

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

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Preface

Welcome

It is a pleasure for us to welcome you to the XIVth Workshop on Scanning Probe Microscopy and Organic Materials here in Munich. The goal of this workshop is to bring together young materials scientists, physicists, biologists and engineers from all over the world working on different questions related to nanoscale organic materials. There will be sessions on biology and biology-oriented aspects of materials science such as biomaterials and biomineralization. Other sessions will be devoted to organic adsorbates, tribological and optical properties of nanoscale materials. Cross sectional aspects will be addressed in the nanobiotechnology session. The different sessions are cross-linked by the common nanoscientist's need to see, quantify and assemble matter on the nanometer scale. For this purpose scanning probe microscopy is the most prominent nanotechnology tool.

We hope that you will enjoy stimulating and fruitful discussions and that you will relish the atmosphere of the Deutsches Museum and of the city of Munich as it prepares for a very special time of the year.

We would like to thank the sponsors who have contributed greatly to making this workshop possible. We are also indebted to the Excellence Network NanoBioTechnology (ENNaB) and the Deutsches Museum in Munich for their support and commitment to this event. Finally, we thank the members of the advisory board for their support and numerous people at the University of Munich and at the Deutsches Museum for their helping hands.

Munich, 14th September 2005



Dr. Robert Stark
Scientific Program



Prof. Wolfgang Heckl
Host



Dr. André Kempe
Website & Registration

Organization

Organization

Dr. Robert Stark, Ludwig-Maximilians-University Munich

Prof. Dr. Wolfgang M. Heckl, Deutsches Museum and Ludwig-Maximilians-University Munich

Dr. André Kempe, Science PR

ENNaB, Excellence Network NanoBioTechnology, Munich

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Program	Talks
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Wednesday, 14 September 2005

10:00-12:00 Registration

12:00-12:10 Welcome & Introduction in Ehrensaal R.W. Stark, W.M. Heckl

Biomaterials

12:10-12:30	AFM stethoscopy: A new diagnostic tool?	M. Bauer (LMU)	p. 24
12:30-12:50	Investigation of the structural and physical properties of synthetic amyloid fibrils	P. Mesquida (UCL)	p. 45
12:50-13:10	SFM imaging of single molecules of atactic poly(sodium 4-styrenesulfonate) adsorbed from ethanol-water solutions	N. Severin (HU Berlin)	p. 51
13:10-13:30	Cooperativity in coiled coil formation studied by molecule force spectroscopy	T. Bornschlöggl (TUM)	p. 27
13:30-13:50	Temperature softening of a protein in single-molecule experiments	M. Schlierf (TUM)	p. 48

Nanobiotechnology

13:50-14:30	Invited: Nanopatterning by induced controlled dewetting of supramolecular thin films by atomic force microscopy	M. Cavallini (ISMN Bologna)	p. 18
14:30-15:10	Coffee break		

Biomineralization

15:10-15:30	Hydrothermal atomic force microscopy	G. Jordan (LMU)	p. 41
15:30-15:50	Micro-thermal analysis: A novel approach for nanomedicine and bionanotechnology	L. Bozec (UCL)	p. 28
15:50-16:10	Scanning force microscopy and spectroscopy in dentistry	N. Schwender (TU Kaiserslautern)	p. 50
16:10-16:30	Insights into the structural properties of collagen fibres using AFM	M. Wenger (UCL)	p. 54
16:30-16:50	A new AFM chemical dissection technique provides insights into the origins of bone fracture resistance	J. Kindt (UCSB)	p. 43

Nanobiotechnology

16:50-17:30	Invited: Nanodevices based on DNA aptamers	F. Simmel (LMU)	p. 22
18:15-19:15	Special tour in the museum	W.M. Heckl	

Program	Talks
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Thursday, 15 September 2005 (morning)

Biology

09:10-09:30	MAC mode imaging and molecular recognition force microscopy of functionalized s-layer proteins under near physiological conditions	A. Ebner (JKU Linz)	p. 34
09:30-10:10	Invited: Single molecule interactions detected on a surface of cancer cells	M. Lekka (Polish Acad. Sci.)	p. 20
10:10-10:30	A new way of SFM manipulation of ds-DNA on surfaces	W. Zhuang (HU Berlin)	p. 55
10:30-10:50	The native photosynthetic apparatus of <i>Phaeospirillum molischianum</i>	R. Goncalves (I Curie)	p. 37
10:50-11:10	Determination of the elastic properties of <i>Dictyostelium</i> cells using the atomic force microscopy	B. Haupt (UCL)	p. 38
11:10-11:40	Coffee break		

Organic Adsorbates

11:40-12:00	Noise Budget in very low current Scanning Tunneling/ElectroChemical Microscopy	M. Carlá (U Florence)	p. 31
12:00-12:20	Structure and bonding of complex organic adsorbates investigated by STM and STS	R. Temirov (IUB)	p. 53
12:20-12:40	Bimolecular hydrogen-bond networks - mediated coadsorption and networks with in situ tunable cavity size	L. Kampschulte (LMU)	p. 42
12:40-14:00	Lunch		

