DOI: 10.1002/smll.200500480

Nanoscopic Medicine: The Next Frontier

*Reiner Peters**

***T**he extraordinary progress that has taken place in cell science and optical nanoscale microscopy has led recently to the concept of medical nanoscopy. Here, we lay out a concept for developing live cell nanoscopy into a comprehensive diagnostic and therapeutic scheme referred to as nanoscopic medicine, which integrates live cell nanoscopy with the structural and functional studies of nanoscopic protein machines (NPMs), the systems biology of NPMs, fluorescent labeling, nanoscopic analysis, and nanoscopic intervention, in order to advance the medical frontier toward the nanoscopic fundament of the cell. It aims at the diagnosis and therapy of diseases by directly visualizing, analyzing, and modifying NPMs and their networks in living cells and tissues.*

Keywords:
- fluorescence microscopy
- live cell imaging
- medical nanoscopy
- nanomedicine
- protein machines

1. Progress in Science and Technology is Revolutionizing Medicine

Traditionally, the human cell is regarded as the one and only source of health and disease.[1] The essence of medicine is accordingly the diagnosis and therapy of the cell. However, striking progress in science and technology is revolutionizing medicine: The human genome has been sequenced, high-throughput techniques for the identification and sequencing of cellular proteins have been developed, classical resolution limits of optical microscopy have been broken, the creation of artificial nanometer-sized structures for medical applications has become feasible. Based on these extraordinary achievements, we have recently considered[2] possibilities for directly visualizing, analyzing, and modifying protein complexes in living cells and tissues. Here, we lay out a concept for developing live cell nanoscopy into a comprehensive diagnostic and therapeutic scheme referred to as nanoscopic medicine. Nanoscopic medicine is the pursuit of traditional medical goals on the nanometer level and should be clearly discriminated from "nanomedicine", that is, the application of nanotechnology to medical questions.

[*] Dr. R. Peters
Institute of Medical Physics and Biophysics
University of Münster
Robert-Koch-Strasse 31, 48149 Münster (Germany)
Fax: (+49) 251-835-5121
E-mail: Reiner.Peters@uni-muenster.de

2. Nanoscopic Protein Machines: Providing the Functional Basis of Cells

In recent years, progress in understanding cells has been tremendous. In particular, it has been found that approximately 80% of cellular proteins are organized within complexes.[3,4] Many protein complexes execute their tasks as if they were small machines.[5,6] A paradigm for the machine analogy is provided by the F-type ATPase (Figure 1). Drawing analogies between machines of everyday life and living organisms or, more recently, certain organs, cells, and protein complexes is not new – for a lucid and critical account, see the book by Harold.[7] However, such analogies should not be taken literally. In contrast to macroscopic machines, nanoscopic protein machines (NPMs) are primarily governed by thermal motion, molecular interactions, and friction.[8]

Another highly significant discovery made recently is that NPMs interact extensively with each other. NPMs cooperate in localized (e.g., ribosome) or dissipated (e.g., signal transduction) modules to exert specific, frequently highly complex tasks.[5,9] Moreover, functional modules form extended networks (Figure 2) occupying the whole cell.[10,11] Heuristically, the cell can be regarded as one huge NPM network.

3. Live Cell Imaging Extended into the Nanometer Domain

Since the nineteenth century, the resolution of the optical microscope was believed to be restricted to ≈ 220 nm.[12]

