The use of light- and electron microscopy for studies on the cell- and molecular biology of parasites and parasitic diseases

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Summary

Lightmicroscopical (LM) and electron microscopical (EM) techniques, have had a major influence on the development and direction of cell biology, and particularly also on the investigation of complex host-parasite relationships. Earlier, microscopy has been rather descriptive, but new technical and scientific advances have changed the situation. Microscopy has now become analytical, quantitative and three-dimensional, with greater emphasis on analysis of live cells with fluorescent markers. The new or improved techniques that have become available include immunocytochemistry using immunogold labeling techniques or fluorescent probes, cryopreservation and cryosectioning, in situ hybridization, fluorescent reporters for subcellular localization, micro-analytical methods for elemental distribution, confocal laser scanning microscopy, scanning tunneling microscopy and live-imaging. Taken together, these tools are providing both researchers and students with a novel and multidimensional view of the intricate biological processes during parasite development in the host.

Keywords: parasite, protozoa, host-parasite, electron microscopy, confocal microscopy.

Licht- und Elektronenmikroskopie zur Untersuchung der zellulären und molekularen Biologie von Parasiten und Parasitosen

Lichtmikroskopische (LM) und elektronenmikroskopische (EM) Techniken hatten seit jeher einen grossen Einfluss auf die Entwicklung der Zellbiologie besonders in Bezug auf die Untersuchung von komplexen Wirt-Parasit-Wechselwirkungen. Früher war die Mikroskopie eher deskriptiv, aber neue technische und wissenschaftliche Fortschritte haben diese Situation stark verändert. Die Mikroskopie ist heute analytisch, quantitativ und dreidimensional, mit Schwerpunkt auf der Untersuchung von lebenden Zellen mit Hilfe von fluoreszierenden Markern. Neue oder verbesserte Techniken die uns heute zur Verfügung stehen sind Immunozytochemie mit Gold- oder Fluoreszenzmarkern, Kryopräservation und Kryosektion, in situ Hybridisierung, fluoreszierende Reporter für subzelluläre Lokalisation, mikroanalytische Methoden zur Untersuchung von Elementenverteilungen, konfokale Laser-Scanning-Mikroskopie, Raster-Tunnel-Mikroskopie und lebend-Zell Analyse. Zusammen vermitteln diese Werkzeuge sowohl Wissenschaftlern als auch Studierenden eine neue und multidimensionale Sicht der komplizierten biologischen Prozesse während der Entwicklung von Parasiten im Wirt.

Schlüsselwörter: Parasit, Protozoen, Wirt-Parasit-Wechselwirkung, Elektronenmikroskopie, Konfokal-Mikroskopie.

Introduction

During the past decade most of the spectacular advances in our understanding of the functions of cells, and in particular parasites, have come through a combination of imaging techniques and molecular biology, immunology and biochemistry, all coupled with improved techniques for *in vitro* culture of different parasite life cycle stages. It has become clear that a single protozoan parasite represents an enormously complex organism and is dependent upon a high level of structural, spatial and temporal organization. The ability to transfect parasites has revolutionized the study of these organisms. The function of specific genes and/or gene products can be explored by disruption of the locus or more subtly, by introduction of altered tagged versions. Using transgenic reporter genes (e.g. green fluorescent protein [GFP] or lu-

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ciferase), cell biological processes can be studied in living parasites and in real time. The combination of these *in vivo* markers with an increasingly diverse genetic toolbox has opened many exciting experimental opportunities. In addition, the enormous technical advances in digital imaging have made it possible not only to visualize but also to functionally characterize the role of individual parasite organelles or parasite molecules in the host-parasite relationship.

In this article, we will give an overview on the general concepts of imaging and briefly summarize the basic steps that are required for the preparation of samples to be visualized by light or electron microscopy. We will further highlight the more recent developments in confocal microscopy, with a special focus on the use of transgenic parasites expressing proteins coupled to fluorescent markers for localization studies. Some of these techniques require highly specialized skills as well as equipment, and should therefore be first learned and acquired in a specialized laboratory.

The basics of imaging

Resolving power, contrast and magnification

The effectiveness of any form of microscope depends on (i) the resolving power, (ii) the contrast, meaning the ability to recognize differences in the components of the specimens to be examined, and (iii) the magnification. Magnification is only useful if the user can continue to identify and distinguish more and more

