Biomolecular motors challenge imaging and enable sensing

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ABSTRACT

Biomolecular motors, such as the motor protein kinesin, are simultaneously objects of scientific inquiry and components for nanotechnology. The investigation of the properties of a biomolecular motor is challenging, since it is a dynamic nanoscale object but at the same time soft and fragile. Photonic techniques are well suited to these investigations due to their compatibility with an aqueous environment and their non-destructive character, however their resolution is often insufficient. We adapted Fluorescence Interference Contrast (FLIC) microscopy to the imaging of microtubules transported by kinesin motors (PNAS vol. 103, p. 15812) and achieved nm-resolution in the z-direction. This advance provided insights into the role of the kinesin tail for the functioning of the motor in vivo, but also enabled us to determine the "ground clearance" of molecular shuttles powered by kinesin motors. Kinesin-driven molecular shuttles, in turn, enable the design of highly integrated bionanodevices. Photons are the most suitable tool to communicate with such devices, since they can address molecules and nanoparticles packaged into the devices without the need for a physical connection.

Keywords: kinesin, microtubule, bionanotechnology, remote sensing, fluorescence microscopy

1. INTRODUCTION

One of the goals of nanotechnology is to assemble active nanoscale systems which mimic the complexity and functionality of biological cells. In fact, biology has been cited by Eric Drexler, one of the visionaries of Nanotechnology, as a proof-of-feasibility for complex, and even self-replicating nanomachines[1]. A key aspect of such active nanosystems is their ability to generate mechanical work, ideally from chemical energy sources. While there are examples for synthetic molecules which e.g. rotate in a particular direction as a result of an energy-consuming chemical reaction[2], biomolecular motors, such as the motor proteins kinesin, dynein or myosin, are until now unsurpassed in their speed, reliability and energy efficiency[3].

The realization that the road to a highly functional synthetic molecular motor is going to be long and arduous, despite recent progress[4], led to the idea of hybrid nanodevices, which integrate biomolecular motors into a synthetic environment[5]. The design of such hybrid, biomolecular motor-powered nanodevices started in the mid-nineties from adaptations of the gliding motility assay developed by biophysicists in the late eighties[6,7]. Within a decade, a wide variety of technical approaches relying on different motors and filaments and targeting different applications has emerged[8]. This rapid progress was possible because the interrogation of dynamic, complex, and soft systems has been a persistent challenge in the biological sciences, and optical microscopy has evolved from humble beginnings nearly 400 years ago to meet this challenge. Despite the limitations in spatial resolution, the sensitivity and non-destructiveness of optical methods makes them uniquely suited to observe motor proteins in action.

This paper will (1) introduce motor proteins and their associated filaments, (2) review several advances in the design of hybrid devices which illustrate the power of optical techniques, (3) describe in detail the application of fluorescence contrast microscopy, and (4) summarize the potential impact of integrating biomolecular motors into nanodevices.

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2. BIOMOLECULAR MOTORS

Biomolecular motors include motor proteins from the kinesin, myosin and dynein families[3], the F1-ATPase rotary motor[9], and the bacterial flagellar motor[10]. With the exception of the bacterial flagellar motor which is driven by a proton gradient, biomolecular motors rely on the hydrolysis of ATP to ADP and inorganic phosphate as an exothermic reaction which can be coupled to a mechanical cycle[11]. ATP hydrolysis provides a free energy change on the order of 100 kT per hydrolyzed molecule, and motor proteins can recover in excess of 50% of this exergy as mechanical work[12,13]. Each individual molecule can generate forces of a few picoNewton in a mechanochemical cycle lasting typically milliseconds.

An example of a motor protein, the kinesin-1 motor, imaged by transmission electron microscopy is shown in Figure 1. False-coloring emphasizes the kinesin motor (green) with its two heads and its tail, the vesicle (yellow) serving as a typical cargo for a kinesin motor in a process termed fast anterograde transport[14], and a microtubule (red) which constitutes the immobile track for the moving kinesin motor.