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Program	Talks
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Thursday, 15 September 2005 (afternoon)

Industry Session

14:00-14:20	High-Resolution Imaging and Chemical Characterization of Heterogeneous Materials with the Confocal Raman AFM	Ute Schmidt (WITec GmbH)	p. 49
14:20-14:40	Piezo Technology for Scanning Applications	Konstantin Jerger (PI GmbH)	p. 40
14:40-15:00	AFM in Liquid Environment simultaneous to Advanced Optical Microscopy	Gerd Behme (JPK AG)	p. 25

15:00 - 17:00 Poster Session + Coffee

Nanobiotechnology

17:00-17:40	Invited: Biomolecular assemblies studied by AFM	F. Kienberger (JKU Linz)	p. 19
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Tribology

17:40-18:00	Investigating lateral forces in supported lipid bilayers and protein lipid interactions using nanomechanical cantilevers	I. Pera (IUB)	p. 47
18:00-18:20	Investigation of the statistics of stick-slip friction on graphite	L. Jansen (WWU)	p. 39
18:20-18:40	AFM morphology studies on roughness gradient replicas	T. Drobek (ETHZ)	p. 33
18:40-19:00	SPM as a Surface Analytical Technique in Conservation Science	U. Binder (FHR)	p. 26

19:30 Conference Dinner (in Flughalle)

Program	Talks
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Friday, 16 September 2005

Optical Properties

09:10-09:30	Ultrafast energy transfer processes in highly doped PMMA films	S. Lochbrunner (LMU)	p. 44
09:30-09:50	STM induced electroluminescence measured by a transparent ITO tip	R. Branscheid (MPI)	p. 29
09:50-10:10	Infrared spectroscopy of a single virus made possible by near-field microscopy	M. Brehm (MPI)	p. 30
10:10-10:30	Surface plasmons on metallic nanostructures fabricated by using colloidal gold nanoparticles	M. Goncalves (U Ulm)	p. 36
10:30-11:00	Coffee break		

Nanobiotechnology

11:00-11:40	Invited: High-speed atomic force microscopy	G. Schitter (UCSB)	p. 21
11:40-12:00	Protein Structure by Mechanical Triangulation	H. Dietz (TUM)	p. 32
12:00-12:20	Replicating Fischer Patterns by micro contact printing	A. Gigler (U Ulm)	p. 35
12:20-12:40	Electrical AFM based definition of anchor points for layered biomolecular structures	N. Naujoks (ETHZ)	p. 46
12:40-13:00	AFM and Forensics	S. Strasser (LMU)	p. 52
13:00-13:15	Concluding remarks	R. Stark	

Lunch

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Program

Posters

Poster session: Thursday, 15 September 2005

Posters P1-P20

P1	Dehydration damage on raft-exhibiting supported bilayers: effects of disaccharides and other stabilizing substances	S. Chiantia (TU Dresden)	p. 63
P2	The microstructure of the lingulid brachiopods <i>Discradisca</i> , <i>Discinisca</i> and <i>Lingula</i>	C. Merkel (RU Bochum)	p. 79
P3	The CellHesion - quantifying adhesion forces between single cells	G. Behme (JPK)	p. 60
P4	Pulling adsorbed polymers from surfaces with the AFM: Influence of polymer-surface friction	A. Serr (TUM)	p. 85
P5	Double-layers of bis-di(4-methoxyphenyl)amino-substituted hexa-peri-hexabenzocoronene studied by STM and STS at the liquid/solid interface	M. Ai (HU Berlin)	p. 58
P6	Functional Nanostructures for Electronic Devices with Macrocylic Oligothiophenes	F. Sperka (U Ulm)	p. 86
P7	Modification and imaging of surface charges on polymers using atomic force microscopy	A. Kleiner (U Ulm)	p. 76
P8	High resolution scanning electrochemical microscopy of dna single molecules on mica	G. Aloisi (U Florence)	p. 59
P9	Lateral structuring of oxide surfaces by microcontact printing - A recipe for the reproducible generation of Fischer Projection Pattern like structures	C. Gnahn (U Ulm)	p. 67
P10	Frequency dependence of mechanical properties of thin polymer films	M. Holzwarth (U Ulm)	p. 72
P11	AFM Investigation of Tubular J-Aggregates Decorated with Ag Nanoparticles	D. M. Eisele (HU Berlin)	p. 65
P12	Towards Automatisation of Nanotomography Imaging	C. Dietz (TU Chemnitz)	p. 64
P13	Controlled positioning of biomolecules with AFM	R. Janissen (HHU Düsseldorf)	p. 75
P14	Stand-alone viscosimeter based on a microcantilever and an array detector	A. Winnemöller (CenTech)	p. 93
P15	Hydrophobic recovery of SU-8 after O ₂ -plasma treatment	F. Walther (LMU)	p. 89
P16	Structural studies of oligonucleotides containing G-quadruplex motifs using molecular modeling	F. Jamitzky (MPI)	p. 74
P17	Study of the rehydration process of metaphase chromosomes by high time resolution AFM tracking	T. Heil (CenTech)	p. 69
P18	Probing of the Proteasome-Protein Interaction with Force Spectroscopy	M. Beuttler (MPI)	p. 62