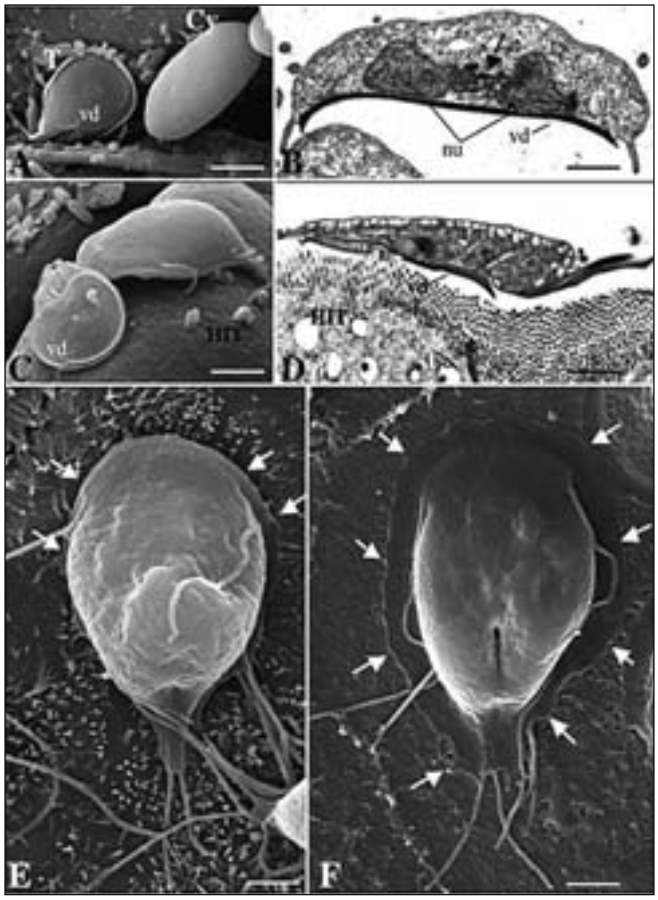

Figure 1. Giardia lamblia and host-parasite interactions visualized by SEM and TEM. Panel A shows the two stages of Giardia, namely a trophozoite (T) and cyst (Cy). Panel B is a cross-section through a trophozoite, exposing the two nuclei (nu) and the cytoskeleton forming the ventral disc (vd). In panels C and D, trophozoites are in close contact with the host intestinal tissue (HIT), whose microvilli cannot be discerned by SEM (C) but by TEM (D). The contact is largely mediated through the vd. Panels E and F are higher resolution SEM images demonstrating attachment of Giardia trophozoites to different cultured intestinal cell lines. Note that attachment of parasites to a microvilli-rich surface (E) is different compared to attachment to a smooth cell surface (F). Smoother surface allows the parasite to establish a much more intimate contact to its host (arrows). Scalebars: 8 µm (A), 2.2 µm (B), 8 µm (C), 2.2 µm (D), 3.2 µm (E), 3.2 µm (F).

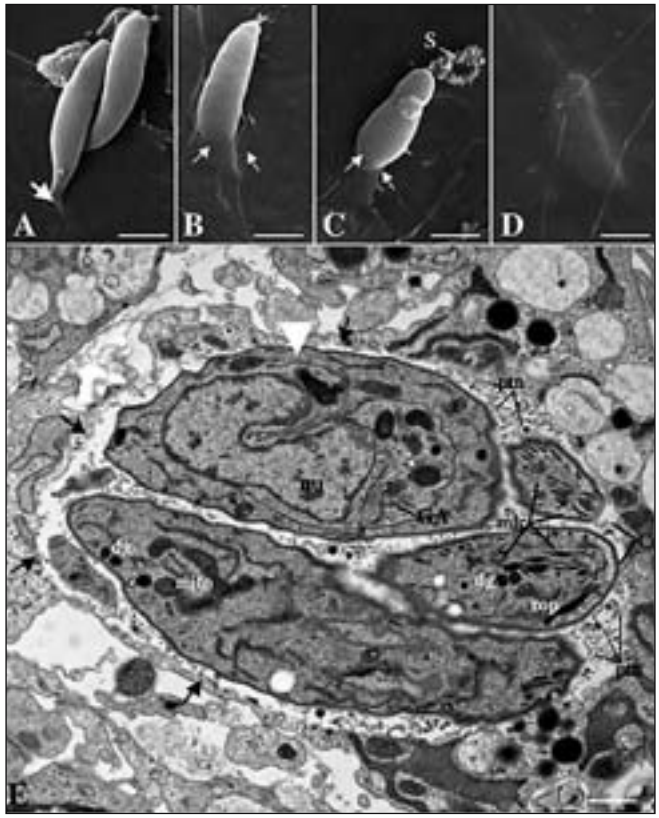

Figure 2. Neospora caninum and fibroblast host cell invasion/intracellular parasitism. Panels A to D show different steps in the host cell invasion process. Tachyzoites first establish initial contact with the host cell, probing the host cell surface with their apical end (arrow in A). Tachyzoites then rapidly invade their host cells by forming a moving junction between tachyzoite and host cell surface membrane (arrows in B and C), glide into the host cell cytoplasm, and simultaneously shed surface- and secretory components (S). Following completion of host cell invasion, the host cell surface membrane remains unharmed (D). Within the host cell (E), tachyzoites reside within a parasitophorous vacuole delineated by a membrane (arrows), and the TEM reveals number of hallmarks such as Golgi Apparatus (GA), mitochondria (mito), dense granules (dg) and apically located conoid (Co), micronemes (mic), and rhoptries (rop). Within the vacuole, tachyzoites are embedded in a parasitophorous vacuole tubular network (ptn), and the white arrowhead in E points toward a tachyzoite that is in the process of dividing by endodyogeny. Scalebars: 2 µm (A–D), 0.5 µm (E).