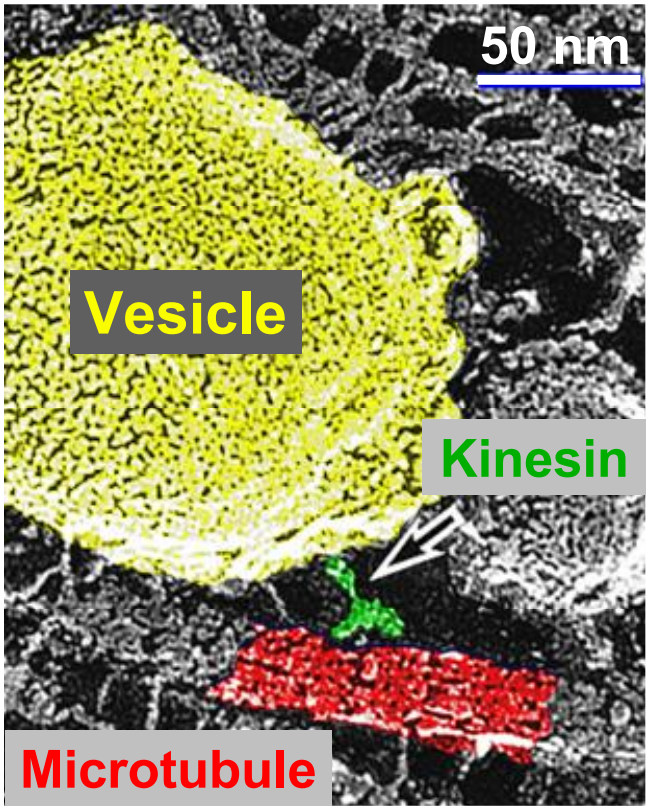

Fig. 1. A kinesin motor protein attached to a microtubule and a vesicle imaged by transmission electron microscopy. The image was adapted from N. Hirokawa's famous paper in Science magazine.[15]

Since the track for the motor – the microtubule – plays a critical role in the design of hybrid nanodevices powered by kinesin motors, a detailed understanding of the microtubule properties has proven to be critical in the design process. Figure 2 from the canonical textbook "Molecular Biology of the Cell" by B. Alberts et al. depicts the hierarchical organization of individual alpha and beta tubulin dimers (A) into protofilaments (B) and finally microtubules (C) with a diameter of 25 nm and a length of typically several micrometers[16].

