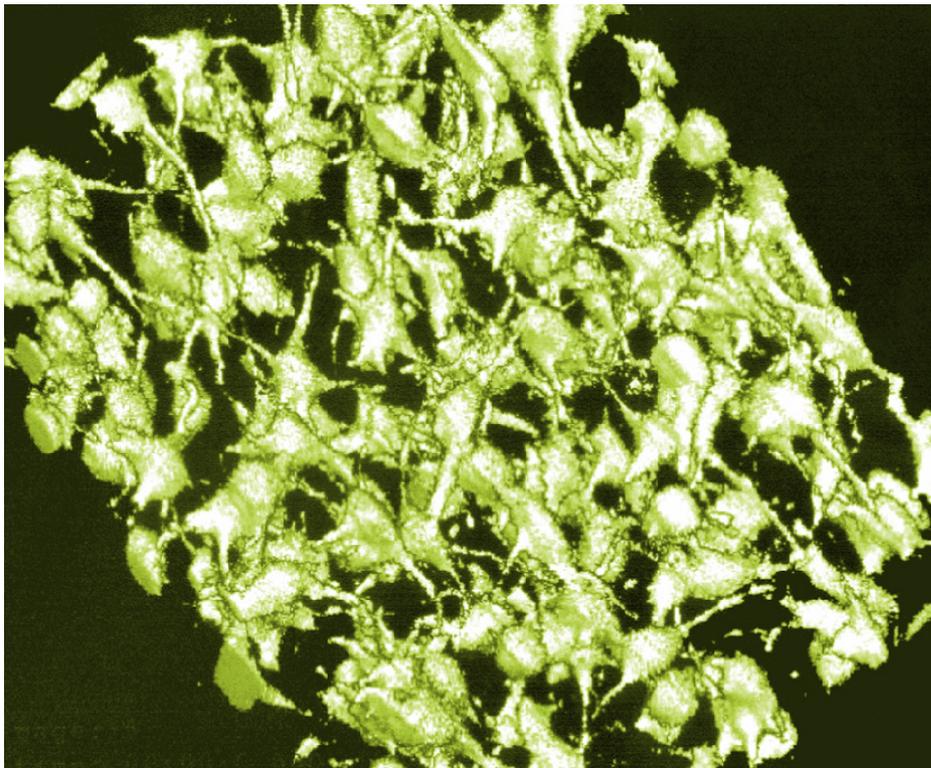
Figure 18. Confocal laser-scanning fluorescence microscopy. 3D-reconstruction of cells of he human cornea, created from a stack of confocal laser scanning micrographs. Photo Live Cell Imaging Center at the University of Rostock, courtesy of Christian Hahnel.
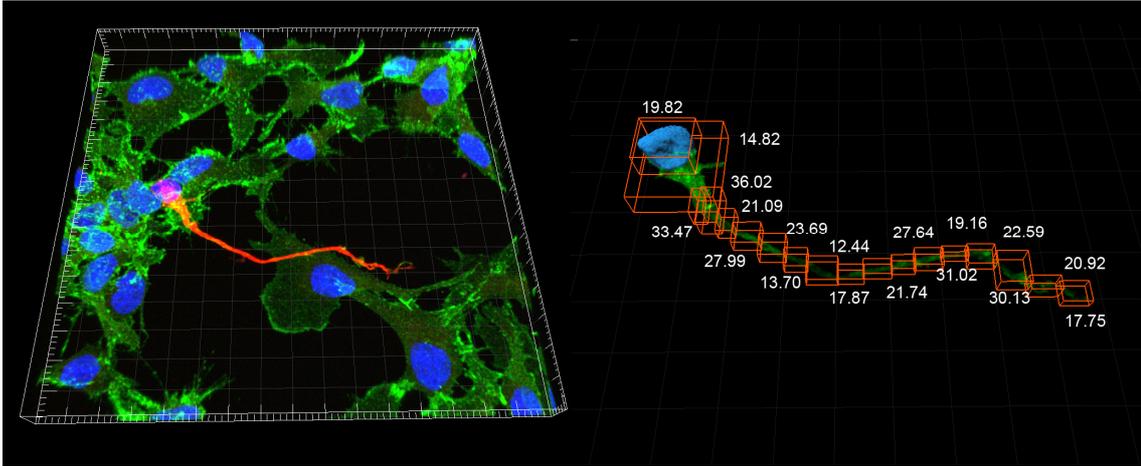
Figure 19. Measuring intracellular components by confocal fluorescence microscopy. Determination of the volume of a neuron between other cells in a neuronal network in vitro using a fluorescent neuronal marker. Once the volume is determined the fluorescence of other components such as the signalling molecule beta-catenin is determined in different fractions of the cell volume (right). Photo Live Cell Imaging Center Rostock, courtesy of Benjamin Bader (2010).

2.5.5 Cytomics and Systems Biology: Charting and modelling of the entire cell

Quantitative fluorescence imaging, especially when performed with confocal microscopy yields information not only on structure (3D) but also on chemical concentration (fourth dimension) and temporal dynamics (fifth dimension) (Figs. 17 and 19). Determining the localization of 100 proteins by immunofluorescence microscopy one obtains the so-called toponome, i.e. the map of proteins and color coded protein families (Friedenberger et al. 2007) (Fig. 20). The global approach to compile quantitative data on many if not all cellular components represents the field of *cytomics*, much like *genomics* which deals with the whole set of genes and *proteomics* which describes the whole set of proteins in a given cell or tissue in a given physiological situation. Taking these data together into a computer data base allows modelling of cells and cellular states as a combination of cell biology and information science: *systems biology*. The abstract graphical representations of molecular interactions, structures, metabolic pathways and signaling cascades which are provided by systems biology are based on high resolution microscopy combined with biochemical measurements. The epistemic role of systems biology images and graphs needs a more detailed description which will be the subject of a subsequent study.

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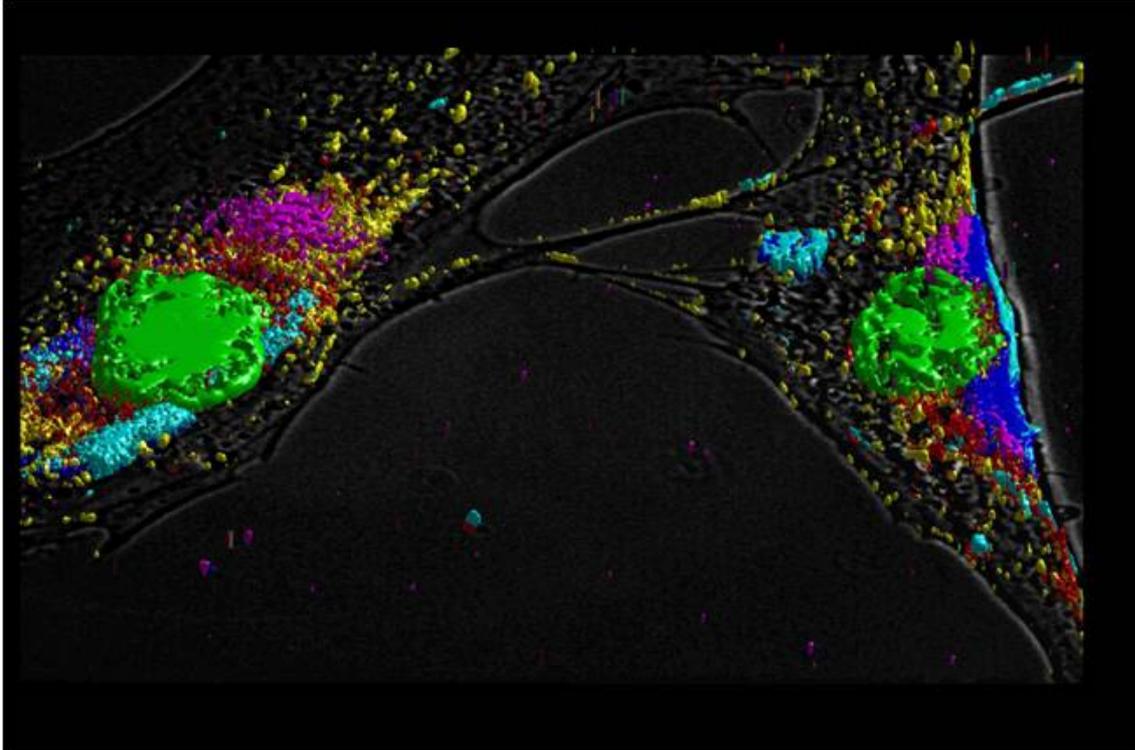
Figure 20. Imaging the toponome of two muscle cells by immunofluorescence. Each color in these two cells encodes the position of a functional group of proteins, which share the same location in the cell. Courtesy of Walter Schubert, University of Magdeburg.

3. Classification and evaluation of microscopic images

3.1 Types of microscopic images

The specific properties of the object that are visualized by the different microscopic techniques, as well as the way they are converted into graphic data vary dependent on the applied technology and must be taken into consideration when observing the resulting micrographs.

Microscopic images can be classified in principle in a way similar to other epistemic images which were obtained without microscopes as described in the work of Klaus Sachs Hombach (2009) on the classification of images: His classification is based on aspects of perception and sign theory. Empirical scientists are more interested in classifications with the potential to recognize error sources. This makes ontology-based and science theory-based classifications necessary. A complete study of this topic is part of ongoing work and beyond the scope of this report. However, considering the above description of different microscopic images obtained with the different microscopies we can already derive the following conclusions:

4. According to the classification of Sachs-Hombach who defines "external" and "mental" images, we see that we have to deal here with external images, which are described to have a material and enduring appearance.

5. Generally, microscopic images are of representing character and are therefore part of Sachs-Hombach´s category "darstellende Bilder".

6. His other category "logical images" (logische Bilder) may be relevant for the graphs and tables in systems biology and for the in silico models of cells and cellular processes. Imaging in the new field of systems biology remains, however, to be discussed in a later study.

7. The finer classification for "representing" images in cell biology discussed here aims at defining categories that are combining images that are created by similar methods and may therefore bear similar problems. To distinguish those is important as they differ in the need of additional knowledge on their ways of origin and possible inherent problems when their epistemic value is to be judged. This knowledge is required for appropriate interpretation and use in building scientific knowledge and for avoiding erroneous interpretations.

8. Terms such as image, artifact, true representation etc may have changed with the advent of completely new methods for the generation of images (photography, x-ray imaging, electron microscopy, digital imaging, computer models) so that also a thorough study of historical ontologies would be desirable in the future.

An overview of the different classes of microscopical images as well as their type of generation and the kind of how we are seeing them is represented in Table 3. One clearly defined class (1.2) of images is seen by simple magnifying instruments where the objects appear similar to our everyday experience, i.e. objects are either

transparent and absorb light or they are opaque and reflect light as in brightfield or darkfield microscopy. Just finer detail is perceivable. Another class (1.3) of images which are inaccessible for our eyes and unknown to our experience comprises the techniques used to create contrast from physical parameters other than from reflection and absorption. With these we make object details visible which have certain material aspects such as birefringence, or phase retardation.

Of a fundamentally different kind are those images which are obtained with "electronic eyes" such as analog or digital cameras (classes 2.1 and 2.2). These allow the modification of contrast and brightness in combination with the possibility to choose microscope settings which are theoretically appropriate but would produce images too bright or too dim to be perceived with our eyes. Abbe's limit of resolution is not valid for electronic eyes, which instead obey Sparrow's criterion of resolution, which allows one to resolve much smaller objects and reach superresolution. Additional techniques introduced very recently use modified laser beams as light source so that even more resolution can be reached and objects of a diameter of one tenth of the wavelength used for imaging can be resolved (see Schermelleh 2010 for review). These techniques also break the previously known limits of light microscopy with respect to resolution, to the light intensity required (up to one million times less than our eyes and photography) and contrast (which can be enhanced over hundredfold). The distinction made between analog and digital imaging, i.e. between the continuous and the discrete type of information seems also to be important and will be subject of a future study.