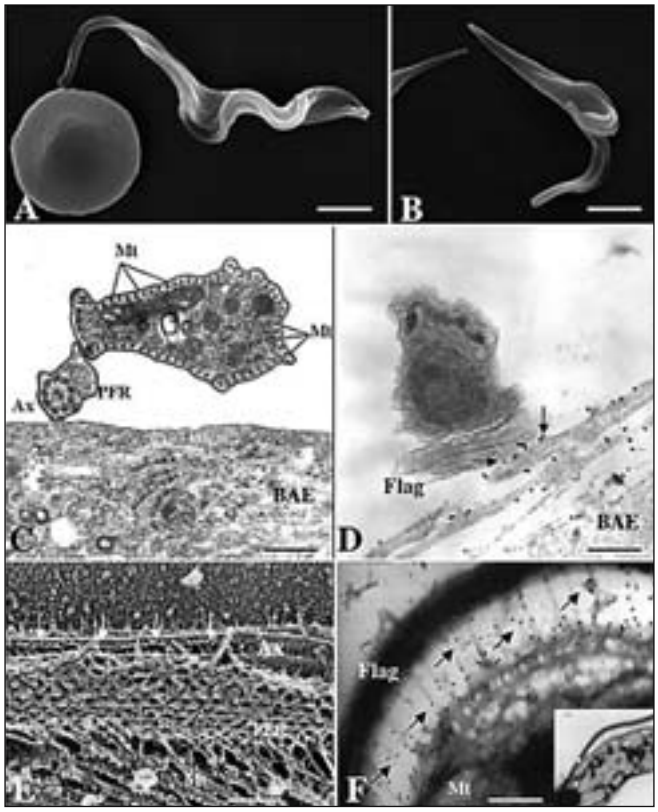

Figure 3. SEM and TEM of African trypanosomes. SEM of Trypanosoma brucei brucei bloodstream form (A) and procyclic stage parasite (B). Note in B the parasite is undergoing cell division. Ec: erythrocyte; scalebars: 3 µm (A), and 5 µm (B). Panel C shows a T. congolense metacyclic form adhering to bovine aorta endothelial cells (BAE). Attachement of parasites is mediated exclusively by the flagellum, and the flagellar cytoskeletal elements such as axonem (Ax) and paraflagellar rod (PFR) are clearly visible. Note the subpellicularly located microtubules (Mt), which form the cytoskeleton of the trypanosme cell body. D shows an adherent T. congolense with a more longitudinally sectioned flagellum, and wheat-germ-agglutinin-gold labeling and silver enhancement shows that the attachment is largely mediated by silaic acid residues (arrows). E presents a high resolution three-dimenional view of a detergent extracted flagellum prepared by quick freeze deep-etch rotary-shadowing TEM. In addition to the classical cytoskeletal elements, dynein-heads and – stalks are clearly visible between the inter-microtubular spaces of the axoneme (arrows). The asterisk indicates flagellum-cell body linking filaments. Similar filaments are visualized by negative staining of whole mount cytoskeleton preparations (F). These filaments are specifically labeled with an antibody directed against a repetitive antigen of T. brucei brucei (arrows). Scalebars: 0.23 µm (C, D), 0.1 µm (E), 0.25 µm (F).