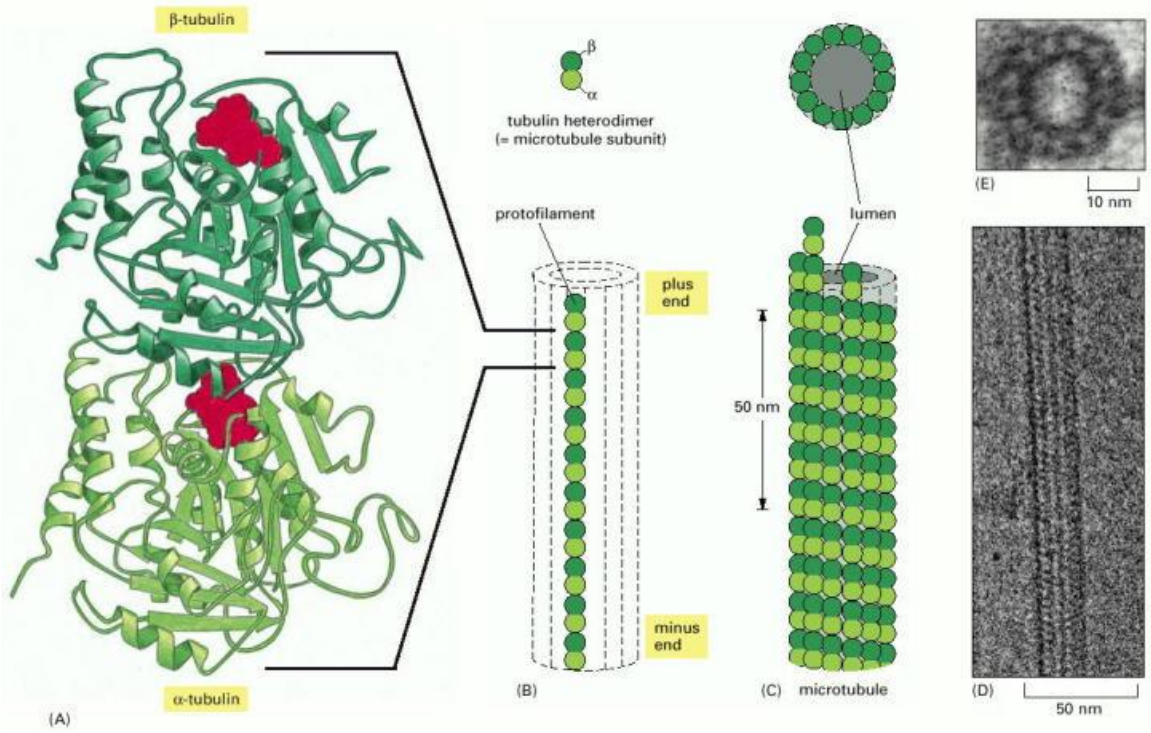

Fig. 2. The hierarchical organization of α,β-tubulins (A) into protofilaments (B) and microtubules (C). The characteristic structure can be observed by transmission electron microscopy (D,E). From Alberts et al. "The Molecular Biology of the Cell"[16].

Specifically, it has been shown that the stiffness of the microtubule, which results in a complex manner from the interaction of the thousands of tubulin subunits[17,18], determines the performance of kinesin/microtubule-based nanoscale transport systems (molecular shuttles)[19-27]. The relationship between physical properties of the microtubule and device performance merits a review by itself, however, the present paper does not attempt to discuss the details here.

While a number of hybrid devices replicates the natural approach of moving motors and stationary microtubules[28-37], an at least equally large number of studies[5,19-24,26,27,38-76] relies on the inverted geometry, in which the tails of the kinesin motors are attached to a surface and the swiveling heads of the kinesin motors bind to microtubules and propel them across the surface (Figure 3). The microtubules are polymerized in-vitro and stabilized against depolymerization with a paclitaxel solution.[77]

Similar considerations have to be made for the myosin/actin transport system, which offers advantages with respect to e.g. transport velocity, and is employed by several of research groups[8,78-98].

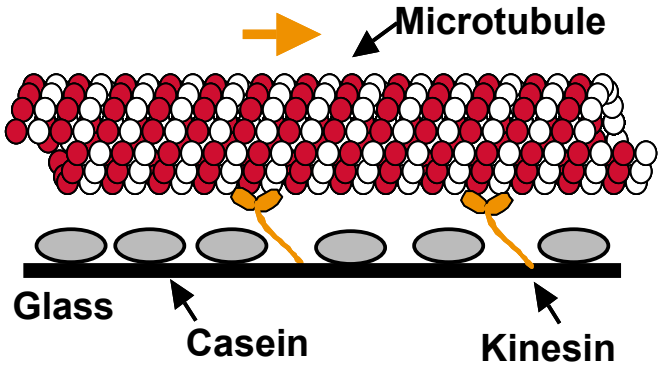

Fig. 3. The "inverted" or "gliding" motility assay relies on non-specifically adhered kinesin motors on a surface pre-coated with casein to translate microtubules.[99]

Optical imaging techniques have always played a key role in the elucidation of motor protein function. Initially, the imaging of fluorescently labeled microtubules was utilized to observe the actions of single motor proteins[100], effectively taking advantage of the large number of fluorophores carried by each microtubule. Lately, single molecule techniques have advanced to the point that individual motors – genetically fused with green fluorescent protein – can be imaged as they walk along their respective cytoskeletal filaments[101,102]. Single molecule microscopy is similarly valuable for the imaging of microtubules, where individual fluorophores attached to widely separated tubulin subunits can serve as markers for the microtubule growth dynamics (speckle microscopy[103])[104].

However, it is not necessary to employ the most cutting-edge techniques with spectacular sensitivity or Abbe-limit defying resolution[105] to realize the key benefits of fluorescence microscopy. Instead, as will be discussed in the next section, the ability to perform non-destructive, frequent measurements over hours has made fluorescence microscopy the central technique for the investigation of active nanosystems integrating motor proteins.

3. KINESIN-BASED MOLECULAR SHUTTLES

One of the molecular motor-based systems which are envisioned to enable a number of different applications is the "Molecular Shuttle", a nanoscale transporter (Fig. 4)[44]. Utilizing the above described gliding geometry, kinesin motor proteins are immobilized in tracks and propel microtubules functionalized with specific linkers for cargo transport. The activation of the motors is externally controlled, e.g. by controlling the availability of ATP.