Posters P21-P34

P19	One Step ahead – Combining AFM and Optical Spectroscopy	V. Walhorn (U Bielefeld)	p. 91
P20	Solid-wetting self-assembly	F. Trixler (LMU)	p. 88
P21	Glass tips completely coated by a thin metal layer - a powerful alternative to aperture SNOM probes	H. G. Frey (U Bielefeld)	p. 66
P22	Tapered pipettes as AFM probes for use in SICM	J. Jägers (CenTech)	p. 73
P23	Intermixed patterns of perylene derivates on Ag(111)	T. Samuely (U Basel)	p. 82
P24	Scanning Ion Conductance Microscopy with Shear-Force Distance Control	T. Schäffer (CenTech)	p. 83
P25	Dynamic Imaging of single DNA-protein interaction using the Torsional Resonance Mode AFM	A. Yurtsever (LMU)	p. 94
P26	High-speed AFM for imaging fast biological processes	A. Grützner (CenTech)	p. 68
P27	Imaging Structural Discontinuities in Myelinated Axons: An Approach to the Properties of Cajal Bands	A. Heredia (CenTech)	p. 70
P28	AFM measures of encapsulated Spondylosium panduriforme alga	R. Bernardes-Filho (U São Paolo)	p. 61
P29	Structural and functional investigation of nuclear pore complex transport mechanisms with atomic force microscopy	A. Sedivy (JKU Linz)	p. 84
P30	Probing the interaction between vesicular stomatitis virus and phosphatidylserine	G. Weissmuller (U Rio de Janeiro)	p. 92
P31	Lithography of polymer thin films by acoustic force microscopy	F. J. Rubio-Sierra (LMU)	p. 81
P32	Frequency Response of magnetically driven cantilever in liquids	J. Preiner (JKU Linz)	p. 80
P33	Combined optical and force microscopy	J. Madl (JKU Linz)	p. 78
P34	Scanning probe microscope for the international space station ISS	P. Hix (LMU)	p. 71
P35	AFM investigations on bioengineered membrane channels	A. Kronenberger (IUB)	p. 77
P36	Nanoantennas and ultrafast spectroscopy: Towards time-resolved near-field optical microscopy	M. Stark (EPFL)	p. 87

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Scientific Program



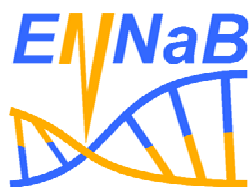
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Host

The logo for the Deutsches Museum München, featuring the text 'Deutsches Museum' in a red serif font.

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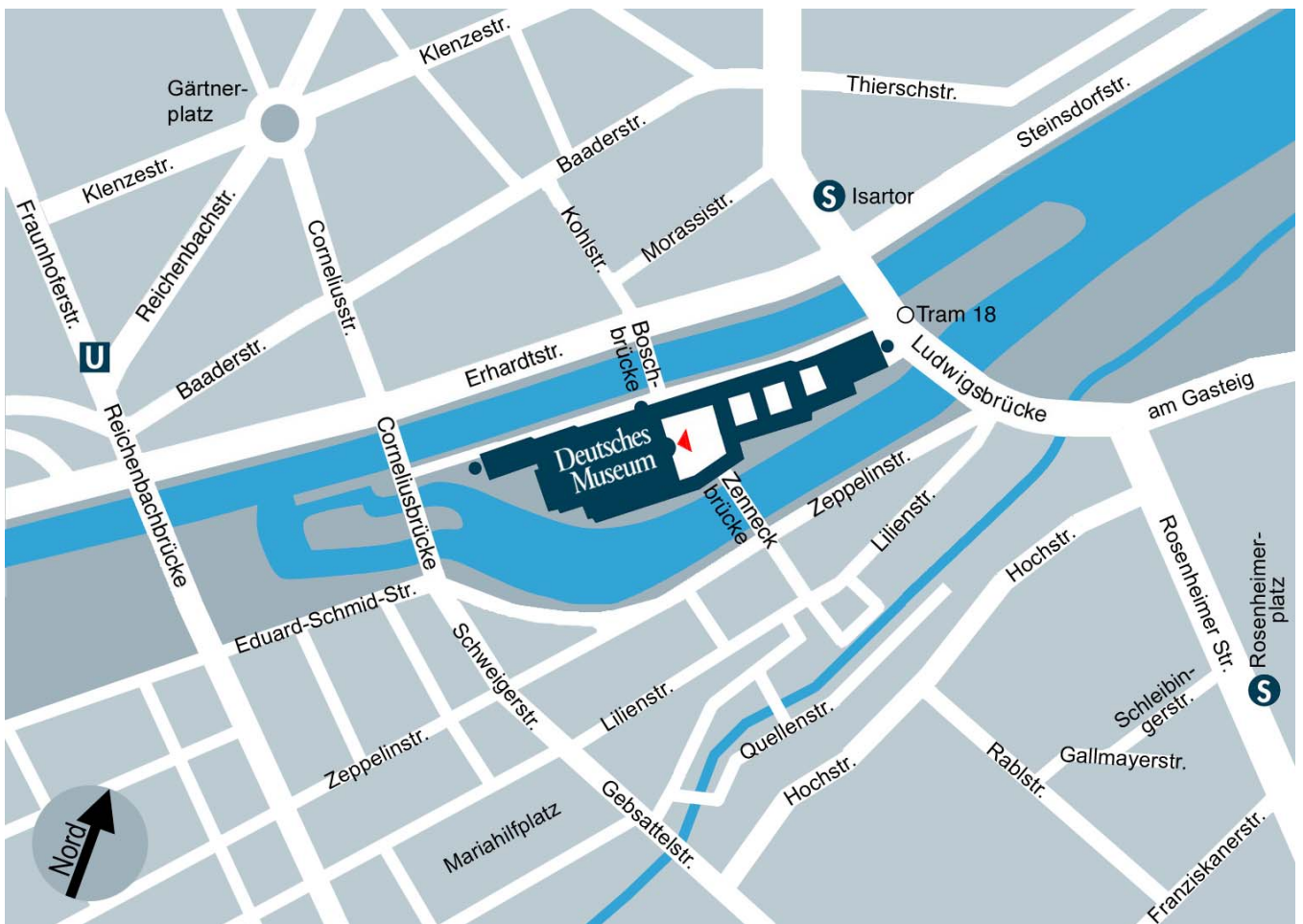
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Location