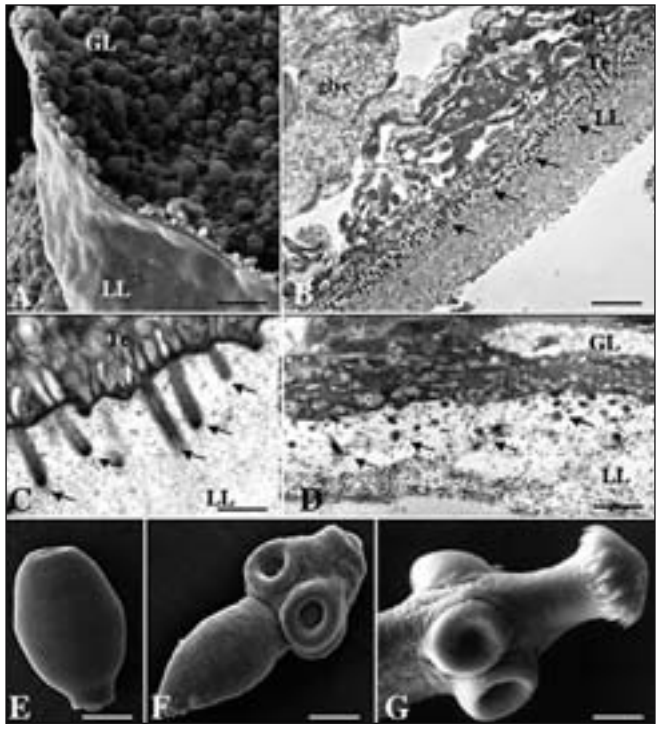

Figure 4. SEM and TEM of Echinococcus sp. Panel A shows a SEM of an E. multilocularis metacestode, exposing the outer, acellular and carbohydrate-rich laminated layer (LL) and the inner cellular germinal layer (GL). The corresponding cross-section in B reveals the microtriches (arrows), microvilli-like protrusions that originate at the tegument and extend into the laminated layer. The germinal layer consists of a number of cell types, most prominently seen here are glycogen storage cells (glyc). In C, a higher magnification of microtriches structure is seen. In D, metacestodes were fixed and processed for immunogold-labeling, and embedded in LR-white acrylic resin. The antibody used is directed against a prominent component of the laminated layer (LL). Note the presence of microtriches, but also that the overall structural preservation is impaired. Panels E and F respresent invaginated and evaginated protoscoleces, and panel G the fully developed scolex of an adult E. multilocularis worm. Note the four suckers and the rostellum (arrows). Scalebars: 200 µm (A), 2.8 µm (B), 0.7 µm (C), 50 µm (F and E), 80µm (G).

elements in the object as magnification increases. The magnification is largely dependent on the resolution and contrast of a given microscope.

Resolution is defined as the closest spacing of two points, each of which can still be clearly distinguished as separate entity when looking through a microscope. This distance is determined by the wavelength of the emissions from these two points. Common emission sources are light in the LM, UV-light for fluorescence work and electrons in the EM.

The shorter the wavelength the higher the resolving power of a given microscope. The wavelength of light limits the resolution of the LM to a theoretical 0.2 µm. With an emission source made of electrons, which have a much shorter wavelength, the theoretical resolution is theoretically down to the width of an atom. In practice, the resolution of a transmission electron microscope (TEM) can be as low as 0.2 nm, or 1000 times better than possible through light microscopy.

However, the basic concepts for visualizing parasitic micro-organisms by LM and EM are strikingly similar: In the LM, light waves or photons are emitted from a light source, and the deflected light creates the image. TEM follows a very similar principle, in that electrons are emitted from an electron gun, through application of a high voltage (20–100 kV), and the degree of deflection is dependent on specimen thickness and stains, which are normally composed of elements of high atomic number (e.g. osmium, lead,

uranium, manganese, gold, tungsten). These stains are the mechanism for achieving contrast. In a scanning electron microscope (SEM), electrons are emitted and accelerated by a voltage of 2–30 kV and are focused and scanned across the surface of a specimen, which is usually coated with a thin layer of gold or platinum. Electrons reflected from the surface are collected by an appropriate detector, amplified and displayed on a screen. Only surfaces can be examined using this type of microscope, but the great depth of focus normally gives an excellent three-dimensional representation, no matter whether small protozoan parasites such as *Giardia* (Fig. 1), *Neospora* (Fig. 2), *Trypanosoma* (Fig. 3) or larger objects such as *Echinococcus* adult and larval stages (metacestodes) (Fig. 4) are investigated.

Approaches for visualizing parasites in the microscope

The two mostly applied approaches for visualizing parasites in the microscope are (i) whole mount preparations and (ii) embedding / sectioning approaches. While the former approach can provide information on the overall three-dimensional shape of a given object, the latter allows a glance into the inside of the object of interest.

Embedding and sectioning (see Fig. 1B–D, 2E, 3C–D, and 4B–D) requires a series of sequential preparative steps to be performed. These include chemical fixation, dehydration of the sample, and embedding in a suitable resin (paraffin, epoxy or acrylic resin). These are followed by polymerization of the resin, and sections are cut on a microtome. Sections for LM are 1–10 µm in thickness, while sections to be inspected in a TEM are 60–80 nm in thickness. Sections are then stained by chemical or enzymatic means, antibodies, lectins and/or other agents, and are inspected by LM or TEM, depending on the thickness of the section (for reviews on methodology refer to (Smith M., 1990; Griffiths, 1993; Hemphill A., 1997). The specimen size that can be processed is limited. Ideally, the processed tissue size for embedding and subsequent LM is not more than 1 cm^3, and maximum 1 mm^3 for TEM.
A large variety of processing methods have been developed. All preparative steps bear the risk of denaturing epitopes, which is a major problem for immunocytochemistry applications. For these applications, a balance must be found between preservation of the structural integrity of the cell or tissues (by fixation) and maintenance of the antigenicity of epitopes (by mild treatments). As a consequence, the structural preservation of specimens used for localization of antibody binding sites is often impaired compared to a specimen that has been fixed and processed for ultrastructural studies. This becomes most evident at the level of electron microscopy. For instance, the integrity of *Echinococcus multilocularis* metacestodes is well preserved in sections fixed and processed for ultrastructural studies (Figs. 4B, C), but less well preserved in sections that are subsequently on-section immunogold-labeled using an antibody directed against a protein of the laminated layer (see Fig. 4D). In some instances, it is possible to perform immunogold-labeling prior to embedding. This is demonstrated in Fig. 3D for a protein on the surface of bovine aorta endothelial cells that serves as a receptor of adhesion to the endothelial cell surface by the *Trypanosoma congolense* flagellum. In this case, gold particles were silver-enhanced. Of course, this approach is only applicable for antibodies whose epitopes are accessible from the outside.
In specialized laboratories, fixation and resin-embedding steps have been replaced by treating the samples with a cryo-protectant and applying rapid freezing techniques (to avoid the formation of ice crystals). Subsequent cryo-ultramicrotomy allows preparing ultra-thin sections of frozen samples to be studied by TEM. Another technique is freeze-substitution, where the rapidly frozen specimen is dehydrated at low temperatures by organic solvents, and finally infiltrated with an acrylic resin which is then cross-linked under UV.