Table 3. Classes of scientific images in microscopy and cell biology

Class	Type of seeing	Type of generation	Type of images
1.	**Seeing with the eye**	**Physiological vision with and without expansion of magnification**	**Seen images**
1.1.	Seeing with the naked eye (or the help of glasses)	Unarmed or unaided vision	Physiological vision
1.2.	Seeing objects in a simple microscope	Magnifying glass or brightfield or darkfield microscope	Increased range of physiological vision
1.3.	Physical - optical contrasting techniques	Fluorescence, polarization, phase retardation, birefringence, interference microscopy	Visualization of physical properties of the object not accessible by physiological vision, image made up of qualitative or quantitative physical parmeter estimation or measurement; demands interpretation

Class	Type of seeing	Type of generation	Type of images
2.	**Seeing with electronics**	**Images are generated electronically at the light microscope but they are not visible with the eye in the microscope** **Analog and digital image improvement**	**Further expanded range of visibility** **Analog or digital electronic images or video films**
2.1.	Analog electronic recorded images to be seen on screen	Topology of light intensity is recorded with analog video cameras and shown as voltage signal over time. Analog contrast enhancement (Video microscopy), surpassing the resolution limit of light microscopy,	Overcoming the limits of classical light microscopy: contrast, intensity, magnification, "the new image of the cell"
2.2.	Digital electronic images to be seen on screen	Topology of light intensity is recorded by digital electronic devices as discrete measurement values of image points over time	Image consists of 2D or 3D measured intensity values
3.	**Imaging by other, non-optical image generating procedures**	**Specialized detectors**	**Images consist of measurement values**
3.1.	Electron microscopy images can be seen on screen or photographs	Electron absorption, - reflection, - dispersion, - refraction or - fluorescence	Highest resolution images, down to atomic resolution
3.2.	Other electromagnetic waves: x-rays, infrared etc	X-ray microtomography, NMR-, IR-, Raman-imaging spectroscopy	Internal structures in 3D (x,y,z), chemical distribution (x,y, energy)
4.	**Seeing with the brain**	**Mental creation**	**Constructed images**
4.1.	Seeing with the brain	Composition of information from many viewed objects of a kind	Composite realistic images (e.g.drawings)
4.2.	Seeing with the brain	Merging of information from many viewed objects with imagination, closing gaps (equipment limitations) by additional biological information	Imaginary realistic images Realistic images with some hypothetical aspects
4.3.	Seeing with the brain (and computers)	Modeling of quantitative image data and other related data, improvement by simulation (often with the aid of in silico techniques)	Systems biology-type schematic

There are certain types of images that are obtainable in an indirect manner (classes 3.1 or 3.2) and by making use of additional information from other specimen or from other fields of biology. Mental activity leads in this case to images, being mainly drawings, which are superior to single images, series of photographs or even selected representative images. Class 4.1. are images which we call composite realistic images. Often microscopists combine information from many fields of view of the same cell or tissue and create a mental image which results in a drawing such as the remarkable images by Radivoj Krstic (1991) (Fig. 21). These contain real image information obtained from sometimes hundreds of observed images but from no other type of information. They provide a very informative, realistic and three-dimensional view of cells and tissues.

If the resolution or contrast of the microscope is insufficient to decide on the fine detail, microscopists of all generations have used additional information (Class 4.2.). It is remarkable to see fine image detail in copper-etched illustrations from previous centuries which are difficult to truly resolve even with contemporary microscopes. It seems as if in those days unlimited time to observe and fully relaxed minds may have contributed to see things barely resolvable by the instruments. Today we can use additional information from physiological, biochemical, and different types of microscopic studies to decide in ambiguous situations when making a drawing of the object which contains more information than a photograph. We call this type the *imaginary realistic images*.

This type of images can also be explained in studying the historic case of Camillo Golgi (1843-1926) and Santiago Ramon y Cajal and how different they understood and drew what they saw in the microscope (Fishman 2007; Stahnisch 2007). Both were neuroanatomists and described the neuronal connectivity in the brain. Golgi had developed a staining technique ("Golgi staining") based on silver salt impregnation of nerve cells which both scientists used. With the silver staining one sees individual black neurons with all their ramifications lying in a clear environment because it stains only a fraction of the neurons. This is indeed advantageous, because otherwise the whole brain sections would have appeared black. By observing many sections, both scientists composed wiring diagrams of the nervous system derived from images of the single stained cells. It might have been that Golgi's variant stained more cells, whereas in Cajal's hands specimens showed the cells more sparsely distributed.
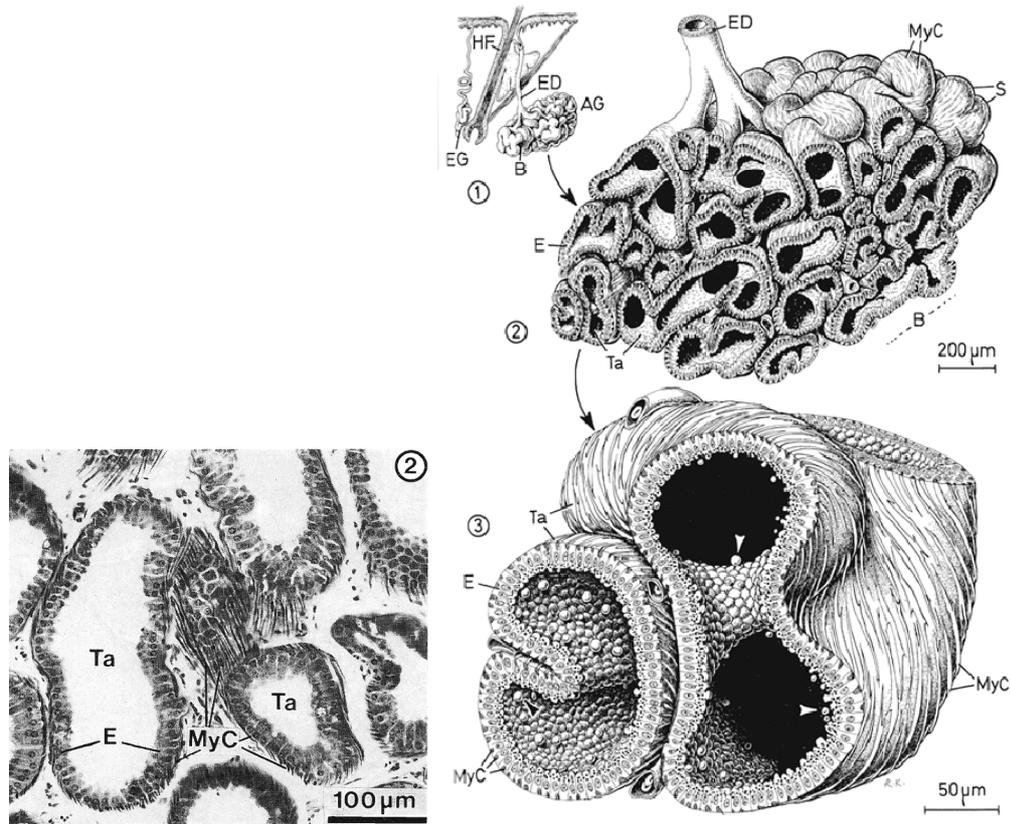
Figure 21. The imaginary realistic image of a human sweat gland. Left: Low magnification brightfield image of a histological section through a human sweat gland. Right: "Imaginary realistic" drawing of the three-dimensional microstructure of the gland at different magnifications, synthesized from a thorough investigation of multiple histological sections by light and electron microscopy. Reproduced from Krstic, 1985.

Already before these studies in the late 19th Century neurobiologists were separated into two parties: The *reticularists* claimed that the nervous system is a continuous multinucleated cellular network (syncytium) with no gaps between nerve cells, while the other, the *neuronists*, proposed that neurons are individual cells separated from one another like cells in other organs. The synaptic separation at the nerve endings or axon terminals is with a separation of 200nm is not resolvable in conventional light microscopy. Golgi was the reticularist and criticized Cajal of making poor preparations with only sparse cells. Cajal shows in his drawings the synaptic gaps between the neurons in their proper places and claimed that the function of the nervous system and the dendritic ramifications of neurons would only make physiological sense if there are gaps. He merged the then very limited information on electrical conduction in neurons and perhaps some imagination with his microscopic observations and drew the results (Ramon y Cajal 1909 and 1911). Both scientists shared the Nobel Prize for Physiology or Medicine in 1906 and when both gave their Nobel Lectures (published later, see: Golgi 1967, Ramon y Cajal 1967) Golgi ferociously attacked Cajal for his poor microscopy and for over-interpreting what he saw. But, as turned out later, Cajal was right and his anatomical atlas contains practically all major connections in the brain

of humans and some animals drawn in a correct and so ingenious way that almost no additions were necessary in the following century up to today. Golgi had accepted only what he saw as reality and even simplified the connections to make them more straightforward as conductors. We will discuss below that this type of "seeing with the brain" is adequate in microscopy and does not violate the requirement of objectivity.

The last class (4.3) contains images generated from a multitude of image data and additional biological knowledge. This merging of information into a holistic view of the cell is only obtainable with the aid of "electronic brains". This "in silico" approach requires the "seeing" not only with our brains but also creating model images with a man-made brain extension, the computer.

3.2 Objectivity

It is well known from text books that the physiological process of vision does not mirror the visual environment pixel by pixel into the brain (e.g. von Campenhausen 1993). Wolf Singer (2009) demonstrates in his review on the relation between vision and perception the physiological problems connected with our mental intake of graphic data. We are surprisingly unaware of the highly self-referential way in which our brain functions, and how strongly it relies on preexisting information. How this can misguide the interpretation of optical information can be demonstrated by the common example of circular, spherical objects on a plane background. If a shadow is seen at the bottom of the objects they are interpreted as convex (see also Figs. 4 and 14). If the shadow is cast at the top, however, they are seen as concave. This is due to the fact that our brain automatically assumes that the light is coming from above (which is mostly the case in a natural environment). The considerations made by Singer remind us to consider precisely the reliability of a given scientific image taking into account the way it has been generated and also the way in which our brain deals with image information.

It would however be wrong to understand the vision process only as a plethora of errors and deceptions. The kind of subjectivity which is learned from childhood on is essential for efficient visual orientation in the visible world. Our brain extracts important, frequently appearing or exciting aspects from experience and uses those to make visual perception faster, more efficient and more informative. We can trust that these advantages of a non-linear transfer of the physical input from the eye to the brain are likewise extremely helpful in seeing the microscopic world. The improvement of the visual information comprises various stages of neuronal processing from the retina, through the thalamic nuclei and the limbic system, where emotional memories may be associated and important aspects may be emphasized, to the visual cortex, where only relevant and interpretable aspects are used as source to construct the optical impression that enters consciousness. We must live with the situation that our brain may eliminate visual information that is so strange that it cannot be put in relation to any known objects. But on the other side it will help us in preferably presenting "interesting" or

"important" aspects of the seen scenes to our consciousness. It seems as if we would rather benefit from such "seeing through illusions" than that we are deceived and falling victim to optical illusions that lead to erroneous information (Gregory 2009). This is probably due to the evolutionary improvement of vision by the brain through millions of years, leading to "better seeing" in almost all situations of daily life while annoying optical illusions surface only in rare instances. This needs to be taken into account when asking for seeing free of prejudice or seeing without the expectation a specific hypothesis.

Today it is often said that evil is introduced in scientific imaging by analog and digital electronic image processing. This is true only so far, as not only these two recently introduced, but also all previous techniques can be misused so that falsified, distorted or completely faked images could be generated. All biological, optical, chemical, as well as all analog and digital steps of image generation are influencing the specimen images and are, therefore, *ab initio* artifactual steps and bear the potential of falsification and distortion so that they need to be applied diligently and with responsibility. But as shown in the above analysis of the microscopy techniques, it is very clear that, if good scientific practice is observed, these methods have proven to be extremely powerful and have furthered science enormously, especially modern cell biology.

Right from the beginning, i.e. when selecting any type of microscopic contrast generating technique the scientist decides about creating more or less artificial images with the aid of instruments translating the physical properties of the object, into image contrast. In addition, when looking at the resulting images we suffer from the limited objectivity of human vision. Microscopic images are under the influence of the scientist's at-will selection of and the search for the actual detail or aspect presently under study before it is depicted. This all comes in addition to the state of the art procedures of digital image processing which microscopists necessarily use.

Almost all the times microscopists were well aware of the desirable virtue of objectivity and they had this probably much more in their minds than other scientists because they knew about the additional problems inherent in microscopy. At all stages of image generation the scientist tries to achieve a most informative image, rich in contrast and most clearly displaying the object properties under study. Henry Baker (1743) wrote:

> „When you look through the microscope, shake off all prejudice, nor harbour any favourite opinions; for, iff you do, `tis not unlikely fancy will betray you into error, and make you see what you wish to see."
>
> „Beim Gebrauch des Microscopii werfe man alle Vorurteil weg, und beherberge auch keine Favorit-Meinung dann wann man noch solche hegen wurde, so wurde die Phantasie sehr in Irrthum führen, und das sehen machen, was man zu sehen wünschte." (Baker, German Edition 1756 (S64-65)

Not only the physical limitation of the methods needs to be known and respected but there are two additional problems. One is that microscopic images in cell biology can *hardly be validated* by independent methods other than microscopic ones. It is however possible and necessary to validate high magnification light microscopic images by electron microscopy and vice versa. The other is the problem of *selecting the field of view* in the microscope or on the monitor that is considered good enough, representative enough, typical, relevant to the actual study and free of distorting artifacts. Here much subjectivity and preoccupation might be introduced as stated already by microscopists of earlier generations such as Pieter Harting (1866) and Otto Bachmann (1883) among many others. At all times have microscopists pointed to this aspect and admonished colleagues to highest responsibility.

In the previous sections on the types of microscope techniques and the classification of the various types of resulting images we have occasionally addressed the question of objectivity. Since this question in relation to epistemic images has been treated in a lucid and exhaustive manner by Lorraine Daston and Peter Galison (1992, 2007) one may ask whether their answers can directly be adopted for microscopic images. Daston and Galison described the basic types of epistemic virtues of objectivity which have been applied in the creation of images over the past centuries and their sequential appearance in history. The earliest of these virtues of objective seeing was "truth to nature" in the early 18th century. This was followed by the era of "mechanical objectivity" between the 1830s and 1890s while in the early 20th century the epistemic virtues "structural objectivity" and "trained judgement" challenged the previous approaches towards objectivity. We think microscopic images need to be treated as a special case of images in a detailed study later but some thoughts can already be mentioned.