Deutsches Museum - Neighborhood

Neighborhood of Deutsches Museum

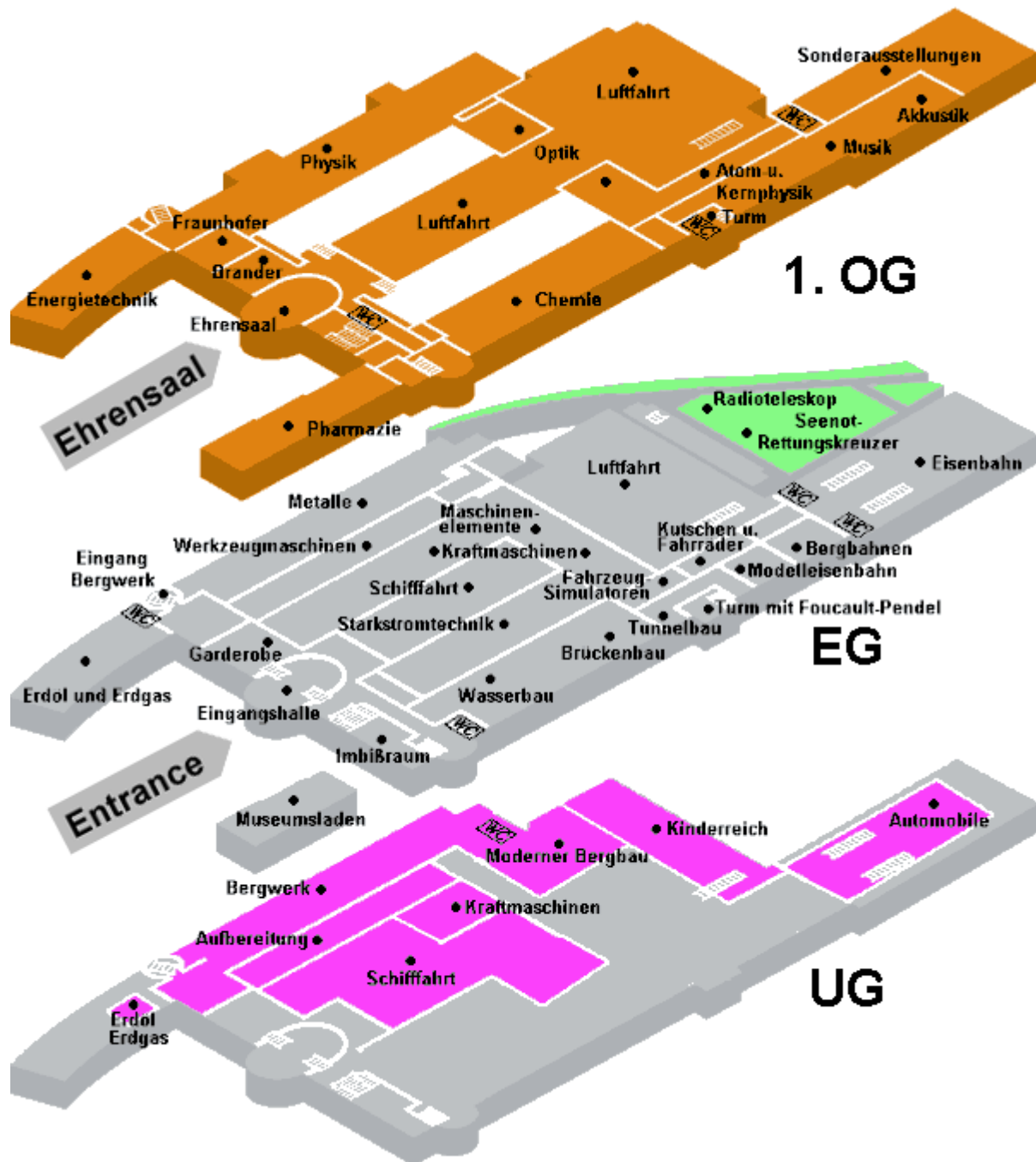


Scanning Probe Microscopy & Organic Materials XIV

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Location

Deutsches Museum – Floor Plan 1

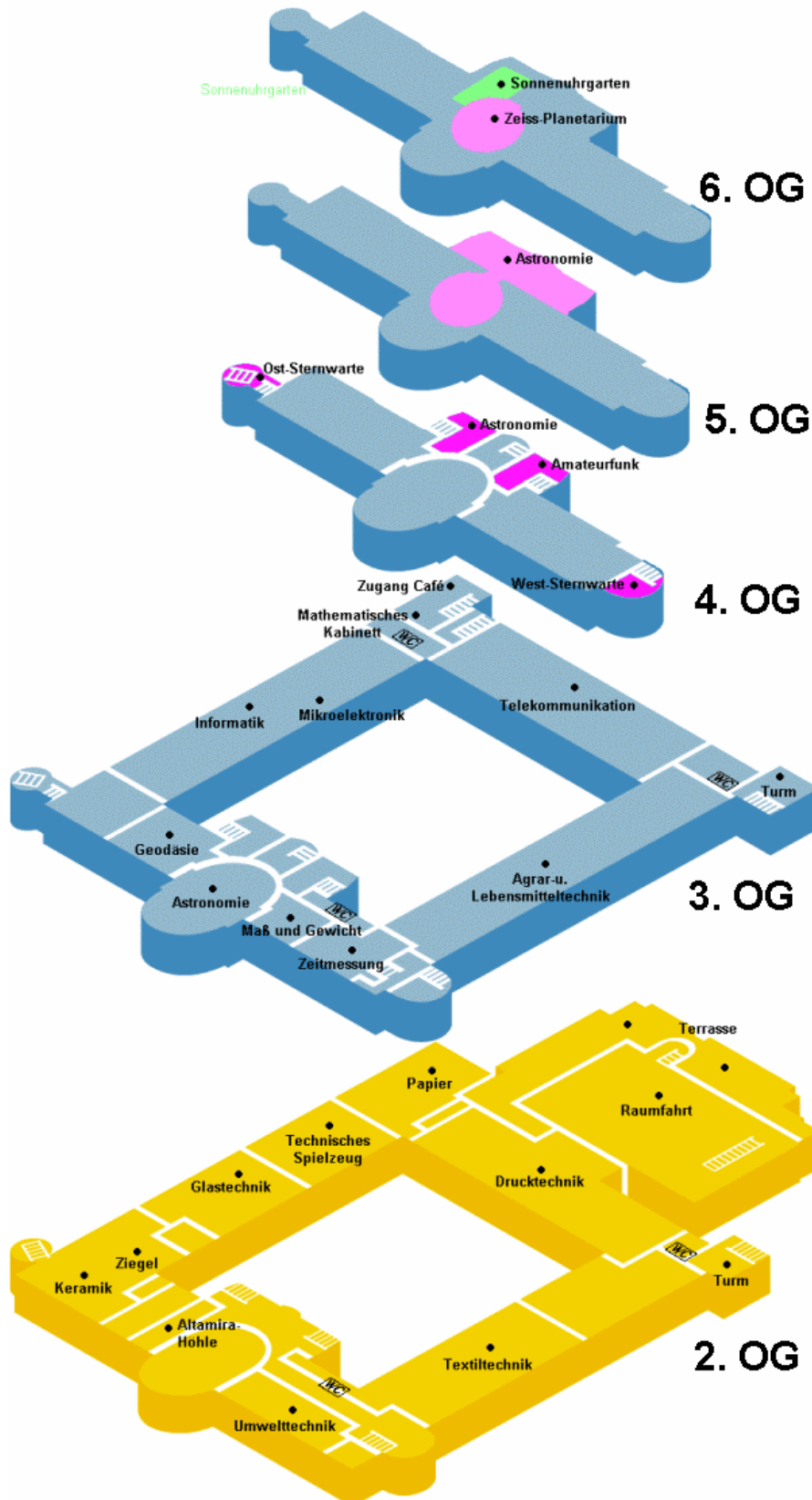


Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Location

Deutsches Museum – Floor Plan 2



Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Abstracts

Invited Talks

Invited Talks

(authors in alphabetical order)

Nanopatterning by induced controlled dewetting of supramolecular thin films by atomic force microscopy

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(3) Department of Chemistry, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh EH9 3JJ UK.