Whole mount preparations are preferentially applied for LM and SEM to investigate protozoan parasites (Fig. 1–3), helminths (Fig. 4) and even larger specimens, but whole mounts can also be used for TEM for visualizing viruses, bacteria or cytsokeletal or organellar preparations of parasites (see Fig. 3F). For whole mount preparations, the specimens are allowed to adhere to a suitable surface, followed by fixation and/or permeabilization. The specimens are then processed for (immuno)staining. SEM is often used to obtain information on the overall shape and surface properties of a given sample or to obtain basic morphological information on cellular interactions such as the host cell invasion process by *N. caninum* tachyzoites (Fig. 2A–D). However, although three-dimensional images are obtained, the resolution is limited, as evidenced by SEM of e.g. *Trypanosoma brucei* (Fig. 3A, B). For improving the resolution, a specialized whole mount preparation method applicable for TEM, the quick-freeze deep-etch method, was established (see Fig. 3E). For this, the sample of interest is rapidly frozen in liquid helium, freeze-dried in a high vacuum chamber, and rotary shadowed with platinum. Viewing the platinum replica by TEM provides a three-dimensional image of very high resolution, and has been used to investigate the cytoskeletal architecture of trypanosomes, e.g. (Hemphill et al., 1991). Other methods include the freeze-fracture techniques, e.g. (Tetley and Vickerman, 1985) and

the wet cleaving technique e.g. (Sherwin and Gull, 1989), which have also been applied to trypanosomes. As a more simple technique, trypanosome cytoskeletons can be stained with uranyl acetate in order to visualize basic structural features in the TEM (see Fig. 3F), such as the thin filaments of the undulating membrane, that connect the cell body with the flagellum. In Fig. 3F, these filaments are labeled with a specific antibody directed against a diagnostic antigen (Sherwin and Gull, 1989).

Staining of parasites does not necessarily reflect reality

The purpose of staining is to achieve contrast and to obtain specificity and localization of intrinsic components. It is important to keep in mind that the method of staining largely determines the final image; therefore different stains often result in different appearance of the same specimen. Hence, rather than reflecting reality, staining produces an image of the object of interest which we finally interpret. In TEM, the biological material as such will produce very little contrast. While aldehyde fixatives have no staining properties, OsO_4 incorporates mostly into membranes. Thus, a heavy metal stain, typically uranyl acetate and lead citrate, has to be introduced into the specimen, and the differential distribution of these heavy metals produces the final image and allows to distinguish between different organelles and cellular components, which are the hallmarks of different parasite species such as *Giardia* (see Fig. 1B, D), *Neospora* (Fig. 2E), *Trypanosoma* (Fig. 3C, D) and *Echinococcus* (Fig. 4B–D). Specific proteins are best localized using antibody-colloidal gold complexes (immunogoldlabeling, see Fig. 3F, 4D), possibly combined with silver enhancement (see Fig. 3D).

Visualizing multidimensional systems

New imaging technology shapes our understanding of host-parasite interactions

Physical interactions of parasites with tissues and cells of their host organisms range from limited to highly complex depending on the system. Due to their small size and large numbers, protozoa often live in close contact with, or even inside host cells and influence their host organism on a cellular, organ and systemic level during their residence. In addition, this host-parasite relationship is subject to dramatic changes as the infection goes its course. Typically, infection

develops from an acute to an often prolonged chronic state to which the host eventually succumbs (e.g. African sleeping sickness) and/or suffers serious sequelae (e.g. Chagas disease). Some parasites, however, are eventually eliminated from the host (e.g., *Giardia lamblia*). Only in a few cases, such as in toxoplasmosis, a stable equilibrium of parasite proliferation and appropriate (*i.e.* non-pathological) host immune response is achieved. The researcher investigating such host-parasite interactions *in vivo* and *in vitro* is confronted with multi-dimensional systems where the parameters and the characteristics of the intimate connections between the two organisms can change dramatically over time. Thus, documenting parasite development in the host even on a basic level remains one of the most challenging problems in cellular biology. Modern molecular biology has proven invaluable for investigating specific mechanisms in a much focused manner. More recently, a series of technological innovations in the wake of the large genome projects made analysis on a systems level feasible. Prominent examples are whole genome sequencing and data mining, array technology and proteomics. The greatest impact on our global understanding of parasite development, however, came from the advances in digital microscopy during the last few years, and the capacity of this technology to provide us with a multidimensional view of events.