Much like in insect or plant taxonomy there is a need in microscopic images in the form of drawings, photographs or digital images to consider what is thought to be "representative" for the type of cell or tissue under study. Taxonomists select one specimen from a population, declare it as "holotypus" and describe it in detail, well knowing that there is variation in the greater number of individuals. In microscopy this need for selection before the imaging step is much more pronounced, because the fraction of the "population" that can be observed is much smaller: only ten or one hundred or at most one thousand out of 100 billion neurons in the brain can be observed, and even more cells are in the liver or other organs. Furthermore, imagine the situation with video microscopy: An image of a live plant cell magnified 10,000x and projected in enlarged version on the screen in a classroom would mean that the cell would have the size of the classroom itself so that the portion that is seen is comparatively small. The situation is even more critical in electron microscopy: At a typical magnification of 50,000x a liver cell would be 1,25m long, wide and high, but only an A4-size photograph is usually observable. Given the thickness of electron microscopic sections of only 100nm, the volume of one EM image would represent

$2,5\mu m^3$ so that one could in theory obtain 6000 different images from the volume of one cell. When assuming that all EM-images ever taken would have been of this magnification and section thickness, one could obtain 600 billion images from the volume of one cm^3, so that it is doubtful if worldwide more than $1cm^3$ of cell volume of all organisms has ever been documented by electron microscopy.

It is therefore evident that the microscopist cannot follow the virtue of "mechanical objectivity" as defined by Daston and Gallison (2007) and take random photographs. There must be a hypothesis about what is under study, the nucleus, the membrane or so, and then the area must be selected. The selectivity problem influences the result probably more than any digital image manipulation. That it is to be seen positively can be seen in the example of coated vesicles in nerve cells. These are transition organelles which are well known in cells from liver and other organs, but they were found to be absent from neurons. But, D. James Morré was convinced that this intermediate carrier organelle for membrane material has such an important role in cell biology that it must exist also in neurons. So he screened hundreds of cross sections through axons and indeed found them everywhere (Morré 1982); they had been considered liver-specific before and nobody had actively searched for in the brain.

The other important question is, to what extent additional, non-microscopic information is allowed to merge into the images without compromising the virtue of objectivity. Do we see only what we expect to see or even what we want to see? Again the debate between Golgi and Ramon y Cajal may be inferred. The "close-to-nature"- or even "mechanical objectivity" approach failed here not only in the part of Golgi but also of his successors. In his impressive Handbook on the Microscopic Anatomy of the Peripheral Nervous System Philipp Stöhr (1957) insisted in his complete impartiality and refused to accept any other prior knowledge but accepted only his own histological sections, which he transferred to drawings showing a continuous reticular system (Fig. 22). Reading his words shows in addition a highly emotional negative preoccupation against the school of the neuronists:

> „Die viel erörterte Frage, auf welche Weise das vegetative Nervensystem mit den plasmatischen Elementen der Erfolgsorgane seine Verbindung findet, bleibt zunächst eine morphologische Aufgabe und ist infolgedessen mit den Mitteln morphologischer Technik in Angriff zu nehmen. Die morphologische Arbeit muss sich darauf beschränken , das zu sehen, was da ist; sie darf aber nicht das sehen wollen, was eine erstarrte Neuronenlehre oder experimentell erarbeitete theoretische Anschauung jeweils vom Mikroskopiker entdeckt zu haben wünschen. Bereitet schon die histologische Technik zur Untersuchung der peripheren Nervenformationen oft erhebliche Schwierigkeit, so fällt es nicht minder leicht, sich am Mikroskop in vollkommener Unvoreingenommenheit mit den geheimnisvollen Strukturen des vegetativen Nervensystems auseinanderzusetzen." From Stöhr 1957, 110
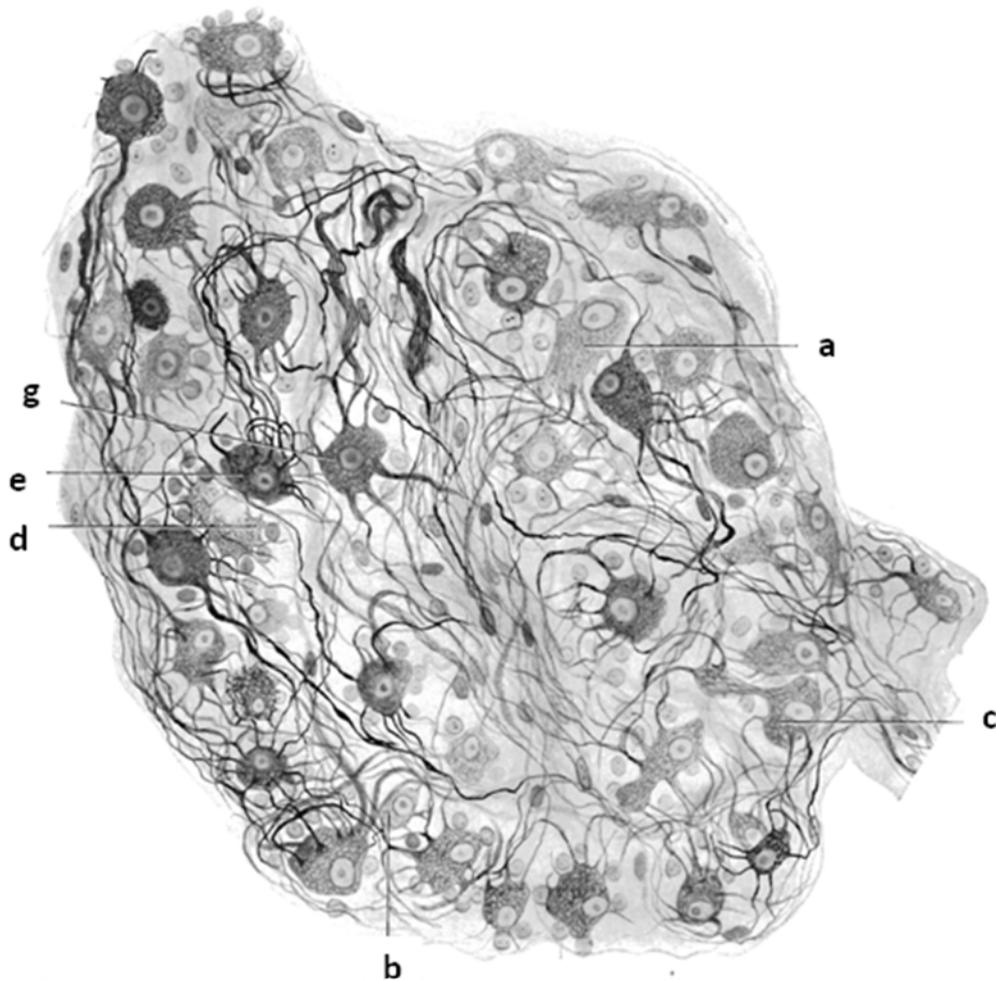
Figure 22. Nerve cell network stained by the Golgi-method that visualizes "neurofibrils", i.e. microtubules and neurofilaments, in black. "Heart ganglion of a 26 year old man. **a** large-, **b** small nerve cell. **c** lobed ganglion cell, **d** degenerating ganglion cell. **e** cell with large-, **f** cell with small extensions. BIELSCHOWSKI-method, magnification 780-fold." Reproduced from Stöhr 1957.

Stöhr was probably the last reticularist and he refused to take physiological information into account. He only ceased to publish his view when in the mid 1950s the first electron micrographs of synapses with their synaptic clefts had appeared.

Other than one might expect, and perhaps different from other fields of science, microscopical science of the 18th and 19th century was already in posession of sound epistemic concepts that respected and even requested that knowledge on properties and functionalities of biological objects be considered when drawing the images. The inability to remove subjectivity from the process of viewing of optical images and the necessity of selecting the fields of view in microscopy had to be seriously considered and the majority opinion requested that cautious consideration of additional practical and theoretical knowledge be an integral part in microscopic work. Harting (1866) argued on this subject:

„Sobald wir uns indessen weiter wagen, sobald wir aus dem vorliegenden Verhalten auf vorausgegangene oder nachfolgende Zustände Schlüsse ziehen, dann gehen die positiven Resultate directer Beobachtung und die Schlüsse unserer subjectiven Auffassung in einander über; wir stehen dann auf dem Boden der Hypothese, die sich in diesem Falle, unerachtet aller Wahrscheinlichkeit, nicht anders als durch thatsächliche Wahrnehmung zur Wahrheit erheben kann."

Harting (1866 Vol. 2:12)

Translation:

As soon, as we proceed, as we draw conclusions from the matter at hand to foregone conditions and those that are yet to come, the results of direct observation and the subjective conclusions of our own conception will converge; we are then standing on the grounds of a new hypothesis, which in this case, regardless of all probability, in no other way than by factual perception can emerge to truth.

The essence of Harting´s statement is not only that microscopists must be cautious at all times, but that theory or subjective conclusion by analogy are fundamental prerequisites for building theories and eventually obtaining truth.

History gives astonishing examples for the high value of this concept. One is the observation of the synaptic gap by Santiago Ramon y Cajal in the late 19th century, a structure he could never have actually seen with the microscpic technology available at the time, but which he included correctly in his drawings. Most likely the necessity of the existence of this entity was obvious to him, either from considerations of neuronal growth in ontogeny or by functional models of the nervous system. The same attitude might have helped early microscopists already two centuries earlier, when they drew images of the flea's bristles and other detail not really observable with the microscopes of this time when tested today. A deeper study on the epistemic virtues of "truth to nature", "trained judgement" or even the most recent type of "nanofactured" or "engineered for presentation" images (Daston and Galison 2009) and their role in microscopy seems necessary.

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

of algae or on developing oocytes and the effects were studied by microscopic observation. Hereby manually displaced intracellular components revealed their ability to relocate themselves (see for example Scarth 1927, Chambers 1931, Kopac 1938, reviewed in Porter 1984 and Frixione 2003). Yet a better glimpse at the active cytoskeleton became possible with the invention of polarization microscopy. This technique allowed visualization of cell components with birefringent properties caused by the condensation and parallel arrangement of submicroscopic fiber structures (Schmidt 1937), a procedure that was later perfected by (Inoue and Hyde 1957). Scientists discovered that birefringence was often found in cell components with obvious motile properties, such as the mitotic spindle or the protozoan cilia, supporting the argument for involvement of the cytoskeleton in cellular- and intracellular movement.

4.1.3 Discovery of axonal transport

Movement of intracellular material along the axon of a nerve cell was described as „axonal flow" as early as the beginning of the 20th century from simple light microscopic observation by F.H. Scott. He concluded that material necessary for the function of neurons must be produced in the cell body and then transported along the axon to the synapses (Scott 1906, reviewed in Lubinska 1964). The fundamentals however had been laid down by Santiago Ramon y Cajal, who had stated that the axon itself elongates from the cell body during development and therefore transport of intracellular material along this cellular tube must occur (reviewed in Lasek 1980). These predictions were first tested experimentally by Paul Weiss and Helen Brush Hiscoe (1948) who observed *damming up* of cytoplasmic material on one side, when they created an artificial constriction on a dissected free but living axon bundle with a silk thread. Cytoplasmic transport along the axon thereby became a solid framework for the study and interpretation of functional and structural data in this respect (see for example Kerkut 1975, Allen 1981, Weiss 1982, Ochs 1982, Schliwa 1984).

Towards the end of the 1960s the technology of autoradiography and especially radioactive tracing were introduced to characterize axonal transport. This procedure begins by exposing the region of the cell bodies of a living nerve, for example the olfactory nerve of the pike or the optical nerve of the rat, to radioactively labeled amino acids, for example ^{3}H-leucine. The substances are taken up, integrated into cellular proteins and transported down the axon. After a certain period of time the nerve itself is dissected out and cut into 1mm segments which are then analyzed by counting the radioactive decays in a scintillation counter. Thereby the concentration of labeled protein at a given position along the axon, and after a certain amount of time could be determined. The pattern of labeled protein over the length of the nerve revealed different classes of transport velocity (see for example Taylor and Weiss 1965, Grafstein and Forman 1980, Lasek 1968, Ochs 1969). A slow component was specified moving at

approximately 1-4mm/day, while the fastest material was found to be transported with velocities of up to 410mm/day. The fast component appeared to be more diverse, yet intermediate components could soon be determined (Willard 1974, Black and Lasek 1979, Lasek 1980). Each rate component contained a highly specific set of transported proteins or organelles.

In this period several properties of axonal transport were identified:

1. Transport does not occur by axoplasmic bulk streaming as was previously proposed by Weiss and Hiscoe (1948), but is observable as movement of individual particles while other surrounding objects remain stationary (Forman 1982).

2. Transported material is composed of different sets of substances, which each travel at a characteristic velocity.

3. Transport activities in the axon are independent of the activities in the soma and at the synapse.

4. The necessary energy is provided by local metabolism in the axon (For summary see Samson 1971, Lasek 1980, Weiss 1982, Ochs 1982). The precise role of the cytoskeletal fibers in axonal transport however was not yet known. Since it was shown that axonal transport can be blocked with the microtubule and mitosis inhibitor colchicine there was a clear indication for the involvement of microtubules (Dahlström 1968, Kreutzberg 1969).

4.1.4 The involvement of electron microscopy

Around 1950 electron microscopy became available to biologists and catapulted cell science into a new era (see section 2.4). The problem of diffraction limitation to resolution in light microscopic images could be circumvented by employing electron- instead of photon beams to produce magnified images of cellular structures. A new dimension of submicroscopic observation opened up and revealed highly resolved images of cellular components to the pioneers of biological electron microscopy, such as Keith Porter (Porter et al. 1945, Porter 1964). The types of cytoskeletal fibers known today, microtubules, microfilaments and intermediate filaments, could be readily identified. However it should be remembered that electron microscopy needed to be carried out under conditions of high vacuum, which made a complete dehydration of the cell material necessary. On the one hand the procedures of fixation and dehydration were quite harsh and the problems of creating visible artefacts by coagulation of proteins were considerable (For a more thorough discussion see Breidenmoser and Weiss (in preparation)). On the other hand, the processes of intracellular motility were still not directly observable and had to be reconstructed from arbitrarily selected series of snapshots showing the cells´ interior in a "frozen" state.