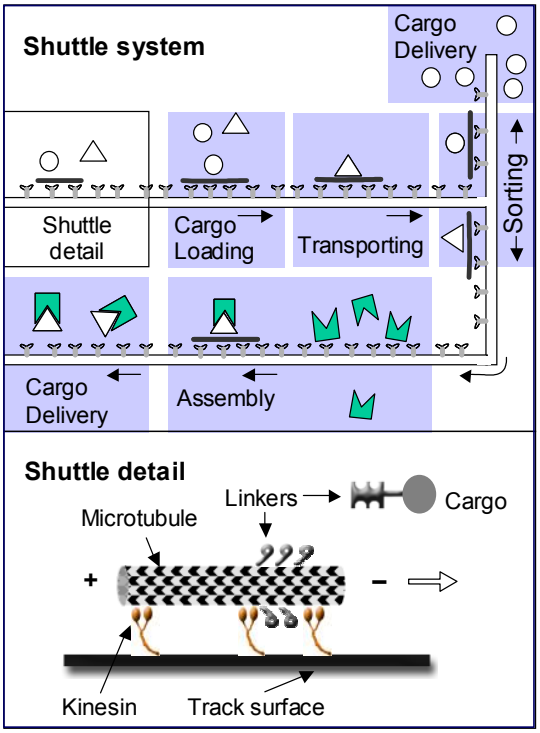

Fig. 4. The concept of a Molecular Shuttle. Reproduced with permission from ref. [61]. Copyright 2003, American Chemical Society.

This basic concept[58] has proven to be a rich source of interesting research questions in both, myosin/actin and kinesin/microtubule systems. Examples include the collective movement of such shuttles in tracks[24], as well as the computer-aided design of guiding structures[73].

An interesting example which illustrates how combining fluorescence microscopy and molecular shuttles enables new approaches to optical imaging is the utilization of molecular shuttles as self-propelled surface probes for Monte-Carlo Imaging of a surface (Fig. 5)[59]. In this technique, a surface with unknown topography is coated with kinesin motors, and the movement of fluorescently labeled microtubules on the surface is imaged over an extended time period, e.g. an hour. This process results in the accumulation of position measurements of hundreds of microtubules at hundreds of time points, which can be summarized in a map which displays the frequency with which a certain point on the surface is visited by a microtubule. On a planar surface, where microtubule movement is entirely random, this procedure results in a uniformly gray image overlayed by Poisson noise. However, if the surface topography or chemistry modifies the microtubule path, the image will begin to reflect the surface topography. It is critical to permit a sufficient observation time for the topography-related contrast to dominate the inherent noisiness of the sampling process.