Three-dimensional imaging

Fluorescent light-based microscopy has been used for several decades to analyze parasites isolated from infected hosts or maintained in cultures. In immunofluorescence assays (IFA) antibodies are used to detect parasite proteins in structures of interest (e.g. organelles, cytoskeleton). Bound antibodies are labeled with anti-IgG antibodies covalently coupled to fluorophores. Fluorophores are molecules that can be excited with ultra-violet light and emit photons in a characteristic section of the spectrum. For analysis, the sample is illuminated from one side by a light source (UV-lamp or laser), and the emitted fluorescence is captured by a digital camera or photomultiplier. Despite expensive optics, conventional wide-field fluorescence microscopy "only" produces projection images of the x–y plane with a defined focal depth determined by the objective. Even combined with precise stepwise adjustments of this focal plane in the z-direction to encompass the entire structure under investigation, this method is not well suited to generate a useful three-dimensional impression of the sample (Agard et al., 1989). Recently, three major advances have come together to make precise multi-dimensional cellular analysis possible: laser scanning confocal microscopy, highly sensitive digital cameras or photomultipliers, and affordable workstations with

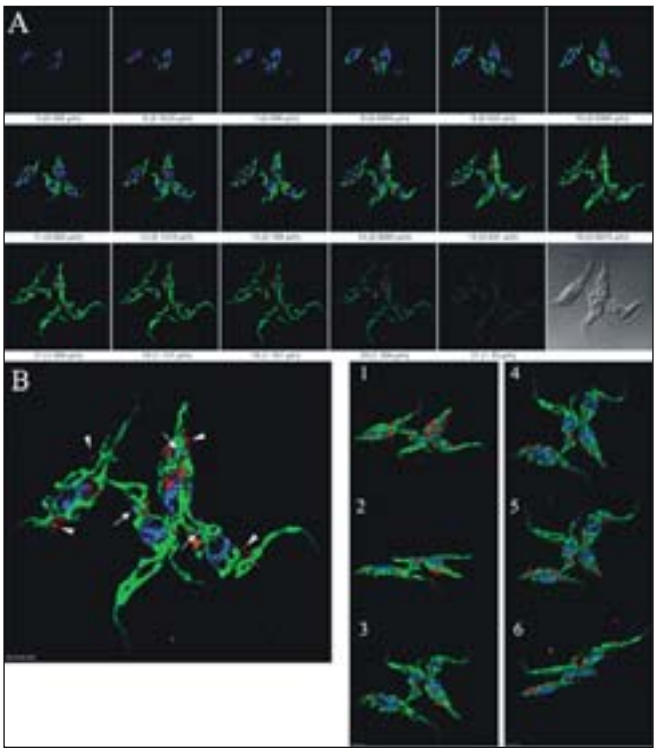

Figure 5. Optical sectioning and three-dimensional reconstruction of Trypanosoma brucei cells. The fixed preparation was labeled with antibodies against a mitochondrial protein cpn60 (green) and a dynamin-like protein (red). The nuclear and kinetoplast DNA is stained blue with DAPI. Panel A shows an image gallery of 21 optical sections produced on a confocal microscope. A representative DIC image of the group of cells is shown at the bottom right. Panel B: three-dimensional reconstruction of the image stack. The resulting virtual volume object was further processed using the isosurface function to render the structures as plastic and partially transparent objects. Arrowheads point to selected accumulations of dynamin-like protein. Arrows indicate the position of the kinetoplast DNA which is embedded in the mitochondrion. The object can be rotated in all directions to examine details and relative positions of the different structures (still frames 1–6).

sufficient processing power and storage capacity to control fast data acquisition and to handle the volume of image data for subsequent mathematical processing. Confocal microscopy was invented in the 1950s, but sensitive and precise digital data acquisition and numerical representation of an image was developed much later and probably had the largest impact on multidimensional imaging technology (Roux et al., 2004). Modern confocal microscopes scan the biological sample line by line with lasers which produce light of precise wavelengths. By sending the emitted fluorescence light through a small hole in a disk (pinhole) before it arrives on the detector, most out-of-focus light, *i.e.* signals which originate from above or below the focal plane, is eliminated, effectively generating a precise optical section. The diameter of the pinhole can be varied and used to adjust the focal depth. Thus, by focusing through the sample and recording a series of images, equidistant optical sections (optical tomogram) are produced. Figure 5A is