The cytoskeletal fibers were classified into three categories:

1. The hollow microtubules (MTs) of 25nm diameter and a length of up to 1mm, running parallel to the axon.

2. Microfilaments (MFs) with a diameter of 7nm and a length of 10-100nm forming a 3D-network throughout the axon.

3. The intermediate filaments (IFs) of 10nm and an intermediate length, which are located separately in bundles (Weiss and Gross 1983, Bridgman at al. 1986).

The Microtubules, that had previously only been *visualized* by specific staining or birefringence, were first reported from various plant- and animal cell systems as diverse as the mitotic spindle apparatus (Harris and Dunn 1962), the protozoan flagella (Gibbons 1961), and the cytoplasm of the cell body (Ledbetter and Porter 1963, Slautterback 1963, Byers and Porter 1964, Porter 1964, reviewed in Allen 1981, Schliwa 2002). Towards the end of the 60s combinations of phase contrast, differential interference contrast and electron microscopy also verified the existence of MTs and MFs (Goldman and Follett 1969). Shortly after, Goldman (1975) discovered that MFs of all cell types were made up of actin, a protein known from previous studies to form part of the contractile apparatus of muscle cells, and was therefore a suitable candidate for supporting motile activity in the axon. The direct visualization of this specific protein which was already known for its function in muscle contraction was made possible by immunofluorescent labeling.

4.1.5 Electron microscopy at its best

In the 1970s the electron microscopic technique made progress due to the development of 1 MeV high voltage electron microscopes (HVEM). Keith Porter's laboratory was one of only three American laboratories which ever operated with this technique specifically for studying biological cells. The most important feature of HVEM was the possibility to study relatively thick specimens. Porter could for the first time investigate whole cultured cells without the need of sectioning them. They were flatter than most cells in situ, but with a thickness of a few μm much thicker compared to the 100nm sections used for TEM before. Moreover, he was able to resolve much finer details in these cells than by using conventional electron microscopy providing hitherto unknown images of the cytoplasm and the cytoskeleton (see Fig. 7).

During the mid-1970s, Porter and his colleagues started to use HVEM for studies on the cytoplasm. John Wolosewick and Keith Porter (1976) were able to receive three-dimensional information on the organization of cellular components. They detected well-known structures like the endoplasmic reticulum, ribosomes and (with some difficulties) the Golgi complex. Furthermore, fibrous elements of the cytoplasm like MFs and MTs could be identified. But the most important effort of their HVEM-investigations seemed to be the discovery of formerly unknown 3-6 nm thick components of the cytoplasm that were named *microtrabeculae*. They became

conspicuous as a three-dimensional lattice, which was a constant feature of all parts of the cytoplasm and connected ribosomes, MTs and MFs (see Figs. 7 and 8). The microtrabecular lattice had become readily apparent for the first time by using HVEM, but Wolosewick and Porter claimed that it already had been visualized in thin-sections of cells with conventional electron microscopy by other scientists, who had associated it with MTs or actin filaments (Ishkawa et al. 1969, Goldman 1975).

The unexpected discovery of the microtrabeculae raised doubts that they exist in the living cell. Therefore, Wolosewick and Porter (1979) tried to prove the real nature of the microtrabecular lattice by using several different preparation methods. They used chemical fixation as well as fixation by freezing; and while critical-point drying was their favorite method of dehydration, they also used freeze-dying. Additionally, Mark Ellisman and Keith Porter (1980) added the preparation technique of freeze-etching and rotary-shadowing to the set of techniques, whereas Porter and Anderson (1982) investigated the lattice in greater depth by using a greater variety of fixation- and freezing procedures. The microtrabecular lattice was visible with every preparation method. Moreover, Porter and Tucker (1981) reported that lattice morphology similar to the images obtained by HVEM could be observed using conventional electron microscopy.

4.2 Explanatory schemes for the mechanism of axonal transport

At the end of the seventies, the ultrastructural images had gained a predominant role in cell biological thinking. The wealth of structural information and the fact that structure could now be seen on the level of molecules was undoubtedly overwhelming. The gap between the knowledge of axonal transport dynamics gained from autoradiography and light microscopy on the one side and between the ultra-structural images of dried cells on the other side, failed to uncover the secrets of the actual transport mechanisms. However, it led to the development of many different theories which were set out to explain axonal transport (see Fig. 22, Table 4)). For reasons of simplicity, we will focus on the two representative ones.

Table 4. Overview of the majority of possible and formulated hypotheses to explain the force generating mechanism of axonal transport.

	Mechanism	Structures involved	Action	Transport mode	Judged from present knowledge
A. Modes involving specific interactions					
1.	Material bound to carrier (I)	FGE on carrier organelle, cytoskeletal element	contractile or shear force generation	specific to organelles or carriers	Not confirmed
2.	Material bound to carrier (II)	FGE on cytoskeletal elements	contractile or shear force generation	specific to organelles or carriers	Not confirmed
3.	Material bound to carrier (III)	contractile cytoplasmic matrix	matrix contraction waves	specific to organelles or carriers	Only confirmed in pigment cells
4.	Longitudinal cytoskeletal pushing (oscillation)	MT or NF and attached contractile, elastic filaments	rapid forward motion of MT or NF with elastic return	drag of attached organelles	Not confirmed
5.	Lateral movement in the plane of membrane	membrane, submembranous MFs	contractile elements pull specific intramembrane components bound to them	intramembrane flow (shown to be slow)	Only in Heliozoan axopods

	Mechanism	Structures involved	Action	Transport mode	Judged from present knowledge
B. Non-specific modes					
6.	Axonal peristalsis	subaxolemmal MFs or satellite cells	constriction waves	hydrodynamic flow of axon contents	Excluded
7.	Endoplasmic reticulum transport	SER, possibly MFs	force generation within SER? movement of SER and/or inside SER	hydrodynamic or contractile	Excluded
8.	Filament contraction-relaxation	cytoskeletal filaments	longitudinal filament oscillation	simultaneous streaming of the entire cytoplasm with different velocities and in both directions	Not confirmed
9.	Microperistalsis	radial contractile elements in MT or of surrounding NF domain	waves of MT domain contractions	hydrodynamic pressure propulsion in MT domains	Not confirmed
10.	Endoperistalsis	MTs and contractile MT cross-links	lateral MT undulation, leading to constriction waves along MT domains	hydrodynamic pressure propulsion in MT domains	Not confirmed
11.	Microstreams	MTs with attached FGEs	shear force generation along MT	local streaming along cytoskeletal elements	Not confirmed, FGEs are located on organelles

Hypotheses involving specific interactions include those postulated by Schmitt 1968, Ochs 1971, Cooper and Smith 1974, Heslop 1974, Kerkut 1975, Schwartz et al. 1979, Porter 1979, Stearns 1980, Pollard 1981, Ellisman 1982; hypotheses involving unspecific interactions by Biondi et al. 1972, Droz etal. 1975, Gross 1975, Edelman 1976, Odell 1976, Weiss and Gross 1982. Abbreviations: FGE, *force generating enzyme*; MF, *microfilsment*; MT, *microtubule*; NF, *neurofilament* SER, *smooth endoplasmic reticulum*.
Modified from Weiss and Gross 1983. Abbreviations see at the end.

4.2.1 The contraction hypothesis

In 1977, Randolph Byers and Keith Porter published a paper called *Transformations in the Structure of the Cytoplasmic Ground Substance in Erythropores during Pigment Aggregation and Dispersion*. The aim of this paper was to combine the discovery of the microtrabecular lattice with light microscopic observations of granule movement in pigment cells to build a powerful hypothesis of organelle transport in the cell. HVEM confirmed the observation of D. Bickle et. al. (1966) and Lorna Green (1968) that microtubules are radially orientated along the direction of pigment movement. Moreover, fine filaments were seen between granules and MTs which composed the microtrabecular lattice and formed arms or bridges between them. However, Byers and Porter denied that this observation suggests that MTs provide the motile force but claimed that they are just a structuring and orientating framework of the cell that provides direction for granule movement. Light microscopic observations were crucial to separate the pigment granules in dispersed, aggregated, aggregating and dispersing state, so that the cells could be fixed for electron microscopy in each of these four conditions. Investigations with HVEM showed a crucial difference between the microtrabecular lattice in the dispersed and aggregated states of pigment movement:

> "During aggregation, it appears that part of the microtrabeculae shorten and translocate with the pigment granules while another part is left behind, attached to the upper and lower cortices of the cell. The microtrabeculae that were once components in a fine three-dimensional lattice suddenly transform or become formless blobs on the inner surfaces of the cell cortex. On the other hand, as dispersion initiated, there is a lengthening and restructuring of the microtrabeculae out of the cortices and from the centrosphere, and the embedded granules seem to follow. Much of this continuum, or lattice substance, is reconstructed before the granules arrive and the fact that pigment dispersion is relatively slow and is saltatory in character may reflect complexities involved in the reconstruction process." (Byers and Porter 1977).

For that reason, Byers and Porter suggested that pigment migration is caused by contraction and expansion of the microtrabecular lattice. Moreover, they admitted that the molecular events of the contraction process were entirely unknown to this time. Further studies were supposed to clarify the role of the microtrabecular lattice for pigment migration and axonal transport. Luby and Porter (1980) calculated the energy requirements for the contraction process of the microtrabecular lattice, and the results were found to be compatible with their hypothesis for pigment cells, that the lattice, together with radial MTs, was the vehicle for pigment translocation. Ellisman and Porter (1980) supposed that Ca^{2+} might control the connectivity of microtrabecular cross-linkages. The role of Ca^{2+} and Mg^{2+} for axonal transport was investigated in greater detail by Mark Stearns (1981). He observed that microtrabeculae became shorter and

thicker if Ca^{2+} concentration was increased in the cell, yet longer and thinner if concentration of Mg^{2+} was increased. For this reason he supposed that Ca^{2+} ions are causing the contraction of the microtrabecular lattice whereas Mg^{2+} ions promote elongation (Fig. 23).
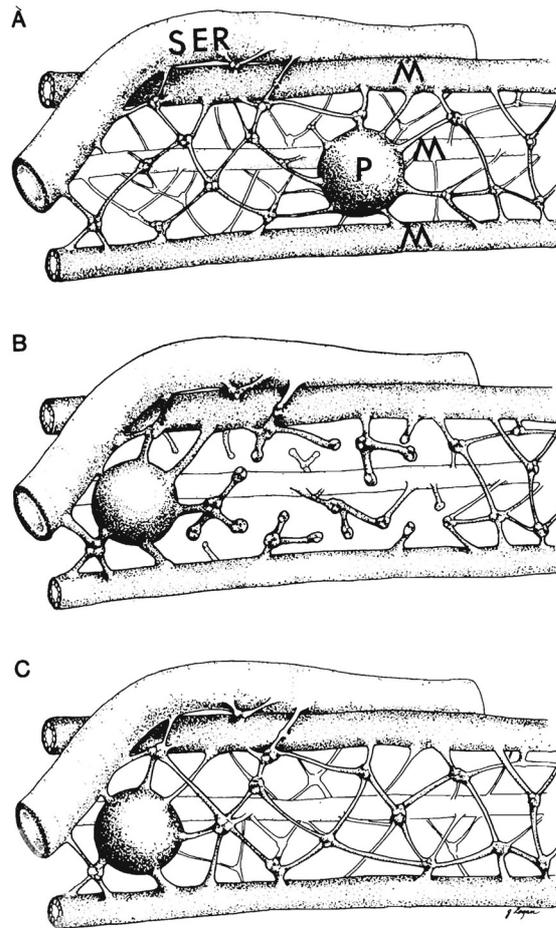

Figure 23. Model of the microtrabecular lattice-dependent contraction hypothesis. "The figure is a highly diagrammatic description to demonstrate possible mechanisms regulating the saltatory motion of particles along axons. **A**. shows a transport channel consisting of three microtubules (m), a particle (p), and nearby SER crosslinked by lattice filaments in which they are suspended. **B**. The onset of particle motion is triggered by a localized release of Ca^{2+} ions by the SER: Minute contraction of the lattice in the direction of motion serve to move the momentarily detached particle. **C**. The sequestering of Ca^{2+} ions results in an energy dependent expansion of the lattice and a momentary attachment of the lattice filaments in preparation for the next saltation event. This sequence of contraction and expansion events is thought to occur in a rapid cyclic fashion along microtubule lattice channels to produce particle transport." Reproduced from Stearns, (1980)

To sum up, the contraction hypothesis was based on the following assumptions:

1. All elements of the cell are embedded in the microtrabecular lattice.

2. Active contraction of microtrabeculae on one side and elongation followed by detachment on the other side cause directed movement of a given particle through the cytoplasm.

3. The activity of the microtrabecular lattice is regulated by moving waves of calcium- and magnesium ion concentration.

This results in the following predictions:

1. Due to the dense microtrabecular lattice (Fig. 8) transport in cytoplasmic channels clear of microtrabeculae is excluded.

2. Cytoplasmic streaming in connection with vesicle transport cannot occur.

3. A highly regulative system of recognition mechanisms between microtrabeculae and different types of transported vesicles is necessary to allow different transport velocities and directions.