Abstract

We propose an innovative nanolithographic scanning probe method based on Spatially Controlled Dewetting (SCD) by Atomic Force Microscopy. The process is based on new property of molecular thin solid films and opens interesting perspectives for new fabrications based on dewetting. In SCD, dewetting can be induced and controlled both in space & time by Atomic Force Microscopy [1].

In order to illustrate this control we used SCD to generate, in few seconds, highly ordered patterns with a resolution of 40 nm and to write some digital information on organic thin solid films.

The films were grown by drop casting onto a variety of technologically relevant substrates such as highly oriented pyrolytic graphite and mica. As prototype materials we used Rotaxanes [2], which are a novel class of supra molecules consisting of a macrocycle interlocked onto a linear chain by spontaneous assembly. Bulky groups bind the chain at the end.

[1] M. Cavallini, F. Biscarini, S. Léon, F. Zerbetto, G. Bottari, D. A. Leigh *Science* 299, 531 (2003).

[2] J. -P. Sauvage, C. Dietrich-Buchecker *Molecular Catenanes, Rotaxanes and Knots*, Eds. (Wiley-VCH, Weinheim, 1999).

Scanning Probe Microscopy & Organic Materials XIV

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Nanobiotechnology

Invited Talk

Biomolecular assemblies studied by atomic force microscopy

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Abstract

In recent years, considerable attention has focused on biological applications of the atomic force microscope (AFM), in particular on high-resolution imaging of individual biological molecules and complexes [1]. The AFM is unique since it not only allows to image individual molecules under near-physiological conditions, but it can also monitor and visualize dynamic processes at the single molecule level [2]. In dynamic force microscopy (DFM), the cantilever vertically oscillates during the lateral scan and touches the sample therefore only intermittently at the end of its downward movement. This imaging mode significantly reduces frictional forces, thus it yields high-resolution images of single molecules which are only weakly immobilized on a support [3, 4]. Furthermore, the capability of AFM to measure forces in the pico-Newton range has opened the possibility to investigate inter- and intra-molecular forces at the single molecule level [5]. In particular, the interaction between tip-bound ligands and surface-bound receptor molecules can be analyzed in terms of affinity and rate constants. Moreover, force spectroscopy experiments yield details on structural parameters of the binding pocket, on the molecular dynamics of the recognition process and on the energy-landscape of the interaction. By combining DFM imaging with force spectroscopy, receptor sites on a surface can be localized with nanometer positional accuracy, rendering possible to acquire topographical images simultaneously with recognition images [6].

[1] F. Kienberger, C. Rankl, V. Pastushenko, R. Zhu, D. Blaas, P. Hinterdorfer, *Visualization of single receptor molecules bound to human rhinovirus under physiological conditions*, *Structure* (2005), in press.

[2] F. Kienberger, H. Mueller, V. Pastushenko, P. Hinterdorfer, *Following single antibody binding to purple membranes in real time*, *EMBO Reports* 5 (2004), 579-583.

[3] F. Kienberger, R. Zhu, R. Moser, C. Rankl, D. Blaas, P. Hinterdorfer, *Dynamic force microscopy for imaging of viruses under physiological conditions*, *Biol. Proced. Online* 6 (2004), 120-128.

[4] F. Kienberger, R. Zhu, R. Moser, D. Blaas, P. Hinterdorfer, *Monitoring RNA Release from Human Rhinovirus by Dynamic Force Microscopy*, *J. Virology* 78 (2004), 3203-3209.

[5] F. Kienberger, G. Kada, H. Mueller, P. Hinterdorfer, *Single Molecule Studies of Antibody-Antigen Interaction Strength Versus Intra-molecular Antigen Stability*, *J. Mol. Biol.* 347 (2005), 597-606.

[6] A. Ebner, F. Kienberger, G. Kada, Stroh, C. M., Geretschläger, M., Kamruzzahan, A. S. M.; Wildling; L., Johnson, W. T., Ashcroft, B., Nelson J., Lindsay S. M., Gruber H. J., Hinterdorfer, P., *Localization of single avidin biotin interactions using simultaneous topography and molecular recognition imaging*, *Chem. Phys. Chem.* 6 (2005), 897-900.

Single molecule interactions detected on a surface of cancer cells

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Abstract

Cell functioning is defined by a number of proteins present in cell membrane. The way how they interact is crucial in nearly all cellular activities. The proteins participating in molecular recognition events are also associated with many serious human diseases including also cancer. It is known that a cell membrane of cancerous cells expresses the altered pattern of diversity of molecules that are responsible for cell functioning, e.g. for their interaction with other molecules (i.e. receptor proteins), present either in an extracellular matrix or in cell membrane of the neighbouring cell.

Thanks to its high force resolution (down to tenths of piconewtons), the atomic force microscope (AFM) offers a unique possibility to measure forces occurring between molecules being in their native conditions (e.g. receptors that are embedded in cell membrane and act with ligands). The strength of a single molecular complex, measured under given experimental conditions, can be used as a parameter describing its state both qualitatively and quantitatively.