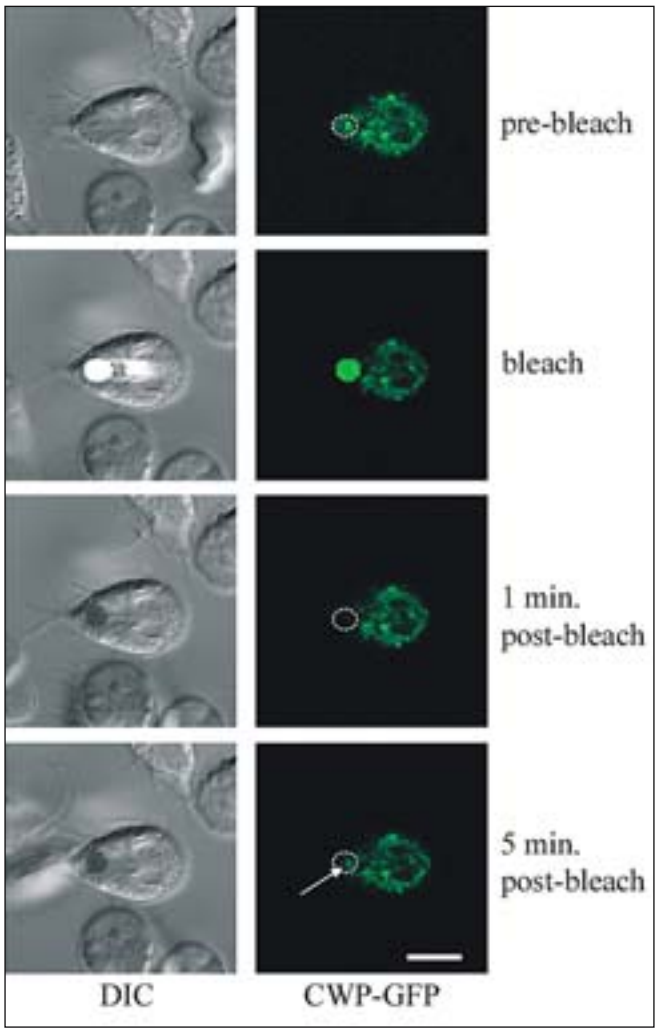

Figure 6. Live cell microscopy using green fluorescent protein-tagged cyst wall protein to label endoplasmic reticulum (ER) and knob-like encystation-specific vesicle (ESV) compartments. Single interference contrast (left) or fluorescence (right) frames are shown for different time-points. The adherent cell was observed for >20 min. (top) before permanent photo-bleaching of all GFP molecules in one ESV and the immediate surrounding ER as indicated by the circle (second row). The post bleaching image after 1 minute shows the absence of fluorescence in this area. The cell was imaged for another 20 min. to document appearance of imported fluorescent reporter from the ER. Recovery of fluorescence in the bleached ESV compartment after 5 min. is significant (arrow, bottom row). Scalebar: 5 µm.

an example for confocal immunofluorescence imaging showing three African trypanosome cells in a fixed preparation. Each planar x–y section in the figure is a numerical representation of the sample in a specific depth. The raw data (serial image stack) can be further enhanced with so-called image deconvolution (Swedlow, 2003). These algorithms are designed to correct two systematic problems of fluorescence microscopy: spherical aberration (SA) and recording of out-of-focus signals. SA is an optical effect which causes apparent elongation of objects in the z-axis. Deconvolution programs calculate so-called pointspread functions to correct the distortion. The overall effect on raw image stacks is that scattered light from out-of-focus objects is eliminated resulting in a much higher signal-to-noise ratio. These enhanced tomograms are reconstructed to virtual three-dimensional volume objects (Figure 5B). The principal advantage of reconstructed serial image stacks over conventional wide-field microscopy is that the sample can be examined in all dimensions (Figure 5B, 1–6) (Liu and Chiang, 2003). This means that the exact relative positions of labeled structures can be determined and the degree of co-localization, if any, can be quantified. For example, a dynamin protein, which is stained red in Figure 5B, is closely associated with the green labeled mitochondria but does not co-localize. On the other hand, the kinetoplast DNA (smaller blue bodies) appears embedded in the proximal end of the mitochondria.