4.2.2 The microstream hypothesis

Another explanation of axonal transport is the microstream hypothesis, originally presented by Guenter Gross (1975) and further developed together with Dieter G. Weiss (Weiss 1982, Gross and Weiss 1983) (Fig. 24). This explanatory framework received only little attention in the scientific community. It proposed that the MTs support the mechanism of particle movement in the axon with the help of cytoplasmic streams. The microstream hypothesis drew a more dynamic picture of the living cell than the contraction hypothesis because MTs were not considered as enmeshed static elements of the cytoskeleton but as allowing flows and movement of small organelles in their vicinity through the cytoplasm. Microtrabeculae were considered as mere artifacts created by the preparation methods for HVEM and not existing in the living cell. The microstream hypothesis also fitted the demands for cytoplasm viscosity and energetics for transport of small particles in liquid medium calculated by Gross and Weiss (1982).
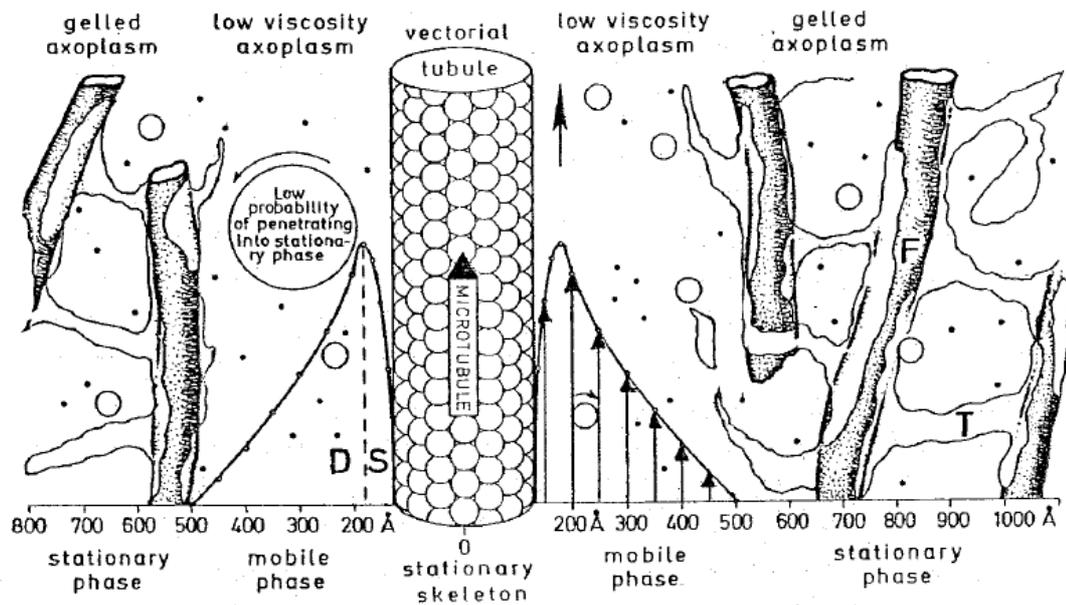
Figure 24. Schematic drawing of the valocity profile around a microtubule, as proposed by the microstraem hypothesis. The streaming zone extends over aproximately 40nm away from the microtubule surface with an inner flowing region (S), powered by shear forces generated on the surface of the tubule, and an outer passively flowing zone (D). The stationary axoplasm arond the area of flow is filled with neurofilaments (F) and soluble proteins. The soluble proteins are shown associated to the neurofilaments (F) and thereby forming microtrabeculae, like bridges consisting of proteins associated by weak hydrogen bonds. Transported material is shown as small molecules (dots) or protein macromolecules (rings) in the streaming area. Modified from Weiss (1981).

According to Gross (1975, 287), the microstream hypothesis consists of five assumptions:

1. Material is not transported through a stationary medium but is moved by carrier streams that are called microstreams.

2. The microstreams are located in annular low viscosity regions around the MTs.

3. The streaming velocity is highest in the vicinity of the MT surface and decreases with distance from the tubule.

4. The force-generating mechanism is situated at the microtubule surface and exerts a shear force on the adjacent fluid.

5. The shear force is generated by a vectorial enzyme reaction at an ATPase, situated on the surface of the MT. The vectorial properties result from the association of this enzyme with the oriented MT structure and from the directional release of the electrostatic energy of repulsion residing in the ATP side chain. (Gross 1975, 287)

Later Weiss and Gross (1982) reduced the number of assumptions of the microstream hypothesis, leaving only three assumptions and their consequences:

1. The force generation is non-specific and is exerted on all cytoplasmic constituents present in its vicinity.

2. The vectorial structure that orients and stabilizes the force generating enzymes (FGE) is the MT. The force generation occurs at or near the surface of the MT.

3. Anterograde and retrograde transport are produced by the same type of force generating system working in opposite directions.

(quoted from Weiss and Gross 1982)

Surprisingly, the term "microstream" does not appear in these three assumptions. However, Weiss and Gross (1983) stated that five consequences come as a result of the first assumption and another two consequences from the second assumption. The non-specific force generation is ascribed to microstreams support axonal transport (Fig. 24). The velocity of the streaming region decreases with distance from the MT and into the more highly viscous, gel-like medium consisting of neurofilaments connected by weak hydrogen bridges between their proteins (Leterrier 1982, Weiss and Gross 1983, Bridgman et al. 1986). This leads to a zone of different velocities in which particles can be transported at different rates. The cytoplasmic flow is assumed to be unidirectional and oriented parallel to the microtubule. Particles at one microtubule can therefore only be transported in one direction. Microstreams themselves are established by the mechanical activity of force generating enzymes that are placed on the surface, while the MTs determine the direction of force generation by polarizing these enzymes.

Weiss and Gross stated that mechanisms based on non-specific force generation are simpler than other mechanisms because they don't need molecular information for recognition, selection, binding, orientation etc., which would require a much larger number of assumptions. As Schliwa (1984, 3-4) states, at least five transport groups, each with different velocities were known in the early 1980s. While specific force generating mechanisms had to explain each kind of transport separately, microstreams were able to explain all kinds of transport at once. The differences in particle speed can be explained as caused by differences in distance to the MT surface and on the basis of different partitioning behavior between the stationary and streaming phase (Gross and Weiss 1977, Stewart et al. 1982). Hence the microstream hypothesis "is presumably the simplest form of active intracellular transport that can be conceptualized" (Weiss and Gross 1982, 363).

4.2.3 The renaissance of light microscopy and the evaluation of explanatory concepts

In 1981 another large technological step was taken in cell biology that brought an extensive improvement of resolution in light microscopic observation of unfixed living cells (see section 2.5.2): Allen Video-Enhanced Contrast Differential Interference Contrast (AVEC-DIC) microscopy (Allen et al.1981a, Inoue 1981). Microtubules and transported vesicles could now be observed in live cells and extruded cytoplasm and their motile activity could be documented (reviewed in Allen 1986, Shotton 1987, 1988).

A series of investigations was initiated immediately to observe, measure and quantify the process of axonal transport that was now directly observable in living cells. Video microscopic studies of the giant squid axon revealed the fast linear motion of vesicles of approximately 30-50nm diameter and also the slower movement of the larger mitochondria (400 to 800nm) along parallel axonal fibers (Allen 1982a,b, Brady 1982). Transport of vesicles and organelles along MTs was analyzed in frog keratocytes (Hayden et al. 1983). Experiments were directed at the more precise determination of the role of cytoplasmic and cytoskeletal components in axonal transport by using extruded squid axoplasm and performing videomicroscopy on samples containing MTs, vesicles and all soluble cytoplasmic components. The video data showed clearly that vesicles move along MTs in both directions and can even switch from one MT to another (Allen 1984, Hayden 1984, Allen et al. 1985, Koonce and Schliwa 1985, Schnapp 1985).

4.2.4 The confutation of the contraction hypothesis

In the early 1980s, the contraction hypothesis of Porter and colleagues ran into serious trouble when it was undoubtedly proven that the microtrabecular lattice (MTL) does not exist but is an artifact created by insufficient dehydration during sample preparation. Already in 1980, Hans Ris had discovered that the MTL is created by incomplete application of critical point drying (CPD). He investigated fibrous proteins as a model system to determine the influence of CPD to the appearance of the MTL. A microtrabeculae-like structure appeared not only in animal cells but could also be created in artificial actin solutions by incomplete dehydration. In contrast, after a more thorough application of dehydration the MTL neither could be observed in cells nor in actin solutions. Therefore, Ris concluded that "the »microtrabecular lattice« appears to be an artifact introduced during critical point drying most likely by distortion of actin filaments" (Ris 1980, 812). However, his observations were ignored until he gave a more detailed account five years later (Ris 1985).

In another study, Pawley and Ris (1987) examined whether similar results could be received by using freeze-drying procedures as opposed to CPD. A network of tapering filaments similar to the MTL was detected if the cells remained at low temperature in liquid nitrogen for only a short time, but after long and more careful dehydration there was no sign of tapering filaments like microtrabeculae. Therefore, Pawley and Ris supposed that the network structure was again caused by an incomplete sublimation. Combined, their studies demonstrated that the two main procedures of dehydration – CPD and freeze-drying – have created the MTL artifactually both in cultured cells and in model protein systems. In contrast, no lattice is shown if these methods are used adequately. Hence, the only reasonable conclusion is that the MTL observed by Porter and colleagues is an artifact and does not exist in living cells.

But if microtrabeculae are artifacts, why did they appear on images of HVEM? One explanation was given by Heuser and Kirschner (1980), who focused on experimental results of the cytoskeleton after freeze-drying. Initially, they referred to previous studies (Webster et. al. 1978) which had shown a three-dimensional lattice-like structure after preparation with CPD and the use of stereo transmission electron microscopy. However, in these studies the lattice-structure was not composed of novel entities like microtrabeculae but consisted in large parts of actin-filaments. Thus, Heuser and Kirschner investigated the cytoplasm and cytoskeleton to clarify whether the MTL consists of known or unknown elements such as like actin-filaments. They used a conventional 100 kV transmission electron microscope on quick-frozen cells and detected a lattice-like structure which looked in some respect similar to the MTL observed with CPD and HVEM, but different in other aspects. These microtrabeculae observed with HVEM varied in thickness and fused with each other at points of intersection, whereas the cytoskeleton seen after freeze-drying with conventional electron microscopy appeared to be composed of nothing but well separated filaments of uniform caliber, which did not appear to be connected.

Now the question arose whether microtrabeculae are additional components of the cytoskeleton opposed to actin-filaments, IFs and MTs or just another view of these filaments. Heuser and Kirschner preferred the latter explanation and suggested that the MTL observed by Porter and colleagues is largely composed of actin-filaments. They explained why the actin-filaments looked like microtrabeculae by supposing that "after aldehyde fixation, these filaments appeared to be, by comparison with unfixed cells, partially agglutinated and decorated with irregular condensations of what may have been soluble cytoplasmic proteins" (Heuser and Kirschner 1980, 233).

Unfortunately, the results of Heuser and Kirschner were ignored or misinterpreted. As Heuser states in retrospective, "at that time, these conclusions convinced very few people, and Porter and colleagues pushed ahead" (Heuser 2003,586). Not before the mid-1980s Heuser and Kirschner received backup from other scientists, first and foremost from J.J. Wolosewick who had discovered and defended the MTL earlier. Wolosewick and Condeelis (1986) investigated actin gels in cells of

Dictyostelium amoebae and worked out that these cells contain an elaborate filament network that is very similar to the MTL observed with HVEM. They stated that filament networks like this were seen in the previous studies of Porter and colleagues; hence hitherto unknown entities like microtrabeculae had not to be inferred any longer.

4.2.5 The falsification of the microstream hypothesis

The microstream hypothesis was constructed in a way that several predictions could be derived directly from it. Unfortunately, these predictions were not testable at the time the microstream hypothesis was invented and constantly improved. But after the invention of video-enhanced contrast microscopy things changed and some predictions of the microstream hypothesis could be tested directly (Allen et al. 1985a). Later Weiss and Allen (1985) analyzed in retrospective four predictions of the microstream hypothesis:

1. Since MTs have to absorb the recoil of the force generation which is suggested to be attached to them, a reactive force would be expected which would move MTs in the direction opposite to the main direction of the organelle transport. This should occur only if MTs were freed from the cytoskeleton.

2. Transport along on MT is expected to be unidirectional.

3. FGEs [force generating enzymes] should create the streaming by their concerted action, thus force generation should show cooperativity.

4. No crossbridges between MTs and organelles in motion are expected since production of the microstream is suggested to be the primary result of the action of FGEs. (quoted from Weiss and Allen 1985, 234).

In order to test prediction 1 Weiss visited the laboratory of Allen to work with his AVEC-DIC microscope. Microtubule gliding, which was only expected by the microstream hypotheses, was not easy to detect. Only after several weeks of experiments and only when the free microtubules in extruded axoplasm were mechanically broken into shorter segments Weiss was able to confirm it and it was presented at a conference in 1984 (Allen and Weiss 1984) and later published (Allen et. al. 1985). In video-microscopic experiments it was possible to observe active gliding of isolated MTs which was in very good agreement with the microstream hypothesis. The microtubules moved very slowly, at less than 1 μm/sec, which means that they need 30-60sec to cross the video screen. Their movement would have gone unnoticed if it would not have been expected and deliberately searched for. Weiss and Allen fortuitously had one of few then existing time lapse video recorders, which made the detection of slow movements easier. Indeed, it turned out that the other laboratories of Raymond Lasek and Thomas Reese who were working with the same preparations at the same time had for one year overlooked the gliding microtubules despite they were also present in their

video recordings. The microstream hypothesis had made a novel prediction which could be regarded as strong evidence for its validity (however see section 5.1 for discussion).