The strength of different adhesive complexes including ligand - receptor and protein - carbohydrate type of interactions was studied. The measurements were performed for interactions involving integrin, cadherin, and carbohydrates present on surface of living cancer cells. The analysis of force histograms and comparison with control measurements confirm the altered binding of proteins that appear to be sensitive to pathological cell state.

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Nanobiotechnology

Invited Talk

High-speed atomic force microscopy

Authors

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Abstract

This paper discusses some key challenges for the next generation of high-speed atomic force microscopes (AFM). For high-speed imaging all AFM components have to be optimized in performance: the force sensor, the scanning unit, and the AFM electronics including the data acquisition (DAQ) system and the feedback controller. i) The force sensor has to be soft and fast in order to minimize the imaging forces and force variations together with a higher sensor bandwidth. This can be achieved by using small cantilevers [1]. ii) The three dimensional positioner (scanner) requires sub-nanometer resolution together with a high positioning bandwidth. To this end a new mechanical design that uses stack-piezos and flexures has been developed. This scanner (15 micron scan range) is designed to have high first resonances in all positioning directions, i.e. it has to be rigid [2], and has been optimized using finite element analysis tools. This scanner furthermore is equipped with strain gauges for closed loop control of the scanning motion. iii) For high-speed imaging also the feedback [3] and piezo drive electronics as well as the data acquisition system [4] have to fulfill high bandwidth and timing requirements. Combining all these improvements, the next generation of AFMs enables imaging speeds of more than two orders of magnitudes faster than current commercial AFM systems.

[1] T.E Schaeffer, M. Viani, D.A. Walters, B. Drake, E.K. Runge, J.P. Cleveland, M.A. Wendman, P.K. Hansma, SPIE 3009, p.48 (1997)

[2] J.H. Kindt, G.E. Fantner, J.A. Cutroni, P.K. Hansma, Ultramicroscopy 100(3-4), p. 259 (2004)

[3] G. Schitter, F. Allgoewer, A. Stemmer, Nanotechnology 15(1), p.108 (2004)

[4] G.E. Fantner, P. Hegarty, J.H. Kindt, G. Schitter, G. Cidade, P.K. Hansma, Review of Scientific Instruments 76(2), p. 026118 (2005)

Nanodevices based on DNA Aptamers

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Abstract

Aptamers are functional nucleic acids which strongly and specifically bind to small molecules or proteins. In some cases they exhibit binding constants similar to those for antibody-antigen interactions. At the same time, they have the advantage of being compatible with standard DNA methodologies. Once the correct sequence for an aptamer is known, it can be synthesized or amplified much more easily than proteins. Recently, we have shown how an aptamer for the protein thrombin can be easily switched between two conformations in which it binds or does not bind the protein [1,2]. This allows us to cyclically bind or release the protein. The principle of operation of this molecular switch is based on DNA strand displacement via branch migration which has been employed before for the operation of other DNA based molecular devices. In our original approach, the release of thrombin had to be triggered by a DNA strand whose sequence depended on the aptamer sequence itself. It would be useful, however, to completely uncouple molecular “input” from “output”. We therefore also present experiments on a DNA signal transduction scheme by which the rate of protein release by DNA aptamers can be made dependent on “generic” DNA signals. With this technique, one should be able to produce molecular devices which controllably release proteins in the presence of freely chosen trigger DNA or RNA strands. In the future, it is also conceivable that dynamical protein arrays be produced in which DNA aptamers are arranged on a DNA supramolecular lattice and hold several interacting proteins in close proximity.

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Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Abstracts

Talks

Talks

(authors in alphabetical order)

AFM-Stethoscopy: a New Diagnostic Tool?

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Abstract

The atomic force microscopy (AFM) has already shown its potential by imaging surface structures in the nanometer range and to detect single molecule interactions. Here we demonstrate the possibility to use an AFM as a stethoscope by determining the oscillation of living cells. All experiments were carried out with an optical microscope combined with an UV- and an Ar-Ion laser for stimulation and an AFM for the detection of cell membrane movements. The experimental setup allows the detection of movements in the nanometer range. A storage oscilloscope was used to externally read out signals of the position sensitive photodiode. By Fourier transformation (FFT) data were processed to evaluate the occurring frequencies. The feasibility of this approach is shown on baker yeast cells (*saccharomyces cerevisiae*). This can open a complete new method to investigate living cells.

Scanning Probe Microscopy & Organic Materials XIV

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Industry Session

Talk

AFM in Liquid Environment simultaneous to Advanced Optical Microscopy

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Abstract

JPK Instruments developed an advanced AFM system specifically for biological applications. This presentation gives insight in the design criteria upon which the development is based and shows application examples for life science research.