Introducing the fourth dimension: live cell imaging

The sensitivity and computing capacity of currently available confocal laser scanning microscopy equipment is sufficient to analyze living cells during their development in vitro (cell culture). Because antibodies cannot be used to label internal structures, a different approach to localize representative marker proteins is used in these cases. Naturally fluorescent proteins, which came into use about 15 years ago, present an elegant solution since they can be genetically fused to most endogenous proteins (Piston, Patterson et al., 1999). The most prominent example is green fluorescent protein (GFP), a 238 amino acid protein of the jellyfish *Aequorea victoria*. The original sequence of GFP has been derived and improved *in vitro* by mutagenesis and selection in bacteria to generate an array of GFP variants with different light emission characteristics (e.g. yellow fluorescent protein, YFP) (Daabrowski, Brillowska et al., 1999) for multicolor analysis. Live cell analysis combined with appropriate culture conditions allow investigation of transgenic parasites expressing GFP variants fused to target proteins for a considerable time. Figure 6 illustrates the possibilities for exploration of developmental processes in the protozoan parasite *Giardia lamblia* using GFP-tagged proteins. Four still frames from an extended time-lapse video analysis show GFP-labeled cyst wall protein (CWP) in specialized compartments, called encystation-specific vesicles (ESVs). The example in Fig. 6 demonstrates that live cell imaging can be used to investigate the dynamics and development of organelles in very small protozoa. The present analysis suggested that the knob-like ESVs are stationary compartments and lack motility (data not shown). A more complicated question to address is the dynamics of protein transport from the endoplasmic reticulum (ER) to ESVs at this stage of development. This is done by a technique called fluorescence recovery after photobleaching (FRAP). In this application

all GFP in a selected ESV organelle (Fig. 6, top row) is permanently bleached by a strong laser pulse (Fig. 6, second row). Recovery of fluorescence in the bleached compartment indicates import of newly synthesized GFP-tagged protein from the ER (Fig. 6, third and fourth row). In addition to the common difficulties of live cell imaging such as unwanted bleaching of the sample and phototoxicity, the vigorous motility of the *Giardia* cells presents a special challenge.

Multidimensional microscopy in the true sense is how we will look at host-parasite systems in the future. Currently, the technical limitations in addition to appropriate culture conditions are twofold: frame rate of the detector, i.e. how many high-resolution images can be recorded in a given time-span, and phototoxicity from illumination by the laser beams. The former is a question of computing power alone and will be solved in due course. Highly sensitive (and expensive) detectors that can capture in excess of 60 frames per second are already commercially available. Limiting phototoxicity, however, is more difficult. Current approaches attempt to minimize repeated illumination of the cell or tissue under investigation, for instance by using lasers to scan the sample from the side and detect emitted light from above (e.g. Single Plane Illumination Microscopy, SPIM) (Huisken et al., 2004). The dazzling images from the few operational custom-engineered prototype microscopes are the best demonstration that fully multidimensional microscopy is possible and will eventually become routine.

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Microscopie optique et électronique dans l'examen de la biologie cellulaire et moléculaire des parasites et des parasitoses

Depuis longtemps la microscopie optique et électronique ont eu une grosse influence sur le développement de la biologie cellulaire, en particuliers quant à l'examen des interactions complexes entre l'hôte et le parasite. Autrefois la microscopie était surtout descriptive mais de nouveaux progrès techniques et scientifiques ont fortement modifié cette situation. La microscopie est ajourd'hui analytique, quantitative et tridimensionnelle en se concentrant sur l'examen de cellules vivantes à l'aide de marquers fluorescents. Les nouvelles techniques ou leurs améliorations dont nous disposons actuellement sont: l'immunocytochimie, avec des marquers dorés ou fluorescents, la cryopréservation et la cryosection, l'hybridation in situ, les marquers fluorescents des localisations subcellulaires, les méthodes micro analytiques d'examens pour la répartition des éléments, la microscopie à balayage en tunnel, et l'analyse des cellules vivantes. Ces outils procurent aussi bien aux chercheurs qu'aux étudiants une vue nouvelle et multidimensionnelle des processus biologiques compliqués qui se produisent lors du développement des parasites dans l'hôte.

Microscopia ottica e elettronica per l'analisi della biologia cellulare e molecolare di parassiti e parassitosi

Tecniche di microscopia ottica (MO) e elettronica (ME) hanno da anni un grande influsso sullo sviluppo della biologia cellulare in particolare in relazione ad analisi di complessi effetti di scambio ospite-parassita. All'inizio la microscopia era prevalentemente descrittiva ma i progressi tecnici e scientifici hanno modificato la situazione radicalmente. Oggigiorno la microscopia è analitica, quantitativa e tridimensionale con punti chiave nell'analisi di cellule vive con l'aiuto di marcatori fluorescenti. Tecniche nuove o migliorate che sono oggi a disposizione sono la chimica immunocita con marcatori d'oro o fluorescenti, la preservazione criogena e la criosezione, l'ibridizzazione in situ, i reporter fluorescenti per localilzzazioni subcellulari, i metodi microanalitici per l'esame di ripartizione di elementi, la microscopia confocale laser-scanning, la microscopia a tunnel e l'analisi di cellule vive. Tutti questi metodi forniscono a ricercatori e studenti una nuova visione multidimensionale dei processi biologici complessi durante lo sviluppi dei parassiti nell'ospite.

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