Unfortunately for the microstream hypothesis, the video-microscopic recordings also clearly showed that movement along a single MT can occur in opposite directions (Allen et al. 1985a) as was shown simultaneously for giant amoeba cells (Koonce and Schliwa, 1985). However, this discovery was not immediately accepted as a falsification of the microstream hypothesis. Weiss and Allen (1985, 236) sketched a modification of the microstream concept which allowed bidirectional transport (Weiss 1985). But this was just an ad-hoc maneuver to save the microstream hypothesis. Weiss and Allen (1985) themselves admitted a little later that crossbridge models account better for the bidirectionality than carrier streams.

Dynamic cooperativity (Shimizu and Haken 1983) might have supported the microstream concept but it could not be detected. However, this was not assessed as a contradiction to any model of axonal transport as long as important details concerning the force generating mechanism remained unexplained.

The main stumbling block for the microstream hypothesis was the existence of crossbridges between MTs and organelles or vesicles. The conception of microstreams implied that the transported vesicles are not connected to MTs but would move through the carrier streams near MTs. Therefore, experimental data of a fixed attachment to MTs rejected the microstream concept. As Weiss and Allen (1985) admitted, crossbridges were known for many years from electron microscopic images (see for example Schmitt 1968, Smith 1971), but to reject the microstream hypothesis one would have to know whether organelles *in motion* showed such crossbridges. This problem was studied carefully by Lasek and Miller (1985). They asserted that the structural investigations with electron microscopy suggested that the cytoskeleton was rigidly crosslinked, whereas investigations with AVEC-DIC indicated that the axoplasm is highly dynamic (Allen 1982). Lasek and Miller (1985) followed the earlier view of Weiss and Gross (1983) (see Fig. 24) that electron microscopic images probably did not accurately represent the actual associations within living axoplasm. Crossbridges that were normally weak or transient could permanently have been bound together during the fixation procedure; hence an experiment was needed to rule out this possibility.

Lasek and Miller (1985) isolated single MTs prior to fixation in a way that structures with strong connections were maintained after fixation, whereas structures that were weakly or not at all connected would remain separated. With electron microscopy they observed that crossbridges connected isolated MTs and vesicles and concluded that these crossbridges were real and might be the force generating unit for vesicle transport (ibid, 202). More detailed studies were made by Vale (1985abc) and Miller and Lasek (1985). Vale and colleagues separated extruded axoplasm into soluble and insoluble phases. The complex of MTs, and vesicles was found in the solid phase and observed by video microscopy. The investigators found that particle transport and

also MT gliding depended on the presence of ATP, as well as on the soluble component of the axoplasm. Miller and Lasek (1985) stated that the visible crossbridges might use a soluble factor to propel vesicle transport along the MT, while Lasek and Brady (1985) found the crossbridge itelf to be the ATP-dependent motor, while similar observations were made by Euteneuer et al. (1985).

Weiss and Allen (1985) acknowledged that the close connection of moving vesicles to free microtubules indicated some kind of at least transient binding and possibly the existence of crossbridges. This and the bidirectionality of vesicle movement disproved their fourth prediction. Because this criterion was most important, they announced that the microstream hypothesis must be rejected (Weiss and Allen 1985).
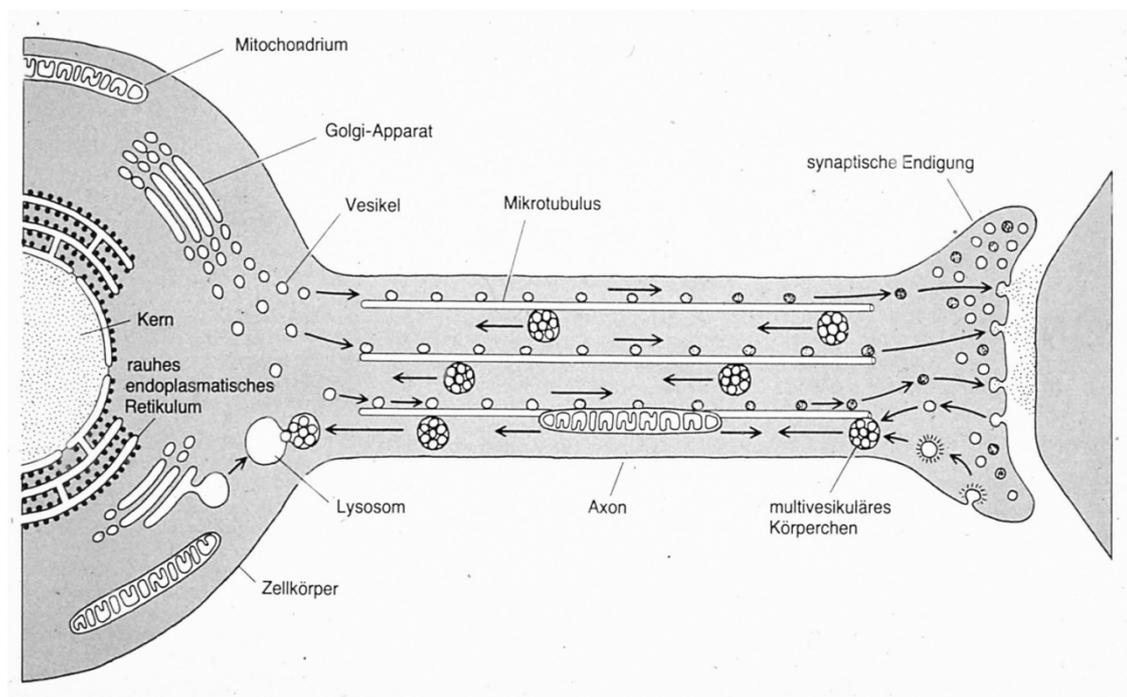

Figure 25. Mechanism of axonal transport as discovered by video microscopy. Organelles and vesicles of various sizes are transported along microtubules in the axon. This way the swift exchange of substances between the cell body (left) and the synapse (right) is accomplished. Modified from Allen and Weiss 1987.

In the same year Ronald Vale and colleagues characterized the novel motor ATPase and called this protein *kinesin* (Vale at al. 1985) and the motor for the opposite direction *cytoplasmic dynein* which turned out to be closely related to the long known ATPase dynein of cilia and flagella, was discovered by the group of Richard Vallee (Paschal and Vallee 1987). While kinesin is responsible for the centrifugal movement of organelles, in axons the anterograde transport to the synapses, dynein explained the

backward or retrograde movement of material bound to be degraded by the lysosomes of the cell body. This made the molecular basis of axonal transport clear (Fig. 25). The cytoplasmic movements in squid axons turned later out to have one more mechanism for the local distribution of the membrane precursor and the synaptic vesicles close to the membranes which is actin filament-dependent and works with a variant of the muscle motor enzyme *dynein* (Kuznetsov et al. 1992). The mentioned studies clarified the views on the molecular mechanism of axonal transport (Fig. 25). Once the three motor enzymes had been described it turned out that was had been found in axons to explain axonal transport is indeed a general principle for distribution of material in all animal cells (and with some variation also plant cells).

The new video-microscopy was the key technique that allowed this scientific progress. The isolation, purification and measurement of the enzyme activity of the three cytoplasmic motor enzymes could not have been achieved without the invention of video microscopy, because these enzymes hydrolyze ATP like many other enzymes, but produce no measurable product except motion, which could only be measured microscopically. The video-microscopic motility assay to find and enrich the enzymes in cell extracts was the microtubule gliding assay and later the actin filament gliding assay that were based on the discovery of MT gliding, which in turn was discovered in the attempt to prove the microstream hypothesis.

5. Philosophical reflections

5.1 Novel predictions and scientific truth: The case of the cytoskeleton

Scientific realists claim that successful theories are at least approximately true. As Hilary Putnam states,

> "Realism is the only philosophy that does not make the success of science a miracle" (Putnam 1975, 73).

This point of view is often called *No-Miracles-Argument (NMA)* or *Ultimate Argument*. Jarrett Leplin, a rigorous advocate of scientific realism, propounds this argument in the following terms:

> "Certainly, a major motivation for epistemic realism is the conviction that unless there is some truth to a theory, there must be some limit to how successful the theory can be. If not impossible, it is at least improbable that a theory wholly misrepresentative of the structure of the world will be highly successful to exacting standards. It would require a colossal coincidence for the theory to work out exactly right so far as we can tell, while being completely wrong at the level of deep structure" (Leplin 1997, 26-27).

To understand this view, one has to explain first what successfulness means. A common answer is that a theory is successful exactly if the theory correctly predicts novel results. Realists claim that the prediction of novel results which are validated by experiments has to be explained by philosophy of science and the only reasonable explanation of a novel result is the truth of the theory which has predicted it successfully. However, novelty itself is an ambiguous term as well. Whereas Imre Lakatos suggests that novelty is temporal newness (Lakatos 1978, 70), Elie Zahar wants older results to count as a novel success too if they are not part of the problem situation which caused the construction of the theory in question (Zahar 1973; 1989). Likewise, Leplin distinguishes between new results and novel results:

> "'Novel' is used, rather, for newly explained or predicted results that are different, unusual, or anomalous with respect to background theory, and that may or may not be newly disclosed in the course of predicting them" (Leplin 1997, 43).

A result that is novel for one theory needs not to be novel for another. Instead, it is highly important if the result has epistemic significance to the theory and if the theory already refers to the results due to available knowledge. In such cases the prediction of a result by a wrong theory would not be miraculous.

Our case study about the history of the cytoskeleton, theories of axonal transport and the revolution of light microscopy raises two critical requests to the realist picture of novel predictions: First, *what if a theory successfully predicts a novel result, yet the result later turns out to be an artifact?* Second, *what if a theory successfully predicts a novel result, yet the theory later turns out to be false* (whereas false means that the theory is not even approximately true)? Let's take a look in detail on both questions.

In 1968, Lorna Green, who was a PhD student of Paul Weiss, published a paper called *Mechanisms of Movements of Granules in Melanocytes of Fundulus heteroclitus*. She has investigated granule movements in marine teleosts with light microscopy to study the mechanism of pigment motion. Her observations indicated that granules are not able to propel themselves actively; hence Green assumed that they must move passively. If granules do not move by themselves, there would have to be a transport mechanism. Green excluded electrical forces and and suggested another explanation:

"The most likely assumption is that the forces to move the granules arise within the granule-bearing matrix of the cytoplasm. In fact, all observations can be interpreted by assuming that the granules are in a structured continuum. Despite the appearance of flow in the distal regions of the arms, the granules are not in a fluid medium. […] There is much evidence that the continuum 'contracts' during concentration and 'expands' during dispersion. […] The granules appear then to be fixed like beads in a structured continuum of whose movements they are the passive markers. […] The granule-moving system is a structured continuum whose state, expressed in the spatial distribution of the granules, is the function of a dynamic equilibrium between concentrative and dispersive forces" (Green 1968, 1183-1184).

Green refused hypotheses of granule movements which assumed a fluid or sol-gel cytoplasm because they did not take into account the submicroscopic constituents. Additional electron microscopic investigations made by Green showed that the cytoplasm consisted of a matrix containing the endoplasmic reticulum and microtubules. Green suggests that either the MTs or the smooth endoplasmic reticulum produce the force for the granule-moving apparatus, yet she acknowledged that the nature of the motile mechanism was largely unknown at that time (ibid, 1185).

Nine years later, Byers and Porter investigated pigment migration in a squirrelfish with HVEM. They suggested that the microtrabecular lattice must have a significant role in mediating pigment migration and believed that a contraction process of the lattice enabled the pigment movement similar to the mechanism suggested by Lorna Green:

"It appears that the microtrabeculae are intimately involved in mediating pigment migration, yet the molecular events which bring about the

transformation of the microtrabeculae, i.e. their shortening and lengthening, is entirely unknown. Clearly, the structural changes observed are compatible with Green's hypothesis [...] that there is an expansion and contraction of a continuum in which the granules are supported" (Byers and Porter 1977, 556-557).

Porter and Mark McNiven (1982) took a more detailed look at the hypothesis of Green:

"Lorna Greene (1968) in a very interesting paper reporting observations mostly on live melanophores, showed impressive prescience in suggesting that the pigment is contained in 'a structured continuum' and that the distribution of granules at any instant 'is the function of a dynamic equilibrium between aggregative and dispersive forces.' Though Greene did not have the observations reported here, she came very close to our current views. That there is a continuum, of which the pigment granules are a part, seems incontrovertible. That it is structured as it is and organized even to the fixed position of individual pigment granules was not known in 1968. Nor did Greene at that time have stereo electron microscopic images of the cytomatrix to help her form a picture of the 'continuum.' [...] With the advantages of whole-cell electron microscopy we have moved closer than Greene to understanding the nature of the motive mechanism" (Porter and McNiven 1982, 29-30).

Obviously Lorna Green has made a novel prediction. She not only assumed that there has to be a structured continuum, which was interpreted later as the microtrabecular lattice, but also predicted the biological function of the lattice, namely force generation for granule movement and axonal transport. Both predictions were independent of Green's hypothesis and her background knowledge and could be reconstructed as an inference to the best explanation. Hence, a scientific realist who advocates the novel defense of scientific realism has to assert that Lorna Green's contraction-hypothesis of granule movement is at least approximately true due to the novel predictions made by this hypothesis.