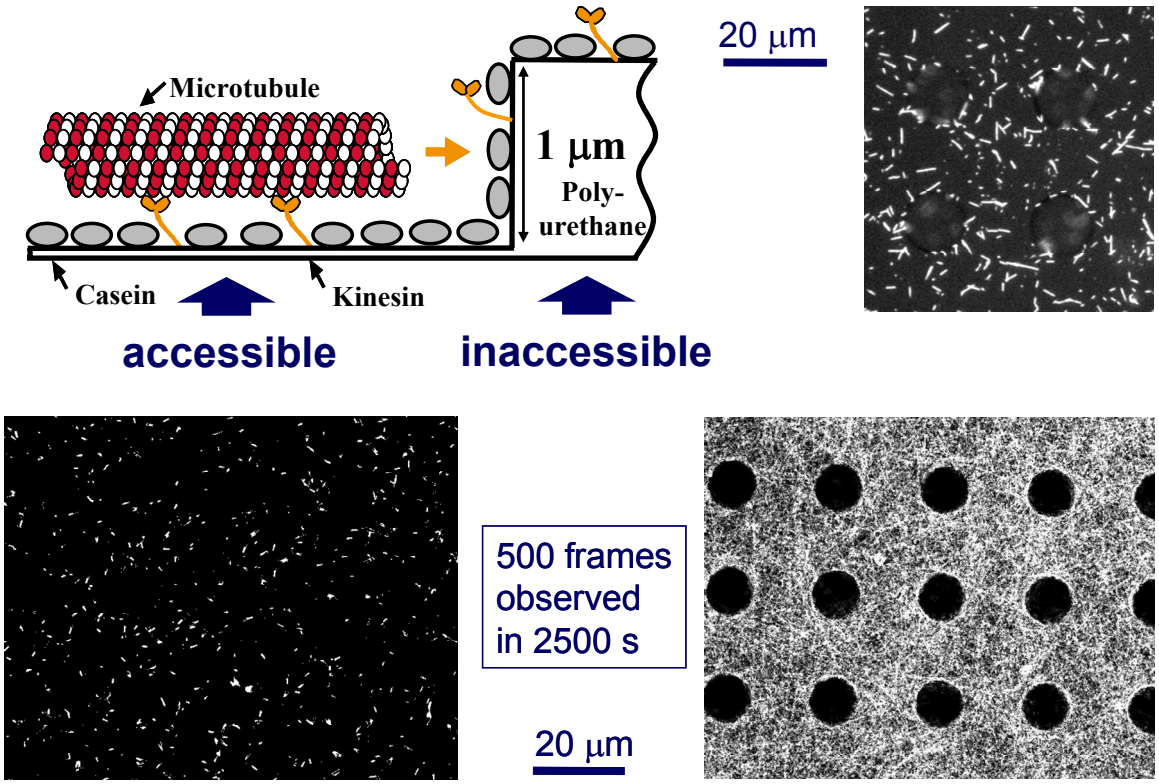

Fig. 5. Utilizing molecular shuttles as self-propelled nanoscale probes for the imaging of a surface with topographical contrast. Posts of 1 micrometer height were replica-molded[106] onto a surface. After coating the surface and posts with motor proteins, microtubules glide on the surface with a preference for avoiding climbing the posts. If microtubules reach the top surface of the post, they are out of focus and can be easily discriminated against by a thresholding filter. The resulting individual images (bottom left) are added to a composite map, which reveals the surface topography – in this case a pattern of posts with 10 micrometer diameter. Reproduced with permission from ref. [59]. Copyright 2003, American Chemical Society.

A second example, which illustrates the happy marriage between fluorescence imaging and molecular shuttles is the utilization of molecular shuttles as a platform for a double-antibody-sandwich (DAS) biosensor. By attaching biotinylated antibodies via streptavidin cross-links to biotinylated microtubules, these microtubules can capture viruses[69] and proteins[74] from dilute solutions. The presence of the respective antigen (or antigen presented by a virus) can be detected by a fluorescently labeled antibody (Fig. 6). Control experiments demonstrate the expected selectivity of this assay.

The significance of these experiment is that a DAS assay can be integrated not just onto a bead, which passively diffuses in the analyte solution, but to an actively moving detection platform, which is potentially able to seek out antigens and accelerate capture as demonstrated by bacteria[107].