© 2006 Wiley-VCH Verlag GmbH & Co. KGaA, D-69451 Weinheim

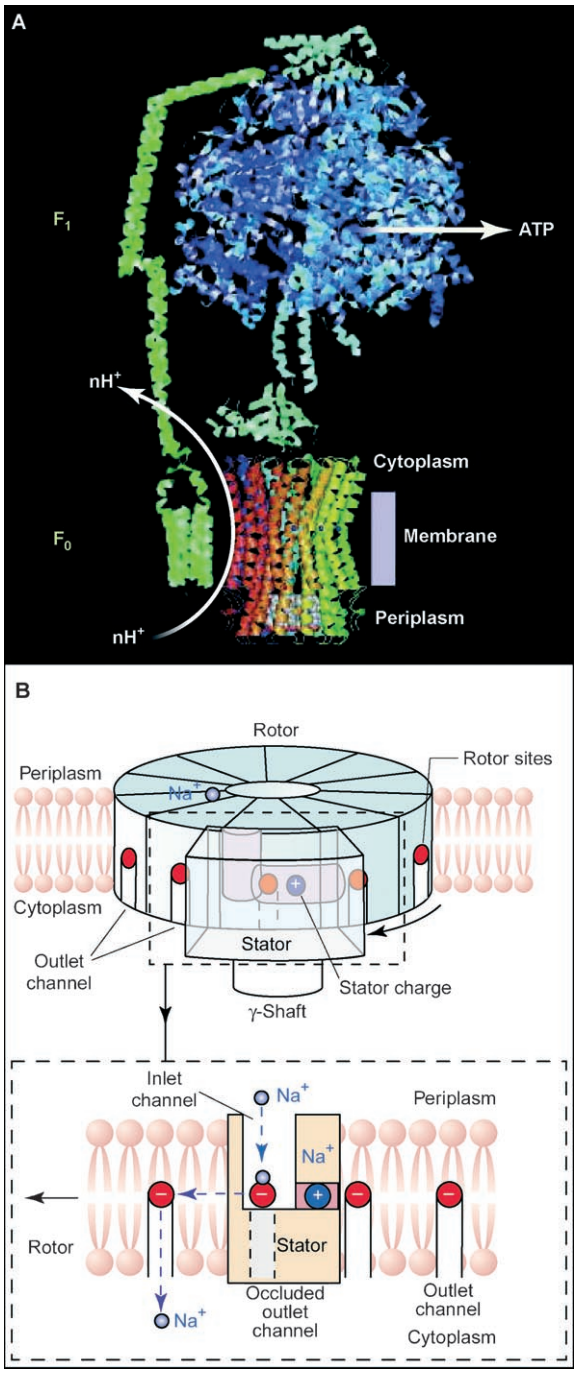


Figure 1. The F_0F_1-ATPase, a paradigm for nanoscopic protein machines. F_0F_1-ATPases are rotary motors that are driven by the transmembrane concentration difference of protons or sodium ions and convert rotary energy into chemical energy, that is, adenosine triphosphate (ATP). F_0F_1-ATPases can also run in reverse, hydrolyzing ATP and pumping ions. A) F_0F_1-ATPases of mitochondria and bacteria consist of two portions.[41] The F_0 portion is made up of a rod-shaped stator (subunits a and b) and a barrel-shaped rotor (10, 11, or 14 c-subunits). The barrel is inserted into the lipid bilayer, while the stator extends from the bilayer into the matrix/cytoplasm. The F_1 portion consists of three α- and three β-subunits, forming a hexamer. The hexamer is connected by a stalk (γ- and ε-subunits) to the center of the rotor. The δ-subunit of F_1 connects the α/β-hexamer of F_1 with the stator of F_0. B) Suggested mechanism[42,43] for the function of the F_0 portion of a bacterial F_0F_1-ATPase: 1) Thermal fluctuations carry a cation binding site on the rotor into the stator; 2) The rotor side is captured due to the attraction of the stator charge; 3) The rotor fluctuates out into the inlet channel; 4) The rotor site is neutralized by a sodium ion; 5) The rotor site loses its hydration shell and enters the hydrophobic membrane phase to the left; 6) The rotor site encounters the outlet channel and releases the sodium ion. (A) reprinted with permission from Ref. [41]. Copyright 2005, AAAS. (B) reprinted with permission from Ref. [43]. Copyright 2003, Elsevier.