Unfortunately (as shown in chapter 4.2.4), the microtrabecular lattice turned out to be an artifact, i.e. not an element of the real cell in vivo but created by the methods of preparation of cells for HVEM. Hence, the hypothesis of Lorna Green has successfully predicted a result which later turned out to be misguided. How can this be possible?   Obviously, the truth of Green's hypothesis cannot be an explanation of the successful prediction of a misguided result. It is rather the other way round: True theories make only true predictions, so if the results of our example are misguided, Green's hypothesis has to be false. For this reason we need another explanation of the

success of Green's hypothesis. But scientific realists believe that truth is the only explanation of the success of science. If this explanation is blocked, realists have to admit that there is either another explanation of its success or a plausible explanation simply does not exist. However, both alternatives are antirealist positions and reject the novel defense of scientific realism.

It would be a weak defense for realists to simply deny the success of Green's hypothesis at all. Obviously there was a successful prediction of a novel phenomenon (although it was a misguided one) and Porter, Byers and McNiven affirmed that Green has successfully predicted their discoveries. Thus, our first question "What if a theory successfully predicts a novel result, yet the result later turns out to be an artifact?" leads to two conclusions: First, truth is not always an available explanation of the success of science. At the moment, there are no philosophical investigations about why false theories are sometimes able to successful predict misguided results. However, our biological example demonstrates that such things can happen, so there are either alternative explanations of the success of science or at least sometimes the success is not explainable. Anyway, successful predictions of misguided results undermine the general thesis of scientific realist that truth is the only reasonable explanation of the success of science. Second, a novel prediction cannot be sufficient to believe in the truth of scientific hypotheses or theories. There has to be a well-founded epistemology of experiment which justifies the experimental results and eliminate the possibility that the predicted results are misguided. Therefore, the novel defense of scientific realism cannot warrant scientific realism on its own.

Let's take a look on our second question: What if a theory successfully predicts a novel result, yet later turns out to be false? Scientific Realists do not believe that this can happen in scientific practice. As Leplin states:

> „The question is why a *false* theory should predict correctly. If the mechanisms or principles a false theory posits are inoperative and unlike those that do operate, why should predictions from them be correct?" Leplin (1997, 16).

Realists claim that only truth can explain the success of science plausibly and if a theory successfully predicts a novel result, it has to be at least approximately true. Indeed, the notion of approximately true is a bit confusing and cannot be taken literally. Stathis Psillos has analyzed a plausible notion that fits well to defend the NMA:

> "A false theory can still be *approximately true*. […] A theory is approximately true if it describes a world which is similar to the actual world in its most central or relevant features. So, what realists need to show is that past scientific theories, although strictly speaking false, have been approximately true" (Psillos 1999, 102).

To counter the novel defense of scientific realism, one has to find examples of theories which are empirically successful, i.e. made novel predictions that have been fulfilled, but were not even approximately true. Unfortunately, most antirealists challenge scientific realism with highly abstract arguments instead of case studies from history of science. On contrary, we want to show that the microstream hypothesis is a counterexample to the novel defense of scientific realism.

As we have already shown in detail, the microstream hypothesis has been falsified due to the existence of bidirectional transport on single MTs and of crossbridges between MTs and vesicles (see chapter 4.2.5). But can it still be approximately true? That is, are the most central and relevant features of the microstream hypothesis true? There are two central features of the microstream hypothesis: The existence of microstreams which cause axonal transport and the existence of force generating enzymes which are placed on the MT and metabolize ATP to generate force. The force generating enzymes were not a genuine element of the microstream hypothesis but were also assumed by many other hypotheses of axonal transport. Therefore, the existence of microstreams and their role in axonal transport has to be considered as the most important feature of the hypothesis and it would be inadequate to state its approximate truth if axonal transport is not caused by microstreams. For that reason, the existence of crossbridges has falsified the microstream hypothesis in a way that we cannot even assert that it is approximately true.

Nevertheless, the microstream hypothesis has successfully predicted a novel phenomenon, namely MT gliding. It was assumed that force generation for axonal transport would produce recoil that has to be absorbed by the structure that carries the force generating enzymes. The recoil forces caused by the enzymes would move the carrier MTs like the streaming caused by a paddle is moving a rowboat. Weiss and Gross (1983, 189-190) assumed that the force generating enzymes were situated at the MT-surface rather than at the cell membrane, the smooth endoplasmic reticulum or the actin filaments because only MTs are long and massive enough to absorb the recoil forces, rigid and present in all axons. They suggested that unanchored MTs would move in the direction opposite to the organelle transport:

> "For every action there is always an equal and opposite reaction. We have stated that the recoil forces to transport force generation should be absorbed by a massive structure such as a long, anchored microtubule in order to maximize fluid flow in the circumtubular annulus. This implies that a relatively short tubule which is not anchored will move in the opposite direction from that of the fluid flow" (Gross und Weiss 1983, 205).

This prediction was not testable at the time, but, due to the development of AVEC-DIC microscopy movements of MTs became directly observable, an object of focus and one of the fist applications of AVEC-DIC microscopy. Gliding of MTs was reported by Allen and Weiss (1984) and described in detail by Allen et. al. (1985a):

> "The translatory movements of microtubular segments are unidirectional and therefore probably related to the growth polarity of the microtubule. […] Since the movement of particles on these segments is predominantly backward, it is reasonable to suggest that this corresponds to the orthograde direction of transport in the intact axon. […] From these findings we conclude that the direction of microtubule gliding would correspond to the retrograde direction in situ. This was one of the predictions of the microstream hypothesis" (Allen et. al. 1985a, 1748).

The microstream hypothesis successfully predicted the gliding movement of MTs. This observation was absolutely novel, it could not have been observed prior to AVEC-DIC microscopy. Furthermore, it was not predicted by alternative theories because the whole experiment only made sense if one could reasonably expect MT movement. Therefore, the microstream hypothesis was heuristically useful:

> "The active movement of MTs was discovered in attempt to test the microstream hypothesis. […] The details of MT [microtubule] movements are in very good agreement with the microstream hypothesis" (Weiss and Allen 1985, 235).

As we can see, the microstream hypothesis is not even approximately true but has successfully predicted the gliding of MTs in the opposite direction of particle transport. Following current knowledge, the gliding of MTs is not caused by microstreams, but by the mechanism of particle movement which uses kinesin as motor enzyme. These two case studies demonstrate that the (approximate) truth of a theory is not always a reasonable explanation for the success of science. Hence, we have presented a counterexample to the novel defense of scientific realism from history to biology. Moreover, we have shown above that even an experimental result which later turned out to be an artifact has been predicted successfully.

However, one should be careful with the following claim, which underlines the realists' argumentation in question:

> "The key argument for scientific realism according to the programme presented here is that *realism as a scientific hypothesis* presents the only *scientifically acceptable explanation for the reliability of scientific methods*." (Boyd 1990)

Many kinds of contemporary scientific realism are naturalistic, i.e. use the same methods and patterns of argumentation as science itself. Two points can be made against naturalistic scientific realism in order to create a kind of metaphysical scientific realism that is immune to empirical counterexamples:

1. Scientific Realism, no matter in which form, is not a scientific but a metaphysical thesis. Realism in no way predicts that a scientific theory T will be empirically successful. However, if T is structured systematically and supported by the results in a non-ad hoc way, then scientific realism enables us to infer that T is approximately true. Hence, the only way of refuting scientific realism is to produce a theory T_0 such that: T_0 is genuinely supported by the results while not being even approximately true. In order to ascertain the second condition, one would have to compare T_0 with the true theory T'. But we never know if we actually possess the true theory T'. Furthermore, would we been able to get hold of T', then the whole controversy would become otiose.

2. Boyd talks about the reliability of the methods, but this comes down to the truth, or approximate truth, of empirical statements. However, according to the naturalistic strategy of scientific realism, the latter are theory-laden and the theories in question have an unknown truth value, hence we cannot even in principle know whether the laws we rely upon are in fact reliable. The explanandum itself disappears; unless we posit, by fiat, the reliability of certain observational results and hence the truth of the underpinning them. This makes, however, circularity unavoidable.

These considerations shed new light on the argumentational approach: The NMA does not explain the success of science due to novel results, or any facts for the matter. Otherwise, the NMA would be a scientific theory, which it clearly is not. The NMA provides a *post-hoc* rational for the following inference: If a theory T systematically explains a whole host of novel ("unexpected") results, it is highly unlikely that this can be possible due to mere chance; it is much more likely that T is either true or (in some sense) truth-like. Hence, the NMA does not explain novel results. It accounts for another state-of-affairs; namely such in which a scientific theory T yields novel – ascertained – facts. Thus the NMA explains the presumed success of T.

The NMA does not yield any facts – novel – or otherwise, because in this case it would be another scientific theory. In order to be testable, scientific realism must be able to pronounce a theory T truth-like before the discovery of any results; but scientific realism cannot do this. It must wait for T to be genuinely supported by novel results to conclude that T is truth-like. In order to predict these results, scientific realism is not needed, the theory T suffices. So there is no situation in which

scientific realism has to be postulated in order to predict novel results. Therefore, scientific realism is genuinely metaphysical.

5.2 Inference to the best explanation: The case of axonal transport

In the early 1980s, many different hypotheses about the mechanism of axonal transport were suggested (see Table 4). Do we have to accept this wide scope of possibilities that implies that there was no reliable knowledge about the mechanism of axonal transport at the early 1980s available? Some scientific realists believe that there is an alternative which can identify the true one of a set of hypotheses. They believe in a non-deductive inference called *inference to the best explanation* (IBE), which was developed and characterized by Gilbert Harman as follows:

> "In making this inference one infers, from the fact that a certain hypothesis would explain the evidence, to the truth of that hypothesis. In general, there will be several hypotheses which might explain the evidence, so one must be able to reject all such alternative hypotheses before one is warranted in making the inference. Thus one infers, from the premise that a given hypothesis would provide a 'better' explanation for the evidence than would any other hypothesis, to the conclusion that the given hypothesis is true" (Harman 1965, 89).

Scientific realists would claim that the available explanatory hypotheses about a phenomenon can be sorted according to likeliness or plausibility with the help of epistemic values like accurateness, consistency and fruitfulness, but also by using probability theory. One of these hypotheses can unambiguously be identified as the most likely and most plausible hypothesis, which is, according to IBE, at least approximately true. On the contrary, antirealists deny the reliability of IBE. In the late 1990s, there was a back and forth discussion about the reliability of IBE between Stathis Psillos and Bas van Fraassen. Just as in our previous discussion about the novel defense of scientific realism, the pros and cons of IBE were discussed in a very abstract way. We would like to investigate again how our case study fits into this discussion.

In his book Laws and Symmetry, Bas van Fraassen brought forward two arguments against IBE. The first one is the argument from indifference:

> "There are many theories, perhaps never yet formulated but in accordance with all evidence so far, which explain at least as well as the best we have now. Since these theories can disagree in so many ways about statements that go beyond our evidence to date, it is clear that most of them by far must be false. I know nothing about our best explanation relevant to its truth-value, except that it belongs to this class. So I must see it as a random member of this class, most of which is false. Hence it

must seem very improbable to me that it is true" (van Fraassen 1989, 146).

Psillos (1996, 43-44) admitted that there are always unborn alternatives to theories which are going beyond the evidence. However, such theories appear to be trivial or absurd and can be ignored legitimately. There are always many possible explanations of a phenomenon, but only few plausible life-options. The argument from indifference is effective only if it can be proved that there are alternative explanations which are at least nearly as good as the best explanation. However, Ladyman et. al. (1997, 309) replied that the mere possibility of equally good explanations undermines the reliability of IBE. Psillos (1999, 223) opposed, because it cannot be proven that the possible alternatives are always good ones. As a result, the discussion about the argument from indifference ended in a stalemate.

Speculations about the possibility of unconceived good alternative explanations can be neglected for our case study about mechanisms of axonal transport. Eleven possible mechanisms existed in the early 1980s, most of them postulated by scientists in actual scientific practice. Realists like Stathis Psillos have to claim that one of these hypotheses is much better supported by evidence than the others and very likely to be true. Antirealists like Bas van Fraassen, on the other hand, can simply state that every hypothesis has an initial plausibility and for every hypothesis its own probability is much lower than the probability that one of the other ten hypotheses is true, so it would be irrational to believe in the truth of one of these hypotheses.

Our case study strongly speaks in the antirealist favor. Not all hypotheses were equally plausible or equally supported by evidence, but none of them was far better than the others. Moreover, these hypotheses were (mostly) not just theoretical possibilities but defended by scientists due to their scientific practice; hence they at least could not be ignored. Although it might be reasonable to say that one of these hypotheses is better than the others instead of a random member of this class, it is not reasonable to infer the truth of that hypothesis via IBE. Furthermore, there is no point from nowhere for deciding which theory is the best. In the early years of philosophy of science, rising from logical empiricism, philosophers believed that a formal algorithm can be developed which is able to choose the best theory of a set of theories in an unambiguous way. However, this viewpoint was strongly criticized by Thomas S. Kuhn in his famous book *The Structure of Scientific Revolutions*. Kuhn claimed that proponents of different paradigms cannot determine objectively which paradigm is the best:

> „The proponents of competing paradigms are always at least slightly at cross-purposes. Neither side will grant all the non-empirical assumptions that the other needs in order to make its case. […] Though each may hope to convert the other in his way of seeing his science and his

problems, neither may hope to prove his case. The competition between paradigms is not the sort of battle that can be resolved by proofs" (Kuhn 1970, 148).