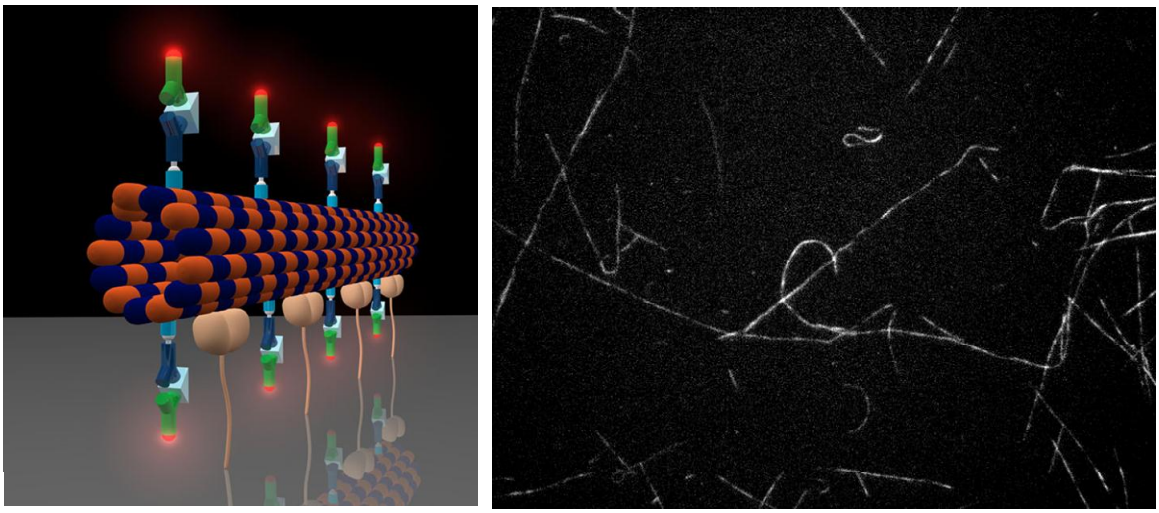

Fig. 6. Microtubules functionalized with antibodies (blue Y) can capture antigens (light-blue prism) from solution while being transported by kinesin motors. Fluorescent antibodies (green Y with red) can indicate the presence of the antigen as shown on the right. Adapted from [74].

The ability of microtubules to glide on a kinesin-coated surface despite being studded with streptavidin-antibody-antigen-antibody complexes is remarkable, given that the size of the each complex is on the order of 20 nm and that some of the microtubules must rotate due to their particular number of protofilaments[11]. This raises the question if larger cargo is also readily transported and what determines the efficiency of transport[108-110]. An interesting corollary intimately connected with optical methods is the question what the "ground clearance" of a molecular shuttle is. This question has been addressed by employing fluorescence interference contrast microscopy, and the approach and results are the topic of the next chapter.

4. FLUORESCENCE INTERFERENCE CONTRAST MICROSCOPY

The challenge in measuring the "ground clearance" of a molecular shuttle arises from the fact that the tails of the 10-50 kinesin motors holding a microtubule are extremely flexible structures with a fully stretched length of about 60 nm[111]. Consequently, the ground clearance has to be measured in the range of 0 to 60 nm with a resolution of less than 4 nm (the diameter of a typical globular protein) without exerting forces which could bias the measurement. Again, only optical techniques satisfy the key requirement of non-destructiveness. Atomic force microscopy has been employed to image microtubules adsorbed to surfaces[112,113], but significant forces are exerted which can even lead to the destruction of the microtubule lattice. Similarly, microtubule/kinesin/cargo complexes can be imaged by cryo-TEM (Fig. 1), but it is unclear if the kinesin length is affected by the staining and freezing process.

While optical microscopy satisfies the requirement of non-destructiveness, its limited resolution is often perceived to be a barrier in the measurement of nanometer distances. However, the Abbe-limit, which is typically larger than 200 nm, defines the minimal distance which two objects have to be separated by in order to be distinguishable. This limitation does not rule out the exact determination of the position of an isolated object, provided its shape is known[114]. For example, the fitting of the image of a point source (e.g. a quantum dot) with a Gaussian can be performed with an accuracy on the order of 1 nm, revealing distinct steps in the gliding of a microtubule on one, two or three kinesin motors[115]. However, measuring the ground clearance requires a precise measurement in the z-direction rather than in the xy-plane as is more frequently the case.

To this end we adapted a technique introduced by Lambacher and Fromherz in 1996 for the measurement of submicron distances in z-direction: Fluorescence Interference Contrast Microscopy[116-119]. This method was originally developed to measure the distance between a surface and the membrane of a neuron, a situation in which the membrane is extended in the xy-plane and can be imaged with a low-numeric aperture objective in contrast to the situation for microtubules

gliding on a motor-coated surface. The nanometer resolution of the method derives from its interferometric nature: The distance is encoded into a brightness variation, and a precise measurement of brightness can be translated into a precise measurement of the distance.

The principle of the technique is shown in Figure 7. The fluorescent object of interest – here microtubules – is placed above a reflecting surface (e.g. a silicon wafer), which serves as a reference plane. The reflected excitation and the reflected emission light interfere with the incoming excitation light and the light emitted towards the objective, respectively. A standing wave pattern forms, with a characteristic minimum of excitation and emission intensity directly at the reflecting surface, and a maximum at approximately a distance of a quarter wavelength. A fluorescent object of a given brightness will thus appear brighter or dimmer depending on its position relative to the surface. Transparent layers of silicon oxide, whose thickness can be accurately determined by ellipsometry, serve as spacers.