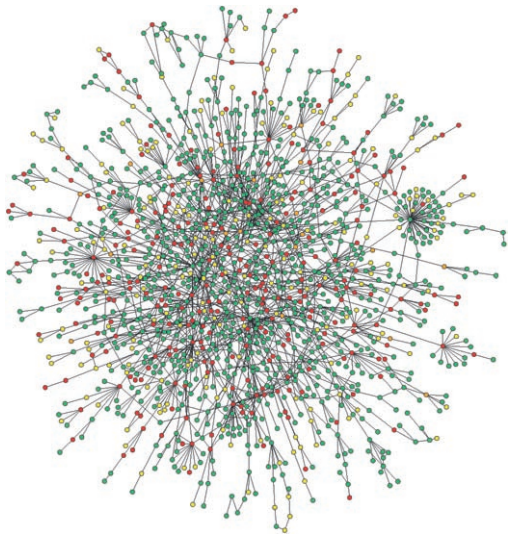
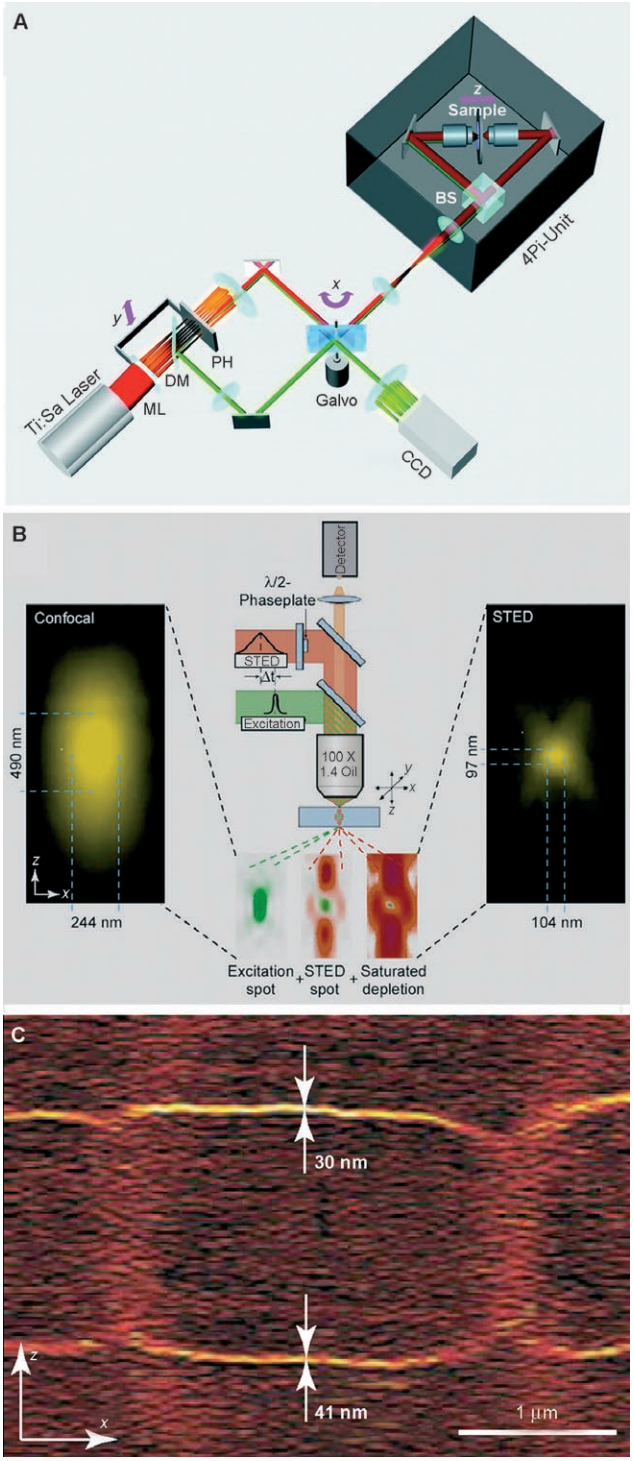


Figure 2. Nanoscopic protein machines form extended networks. Protein–protein interactions in yeast, measured by the two-hybrid technique, are displayed by a diagram in which nodes and lines represent proteins and their interactions, respectively. The diagram reveals that interactions are extensive and follow a hierarchical, nonrandom pattern. Reproduced with permission from Nature Reviews, Ref. [10]. Copyright 2004, Macmillan Magazines Ltd.