The main reason for Kuhn's opinion is his incommensurability thesis, i.e. the claim that proponents of different paradigms speak different languages and see different things by looking at the same object. He claims that "the proponents of competing paradigms practice their trades in different worlds. […] Practicing in different worlds, the two groups of scientists see different things when they look from the same point in the same direction" (ibid, 150).

Applied to our case study, an advocate of the contraction hypothesis cannot even fully understand why supporters of the microstream hypothesis believe in the existence of microstreams, because if he looks at a HVEM-image, he observes that MTs as well as organelles and vesicles are enmeshed in a rigid lattice-like structure, so it is impossible that MTs can transport vesicles due to microstreams without any connections between them. On the contrary, advocates of the microstream hypothesis claim that the numerous movements in the cytoplasm well-known due to light microscopy cannot be compatible with the static framework of the contraction hypothesis; hence in their opinion it is highly implausible that the microtrabecular lattice exists in the living cell, so they regard the contraction hypothesis as completely misguided. Both groups of researchers are using the same experimental data, but they do not see the same things.

Critics of Kuhn stated that his view would lead to relativism. While it is not possible to determine which theory is best on purely logical reasons, there can be good reasons to choose one theory rather than another. In a later essay, Kuhn offered five criteria for theory choice, i.e. accurateness, consistency, broad scope, simplicity and fruitfulness (see Kuhn 1977, 321-322). However, these criteria are imprecise individually and can conflict with each other. Moreover, in some cases, one can reach different conclusions even when applying exactly the same criteria to the same theories. Therefore, Kuhn considers them "not [as] criteria or rules, but [as] maxims, norms or values" (ibid, 330).

Anyway, because there were many life-options of theories about axonal transport in the early 1980s and it could not definitely be determined which one is the best, this example strengthens the argument from indifference and attacks IBE. Over and above, this is not the only argument against IBE. Van Fraassen's second argument against IBE is the *argument from the bad lot*:

"[IBE] is a rule that only selects the best among the historically given hypotheses. We can watch no contest of the theories we have so painfully struggled to formulate, with those no one has proposed. So our selection may well be the best of a bad lot. To believe is at least to consider more

likely to be true, than not. So to believe the best explanation requires more than an evaluation of the given hypothesis. It requires a step beyond the comparative judgment that the hypothesis is better than its actual rivals. While the comparative judgment is indeed a 'weighing (in the light of) the evidence', the extra step – let us call it the ampliative step – is not. For me to take it that the best of set X will be more likely than not, requires a prior belief that the truth is already more likely to be found in X, than not" (Van Fraassen 1989, 143).

In reply to Van Fraassen, Psillos admitted the logical possibility that the true hypothesis has not been considered yet. However, he stated that scientists don't live in a knowledge-vacuum but have *privileged background knowledge* which ensures that an approximately true theory will always be considered if a scientific phenomenon needs explanation (Psillos 1996, 37). Again, Ladyman et. al. (1997, 306) opposed by arguing that the mere possibility of a bad lot is sufficient to inhibit justification of IBE, a point that Psillos (1999, 222) denied. Once again the discussion ended in a stalemate.

Our case study provides a historical example of a bad lot. None of the eleven hypotheses of axonal transport had described the true mechanism and none of them can be regarded as even approximately true. Hence, even if it could have been determined unambiguously which of these hypotheses was the best, likeliest or most plausible one, a proponent of IBE would have chosen a strictly false hypothesis.

To sum up, our case study provides a strong counterexample to the justification of IBE. There was no hypothesis which was much better than the others, it maybe cannot even be determined which one was best, and even the most plausible hypothesis was not true. Therefore, philosophers as well as scientists themselves have to be careful and cannot always trust in a reasonable way that a theory is true just because it is better than its rivals. As a matter of course, this does not prevent IBE to be heuristically useful. Furthermore, we do not want to claim that there are no cases in which IBE can be a warranted kind of inference.

5.3 Scientific revolution or "normal science"?

Most biologists would agree that the discoveries made within cell biology during the early eighties essentially changed our views on the structure of the living cell, and especially our view on the mechanism of intracellular transport. At the beginning of the eighties, many biologists (seemingly for good reasons) believed that the microtrabecular lattice is a real structure of the cell that plays a key role in the mechanism of intracellular transport. Only a few years later, the microtrabecular lattice was exposed as an artifact produced by the preparation techniques of electron

microscopy. Accordingly, all hypotheses about intracellular transport that presupposed the existence of microtrabeculae (e.g. the contraction hypothesis) turned out to be false. Furthermore, due to the development of AVEC-DIC microscopy, it was possible for the first time to actually observe the transport of intracellular materials within living cells. These observations showed that all hypotheses about intracellular transport that had been developed until then (e.g. the microstream hypothesis) are false.

During a relatively short time, the views held by a majority of biologists about the ground structure of the cytoplasm and the character of intracellular transport turned out to be wrong. One philosophical question we set out to answer within this project is if this change in biologists views about the structure of the living cell and intracellular transport, caused by the discovery that the microtrabecular network is just an artifact and the development of AVEC-DIC microscopy, could be called a scientific revolution in Kuhn's sense (Kuhn 1970; 1987).

According to Kuhn, there are two types of scientific development: normal and revolutionary. Normal scientific development is cumulative and happens against the background of a widely accepted paradigm, or (to be more precise) a *disciplinary matrix* (Kuhn 1970, postscript). Ultimately, a disciplinary matrix provides the basic means for scientists to identify, formulate, and solve the theoretical and practical problems they face during their everyday research practice. It encompasses the basic beliefs, key theories, values, and metaphysical assumptions shared by members of a scientific community, plus the methods and instruments they consider admissible for use in scientific research. Typical examples for successful scientific problem solutions and models, as well as analogies and metaphors preferentially used to illustrate central scientific concepts, are further elements of a disciplinary matrix (Kuhn 1970, 1987). Because it is the constituting basis for their research practice, scientists do not challenge or test their well-established basic believes, key theories and methods during normal times, but attempt to extend them and solve all emerging scientific problems in accordance with them (Kuhn 1970, postscript). Problems that cannot be solved within the boundaries of the given disciplinary matrix as well as anomalous experimental results (anomalies) that seem to falsify the basic beliefs and key theories held by the majority of the scientific community, are ignored or temporarily put aside to be solved by future generations of scientists (Kuhn 1970).

Revolutionary scientific development can be characterized in contrast to normal scientific development (Kuhn 1987). It takes place when anomalies accumulate and troublesome problems become so pressing that parts of the scientific community lose confidence in the problem-solving capacities of the established disciplinary matrix. Kuhn calls this situation a crisis (Kuhn 1970). As a result of a crisis, some scientists will revise commonly accepted basic believes, theories, and practices, and start to develop a new disciplinary matrix that has the power to solve the pressing problems and anomalies while still preserving the most important

solutions found in accordance with the old matrix. This eventually leads to a period in which the scientific community is divided into two competing groups: The adherents of the old matrix on the one side, and the adherents of the new developed matrix on the other. Kuhn calls this period a scientific revolution (Kuhn 1970, 1987).

As a result of scientific revolution, the old matrix is replaced by the new one, which becomes the standard for the vast majority of the scientific community. An important characteristic of scientific revolutions is that the conflict between the two competing groups of scientists cannot be solved by rational argument within science. The reason is that a disciplinary matrix fixes the standards for good science, i.e. which theories and beliefs about the world can be considered as basic, which problems scientists should envisage, and which methods and instruments they can legitimately use to solve them. This means that a difference in disciplinary matrix leads to a fundamental difference about how good science should be done. Therefore, neither side will be persuaded by the *scientific* arguments of the other (Kuhn 1970).

To summarize: While normal scientific development occurs in accordance with a widely accepted disciplinary matrix, revolutionary development is characterized by a revision of this disciplinary matrix. For our case the question is whether the identification of microtrabeculae as artifacts and the development of AVEC-DIC microscopy led to a change in the disciplinary matrix of cell biology or not. For the following reasons, we think that it is not the case:

1. A revolutionary change in theory occurs when a key theory that is widely accepted within the scientific community is replaced by a different theory. But, at the time in question, there was no such thing as a widely accepted theory of intracellular transport. Instead, there were many different hypotheses that did equally well fit the available data, some of them more prominent than others (see chapter 4.2). Hence, there was a latent underdetermination problem that was finally solved in the light of newly available data due to the development of AVEC-DIC microscopy. The adherents of the contraction-hypothesis were just wrong in supposing that the images produced by electron microscopy had any decisive power in this case. Due to the lack of a widely accepted key theory of intracellular transport it can be doubted that one can capture our episode of cell biology within Kuhnean terms.

2. A revolutionary change in methods and instrument use occurs when methods and instruments that are considered to be impermissible for use in scientific research become permissible to the majority of the scientific community. But neither did anyone question the general permissibility of the electron microscope after the microtrabeculae turned out to be artifacts, nor did anyone question the permissibility of AVEC-DIC microscopy (the same is true for the use of preparation techniques). Biologists used both instruments, and the development

of a new technique, or the better understanding of an old one, can hardly be called 'revolutionary' in Kuhn's sense.

3. A revolutionary change in basic concepts occurs when the meaning of scientific key terms like "cell", "mass", "atom" and the like changes so drastically that they refer to completely different entities. However, this was not the case. Biologists did not revise their concept of "cell", "organelle", "axon" or even "intracellular transport". Perhaps biologists had wrong opinions about the specific nature of the mechanism that caused "intracellular transportation", but they all perfectly agreed on the reference of the term.

4. A revolutionary change usually leads to a revision of the central problems and questions that science sets out to answer. But in case of cell biology, there was no such change. There was just an open question about the mechanism of intracellular transport that could not be answered, because the instruments needed to make the relevant observations were not available before the development of AVEC-DIC microscopy. So, there was just a technical problem that has been solved within the boundaries of the commonly accepted theories and methods.

5. A revolutionary change is often accompanied by a change in ontology, i.e. the general opinion about what entities exist in the world. After a revolution, the world is considered to contain other basic entities than before. The discovery that there is no microtrabecular lattice within the living cell is not a case of such revolutionary change.

6. The only change in cell biology that resembles a change that occurs within scientific revolutions concerns the metaphors and analogies used to describe the cytoplasm and the activities within the cell responsible for intracellular transport. While some biologists (e.g. adherents of the contraction hypothesis) had a rather static view about the cytoplasm and compared it to a lattice that contracts and elongates, others (e.g. adherents of the microstream hypothesis) had a more dynamic view and compared it to a liquid with streams. Neither view turned out to be right, although the dynamic views have been closer to the truth than the static views. Again, there was no such thing as a standard metaphor, but multiple competing ones.

6. Outlook

High impact research progress in biology is often caused by new possibilities due to advanced techniques of inquiry. The history of cell biology is closely connected to the development of microscopy techniques. Every innovation in microscopy transforms biological knowledge either radically or gradually, letting many scientists focus on new problems and viewpoints. The introduction of electron microscopy to biology has led to unforeseen discoveries of cell biological structures which could not be observed with light microscopy. Biologists had to pay the price for focusing on inquiries on the structure of the cell because the dynamics could not be investigated with electron microscopy. Some researchers exaggerated the structural elements of the cytoplasm so that dynamical aspects were neglected and dynamical hypotheses (like the microstream hypothesis) were considered as mistaken while structural concepts such as that of a microtrabecular lattice were exaggerated. Due to the development of video microscopy, which drastically improved the resolution limit of light microscopy, the dynamics of the cell has been rediscovered and the overemphasis of structural aspects of the cell has been corrected.

While every improvement in microscopy techniques leads to new discoveries and problem shifts in cell biology, we do not regard these processes as scientific revolutions as described by Thomas S. Kuhn. In contrast to physics, cell biology does not seem to have hypotheses or theories which are highly abstract, unifying and a leading instance for norms, rules and problems to further inquiries. Cell biology focuses much more on experiments than physics. There are many working hypotheses and testable assumptions, yet they are not considered as paradigms and can be abandoned quickly if new evidence speaks against them. Moreover, contrary to Kuhn's view, in normal science of cell biology many new phenomena can be discovered.

Nevertheless, the improvements of cell biology due to new research techniques seem to be revolutionizing in some way. Therefore, a philosophical framework beyond Kuhn´s is needed to describe exactly in which way improvements can be regarded as revolutions. We assume that this framework has to investigate the role of experiments for scientific research more profoundly than conventional philosophy of science has done it. Furthermore, epistemological strategies to avoid experimental errors like the microtrabecular lattice have to be developed or reconsidered. In any case, a detailed look on biology can promote and refresh many issues in philosophy of science and vice versa.

7. Acknowledgements

8. Abbreviations

3D: three-dimensional
AVEC: Allen video-enhanced contrast
CCD: charge-coupled device (camera)
CPD: critical point drying
DIC: differential interference contrast
EM: electron microscope
FGE: force generating enzyme
GFP: Green Fluorescent Protein (technology)
IBE: inference to the best explanation
IF: intermediate filament
MF: microfilament
MT: microtubule
MTL: microtrabecular lattice
NF: neurofilament (intermediate filament in neurons)
NMA: no-miracles-argument
SEM: scanning electron microscope
SER: smooth endoplasmic reticulum
TEM: transmission electron microscope
VIM: video-intensified microscopy

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