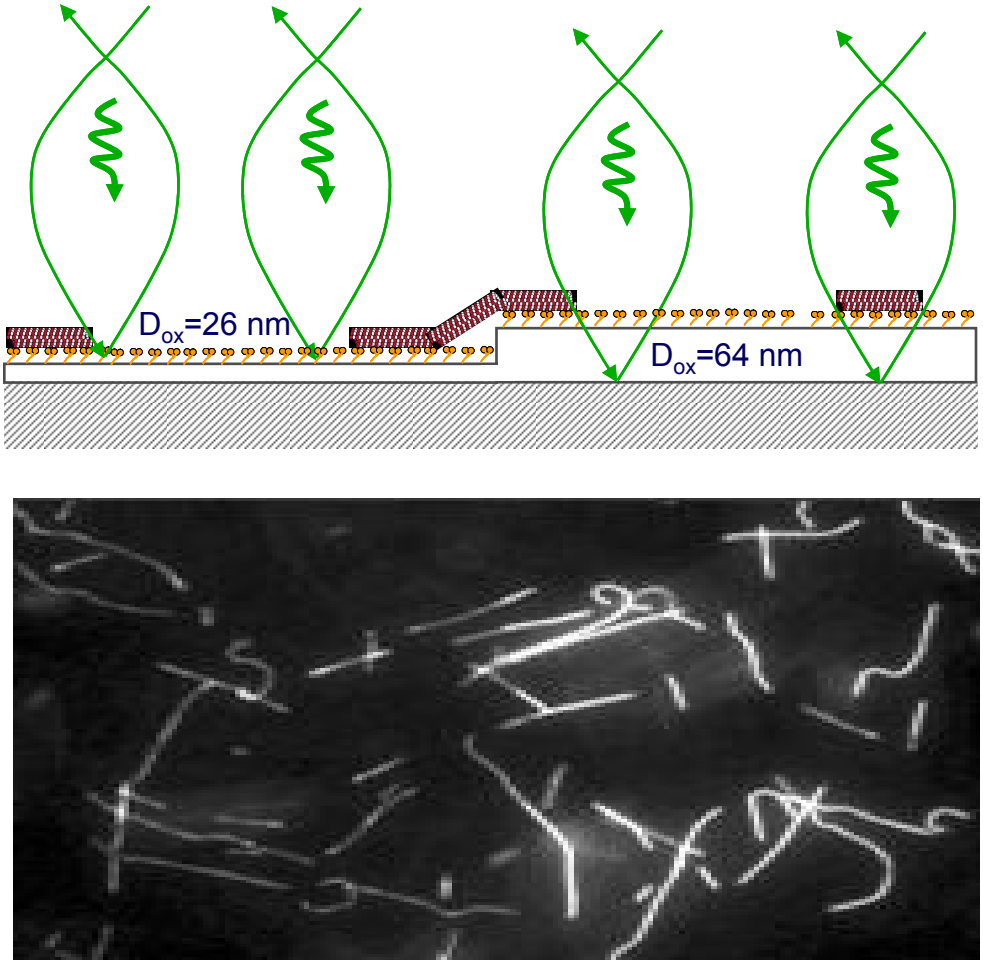

Fig. 7. The principle of Fluorescence Interference Contrast Microscopy. The standing wave pattern for the emitted light is not shown. In the bottom image, it can be seen how microtubules which ascend to the region of the thicker silicon oxide increase in brightness.

Jacob Kerssemakers et al. performed detailed measurements of the ground clearance of a kinesin/microtubule molecular shuttle using fluorescence interference contrast microscopy[56]. They found that the distance between the surface and the bottom of the microtubule is only 17+-2 nm. This result is surprising, since the distance is much smaller than the length of the kinesin molecule but confirms the observations obtained from transmission electron microscopy (Fig. 1) and from earlier measurements of the distance between the surface and microtubules held by single kinesin motors[120]. However, the value of 17 nm can be understood if the tail of kinesin motor is considered to be a freely jointed chain with the length of the segments given by the distances between particularly flexible regions in the coiled-coil tail.

5. SUMMARY

Biomolecular motors are fascinating nanomachines and enable the construction of nanodevices which integrate active movement. However, the design and iterative improvement of such hybrid devices requires analytical techniques with the necessary temporal and spatial resolution. However, it is the non-destructiveness of optical imaging which makes photons the unique tools in interrogating these systems. New approaches, such as Fluorescence Interference Contrast Microscopy enable us to combine millisecond time resolution and nanometers spatial resolution and elucidate key details of device operation.

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