Recently, however, that limit has been overcome through the application of lasers, point scanning techniques, sensitive light detectors, and computers.[13] During the 1990s, the spatial resolution of fluorescence microscopy was substantially improved by confocal microscopy[14] and two-photon microscopy.[15,16] Both techniques provide a lateral resolution of ≈220 nm and a vertical resolution of ≈600 nm, thus allowing thick samples to be sectioned optically. The nanoscopic range first became accessible for fluorescence microscopy by single-molecule techniques.[17–19] Although single molecules are not resolved by these techniques, rather imaged as diffraction-limited spots of about 220 nm diameter, their positions can be determined by curve fitting with high accuracy.[20,21] In living cells, localization accuracies of 20–30 nm have been obtained at a time resolution of a few milliseconds.[22,23]

A true increase in resolution, improving[24,25] and eventually breaking[26,27] classical limits, was recently achieved by 4Pi microscopy[24] (Figure 3A) and stimulated emission depletion (STED) microscopy[26] (Figure 3B). In theory, STED microscopy can yield infinite resolution. In living cells, a resolution of 30 nm[28] has been achieved so far (Figure 3C). Recently, a commercial 4Pi microscope employing beam scanning instead of object scanning has become available.[29]

© 2006 Wiley-VCH Verlag GmbH & Co. KGaA, D-69451 Weinheim



4. Nanoscopic Medicine: The Emergence of a New Medical Specialty

Most diseases, regardless of their various and frequently highly complex etiologies, manifest themselves as defects of cellular proteins. Taking the recent insight into the organization of the proteome into consideration, this means that most diseases result in defects of NPMs and their networks. Assuming that normal human cells contain ≈50000 different proteins and that the average number of protein molecules per complex is ≈10, the number of different physiological protein complexes is estimated to be ≈5000. Furthermore, taking the abundance of pathological protein variants into consideration, the number of protein complexes yet to be analyzed is virtually infinite. Currently, however, the identification and characterization of NPMs is a long and difficult process involving a number of experimental and computational steps.[30] This could be dramatically simplified and enhanced[2] by live cell nanoscopy employing just four consecutive steps: fluorescent labeling, nanoscopy, nanoscopic analysis, and nanoscopic intervention.

The application of live cell nanoscopy to medical questions is, however, more than just another approach to the analysis of NPMs. It absolutely requires, as we believe, the establishment and rapid development of a new specialty, nanoscopic medicine (Figure 4). Nanoscopic medicine integrates nanoscopy with systems biology, functional proteomics, fluorescent labeling, nanoscopic analysis, and nanoscopic modification, and thus is characterized by a high level of complexity and an extraordinary degree of interdisciplinary cooperation. The target of nanoscopic medicine is that of traditional medicine, the diagnosis and therapy of the cell. However, the frontier is moved deep inside the cell towards its nanoscopic fundament.

Nanoscopic medicine must be clearly distinguished from applications of nanotechnology to medical questions, frequently referred to as "nanomedicine".[31,32] Although phonetically similar, nanoscopic medicine and nanomedicine represent opposing approaches. The roots of nanoscopic medicine lie, as described, in medicine while those of nanomedicine are found in nanotechnology. Nanoscopic medicine seeks to analyze and improve the nanoscopic fundament of the cell while nanomedicine aims at the creation of artificial nanometer-sized devices such as particles, tubes, or sieves, if not electromechanical robots, for diagnostic and therapeutic purposes.

Figure 3. Extending live cell studies to the nanometer domain. A) In 4Pi microscopy[44] the infrared beam of a titanium–sapphire (Ti:Sa) laser is directed into the 4Pi-unit and, using the beam splitter (BS), divided into two beams of equal amplitude and phase. The beams are focused by two opposing objectives into the sample. In the common focus, the laser spot that determines optical resolution is reduced by interference to a width of 100 nm in the direction of the optical axis. B) In stimulated emission depletion (STED) microscopy,[28] the pulse of a laser beam (green beam) is focused into the sample and employed to excite fluorescence at a particular spot of the specimen. During the few nanoseconds that the fluorophores are in the excited state, a second laser pulse (red) is delivered which has a ringlike focus. The ringlike pulse is adjusted such that the spot excited by the first laser pulse is sharpened by means of stimulated emission. Thus, the excited spot is trimmed down yielding, in principal, unlimited resolution. C) Combining STED with 4Pi microscopy,[45] a bacterial cell in which the plasma membrane was stained by a fluorescent lipid analog was imaged, obtaining a resolution of ≈30 nm. (A) reprinted from Ref. [44]. Copyright 2002, The National Academy of Sciences, USA. (B) and (C) reprinted with permission from Ref. [28]. Copyright 2004, Elsevier.

 © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, D-69451 Weinheim

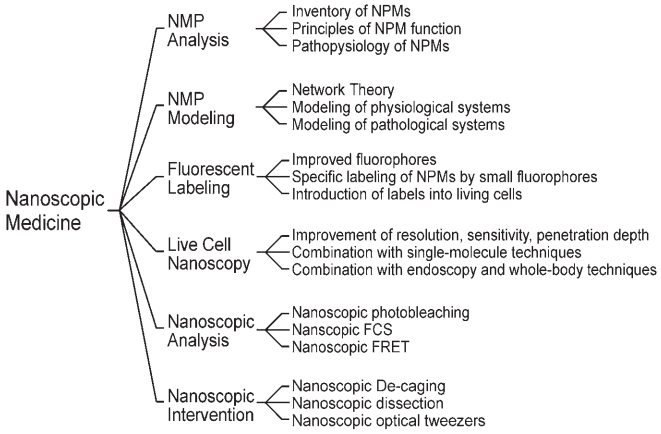


Figure 4. Taxonomy of nanoscopic medicine. Nanoscopic medicine is understood in this article as an emerging specialty, which aims at the diagnosis and therapy of diseases by directly visualizing, analyzing, and modifying single nanoscopic protein machines in living cells and tissues. In order to accomplish this aim, nanoscopic medicine integrates diverse fields of the natural sciences, technology, and medicine.

5. Further Development of Nanoscopic Medicine

Nanoscopic medicine is an emerging discipline that needs massive development before it can be incorporated into medical practice. Considering Figure 4, it is obvious that many of the fields mentioned and their branches are in an early state. Thus, a comprehensive inventory of NPMs and their pathological variants has yet to be established. Only then, general principles underlying the structure and function of NPMs can be established and the effect of pathological condition can be understood. This will also lead to a specification and qualification of the machine analogy.

Concerning network theory, the mathematical modeling of complete cells is currently out of reach. The list of involved components and interactions is far from completion, and mathematical methods for handling extremely large networks have yet to be developed.[11]

There has been substantial progress in the specific fluorescent labeling of NPMs in living cells and tissues. However, current approaches[33] require genetic manipulation and involve rather large fluorophores. The resolution of live cell nanoscopy is currently limited to $\approx$30 nm. An inspection of NPMs for which atomic models are available reveals, however, that NPMs typically have dimensions of 10–100 nm. Exceptions such as the nuclear pore complex (125 nm by 250 nm)[34] exist. This requires an improvement of resolution to 5–10 nm. The application of optical nanoscopy to living tissues and, eventually, the entire human body is clearly a tremendous challenge. Here, promising progress has been made in "molecular imaging".[35] But its resolution is currently far from nanoscopic. The amalgamation of nanoscopy with endoscopy appears feasible but has yet to be accomplished.

A number of techniques for the quantitative analysis of molecular processes in microscopic systems have already been established. However, techniques such as fluorescence microphotolysis (fluorescence photobleaching),[36] fluorescence correlation spectroscopy,[37] and fluorescence resonance energy microscopy[38] have yet to be adapted to the nanometer scale. Similarly, techniques for the photochemical and photomechanical manipulation of microscopic systems are available,[39,40] but have yet to be adapted to the nanoscale.

6. Concluding Remarks

In this article, a concept for a novel specialty, nanoscopic medicine, has been outlined. Nanoscopic medicine integrates live cell nanoscopy with structural and functional studies of NPMs, systems biology of NPMs, fluorescent labeling, nanoscopic analysis, and nanoscopic intervention. It aims at the diagnosis and therapy of diseases by directly visualizing, analyzing, and modifying NPMs and their networks in living cells and tissues. Nanoscopic medicine is at an early stage. Massive interdisciplinary efforts are required before it can be incorporated into medical practice.

Acknowledgements

Supported by the National Institute of Health, grant GM071329, and Deutsche Forschungsgemeinschaft, grant PE 138/19.

[1] R. Virchow, *Cellularpathologie*, Hirschfeld, Berlin, **1862**.
[2] R. Peters, *Trends Molec. Med.*, Online publication date: January 10, 2006, DOI:10.1016/j.molmed.2005.12.002.
[3] Y. Ho, A. Gruhler, A. Heilbut, G. D. Bader, L. Moore, S. L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, L. Y. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A. R. Willems, H. Sassi, P. A. Nielsen, K. J. Rasmussen, J. R. Andersen, L. E. Johansen, L. H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen, B. D. Sorensen, J. Matthiesen, R. C. Hendrickson, F. Gleeson, T. Pawson, M. F. Moran, D. Durocher, M. Mann, C. W. V. Hogue, D. Figeys, M. Tyers, *Nature* **2002**, *415*, 180–183.
[4] A. C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A. M. Michon, C. M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M. A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, *Nature* **2002**, *415*, 141–147.
[5] B. Alberts, *Cell* **1998**, *92*, 291–294.
[6] C. Mavroidis, A. Dubey, M. L. Yarmush, *Annu. Rev. Biomed. Eng.* **2004**, *6*, 363–395.
[7] F. M. Harold, *The Way of the Cell*, Oxford University Press, New York, **2001**, pp. 1–305.
[8] A. Kurakin, *Biol. Chem.* **2005**, *386*, 247–254.
[9] L. H. Hartwell, J. J. Hopfield, S. Leibler, A. W. Murray, *Nature* **1999**, *402*, C47–C52.

 © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, D-69451 Weinheim

[10] A. L. Barabasi, Z. N. Oltvai, *Nat. Rev. Genet.* **2004**, *5*, 101 – 113; H. Jeong, S. P. Mason, A. L. Barabasi, Z. N. Oltvai, *Nature*, **2001**, *411*, 41 – 42.
[11] A. Ma'ayan, R. D. Blitzer, R. Iyengar, *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 319 – 349.
[12] E. Abbe, *Arch. Mikrosk. Anat.* **1873**, *9*, 413 – 487.
[13] A. Egner, S. W. Hell, *Trends Cell Biol.* **2005**, *15*, 207 – 215.
[14] P. M. Conn, *Confocal Microscopy*, Academic Press, San Diego, **1999**, pp. 1 – 663.
[15] W. Denk, J. H. Strickler, W. W. Webb, *Science* **1990**, *248*, 73 – 76.
[16] M. Rubart, *Circ. Res.* **2004**, *95*, 1154 – 1166.
[17] W. E. Moerner, *J. Phys. Chem. B* **2002**, *106*, 910 – 927.
[18] X. Michalet, A. N. Kapanidis, T. Laurence, F. Pinaud, S. Doose, M. Pflughoefft, S. Weiss, *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 161 – 182.
[19] U. Kubitscheck, *Single Mol.* **2002**, *3*, 267 – 274.
[20] U. Kubitscheck, O. Kuckmann, T. Kues, R. Peters, *Biophys. J.* **2000**, *78*, 2170 – 2179.
[21] R. J. Ober, S. Ram, E. S. Ward, *Biophys. J.* **2004**, *86*, 1185 – 1200.
[22] T. Kues, A. Dickmanns, R. Luhrmann, R. Peters, U. Kubitscheck, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12021 – 12026.
[23] U. Kubitscheck, D. Grünwald, A. Hoekstra, D. Rohleder, T. Kues, J. P. Siebrasse, R. Peters, *J. Cell Biol.* **2005**, *168*, 233 – 243.
[24] S. Hell, E. H. K. Stelzer, *Optics Comm.* **1992**, *93*, 277 – 282.
[25] B. Bailey, D. L. Farkas, D. L. Taylor, F. Lanni, *Nature* **1993**, *366*, 44 – 48.
[26] S. W. Hell, J. Wichmann, *Optics Lett.* **1994**, *19*, 780 – 782.
[27] M. G. L. Gustafsson, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13081 – 13086.
[28] S. W. Hell, M. Dyba, S. Jakobs, *Curr. Opin. Neurobiol.* **2004**, *14*, 599 – 609.
[29] T. Szellas, T. Zapf, V. Nickl, *Photonik* **2005**, *5*, 60 – 62.
[30] A. Sali, R. Glaeser, T. Earnest, W. Baumeister, *Nature* **2003**, *422*, 216 – 225.
[31] K. E. Drexler, *Engines of Creation. The Coming Era of Nanotechnology*, Anchor Books, New York, **1986**, pp. 1 – 298.
[32] R. A. Freitas, *J. Comp. Theor. Nanosc.* **2005**, *2*, 1 – 25.
[33] R. Y. Tsien, *FEBS Lett.* **2005**, *579*, 927 – 932.
[34] R. Peters, in *Xenopus Protocols: Cell Biology and Signal Transduction* (Ed.: X. J. Liu), Humana Press, Totowa, NJ, **2005**.
[35] V. Ntziachristos, J. Ripoll, L. H. V. Wang, R. Weissleder, *Nat. Biotechnol.* **2005**, *23*, 313 – 320.
[36] R. Peters, J. Peters, K. H. Tews, W. Bahr, *Biochim. Biophys. Acta* **1974**, *367*, 282 – 294.
[37] D. Magde, E. L. Elson, W. W. Webb, *Biopolymers* **1974**, *13*, 29 – 61.
[38] E. A. Jares-Erijman, T. M. Jovin, *Nat. Biotechnol.* **2003**, *21*, 1387 – 1395.
[39] R. H. Singer, D. S. Lawrence, B. Ovryn, J. Cordeelis, *J. Biomed. Optics* **2005**, *10*, 1 – 9.
[40] Y. G. Lee, K. W. Lyons, T. W. Lebrun, *J. Res. Natl. Inst. Stand. Technol.* **2003**, *108*, 275 – 287.
[41] W. Junge, N. Nelson, *Science* **2005**, *308*, 642 – 644.
[42] S. Engelbrecht, W. Junge, *FEBS Lett.* **1997**, *414*, 485 – 491.
[43] G. Oster, H. Y. Wang, *Trends Cell Biol.* **2003**, *13*, 114 – 121.
[44] A. Egner, S. Jakobs, S. W. Hell, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3370 – 3375.
[45] L. Kastrup, H. Blom, C. Eggeling, S. W. Hell, *Phys. Rev. Lett.* **2005**, *94*, 178104-1–178104-4.

Received: December 2, 2005

 © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, D-69451 Weinheim