The JPK NanoWizard® BioAFM is the only AFM system that enables the true integration of AFM and advanced optical imaging through common inverted optical microscopes. This allows the combination of optical techniques like phase contrast, DIC, fluorescence, TIRF and confocal imaging with the capabilities of an AFM for high resolution imaging, molecular and elastic probing, as well as nano manipulation. Consistent design for operation in physiological solutions, use of state-of the art scanning technology, and atmospheric and temperature control capabilities are key features of the NanoWizard AFM. Application fields include: single molecule fluorescence, cytoskeletal properties, protein interactions, live cell imaging, manipulation, lipid and polymer imaging and drug delivery mechanisms. The application spectrum ranges from the imaging of entire cells down to single molecules and atomic resolution on crystalline samples. Optional add-ons provide capabilities for studying cell adhesion and tip enhanced optical spectroscopy.

SPM as a Surface Analytical Technique in Conservation Science

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Abstract

A fundamental problem in conservation, restoration and storage of cultural heritage is the uncertainty about the degree of deterioration of a material, i.e. the actual condition of the material under consideration. The degree of deterioration often can not be precisely ascertained.

There is a lack of testing procedures to enable the unequivocal characterisation of the condition of artifacts and little research has been carried out to evaluate the relationship between physical and chemical decomposition and the measurable macroscopic changes in the properties of a given material. Artifacts made from metals can be destroyed by corrosive reactions, however, metals can also develop a protective coating by corrosive processes. A technique has been devised which links experimental laboratory investigation of atmospheric corrosion of copper/copper-alloy with field exposure trials, allowing precise studies of the corrosion behavior of copper/copper-alloy under realistic conditions. A weathering chamber is used to investigate the corrosion mechanism of copper/copper-alloy before and after long-term exposure in humid air in the presence of sub-ppm concentration of SO_2 , with observations using surface analytical techniques. These include scanning electron microscopy with energy-dispersive x-ray analysis (SEM-EDX) and scanning probe microscopy (SPM) allowing visualization of the initial stages of the atmospheric corrosion of copper/copper-alloy. This technique can be used to define and locate the individual steps in the multistep corrosion mechanism depending on the microstructure of copper/copper-alloy. This corrosion behavior is similar to that experienced as copper/copper-alloy undergoes atmospheric corrosion after long-term exposure in an urban environment. A pilot study using in-situ exposure conditions with flowing humidified air containing SO_2 indicates that a flow rate accelerates of almost two orders of magnitude the corrosion process of copper/copper-alloy and leads to a statistical corrosion attack.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biomaterials

Talk

Cooperativity in coiled coil formation studied by single molecule force spectroscopy

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Abstract

We report the unzipping and refolding of single coiled coils, measured by atomic force spectroscopy. We investigate two double stranded coiled coils with different lengths (consisting of 33 and 61 amino acids respectively). The unzipping force is applied to the N-Terminal end of the coiled coil in a well defined way. Both coiled coils unfold in a cooperative way near thermodynamic equilibrium. The unzipping force increases from 13 pN for the 33 amino acid coiled coil to 17 pN for the 61 amino acid coiled coil. The refolding behavior is studied too. The cooperative folding of both coiled coils can be explained by the formation of an energetically unfavourable nucleation seed.

Micro-thermal Analysis: a novel approach for nanomedicine & bionanotechnology

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Abstract

There has been a recent report [1] suggesting that collagen may be unstable at room temperature leading to the conclusion that this molecule, the main constitutive protein of the human body and skeleton, may be in a denatured state under normal physiological conditions. In an attempt to understand the thermo-mechanical properties of collagen and its higher order hierarchical arrangements (such as the collagen fibre), a study was carried using techniques developed initially for polymer analysis, known under the generic name of microthermal analysis [2]. The method is based on the combination of atomic force microscopy, scanning thermal microscopy and thermal analysis, with the common feature of having all three techniques accessing information at the micro/nano-scale. Whilst there have been extensive efforts to understand the bulk mechanical and thermal properties of collagenous tissues [3], less is known on the properties of the fibre itself or at the single molecule level. It is known that collagenous structure may undergo a denaturation process (gelatinisation) when submitted to supra-physiological temperatures, but the denaturation pathway remains yet to be elucidated. Our research involves not only understanding the fundamental response of biological samples to temperature, but also developing methods and protocols that could be applicable to disease assessment such as, for example, in tendonitis or osteoporosis.

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Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Optical Properties

Talk

STM-Induced Electroluminescence Measured by a Transparent ITO-Tip

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Abstract

A homebuilt STM combined with an efficient optical detection is used to investigate photon maps of nano-structured gold substrates ("Fischer-Patterns" [1]) and disc-shaped single-particles under ambient conditions. Common metal tips like Pt/Ir or W often are reported to show photon emission consistent with topographical features [2] but presence of the metallic tip "felt" by the sample provokes a strong influence on photon emission.[3] Additional distortions caused by multiple-tip phenomena make a quantitative interpretation almost impossible.[4] Therefore a dielectric tip consisting of indium doped tin oxide has been compared with Pt/Ir. This material is transparent for optical fields and thus leads less tip-induced modifications. On nano-structured triangle patterns plasmon induced field enhancement is observed. A strong optical contrast can be shown even with ITO-covered samples discarding the common model of decaying gap modes formed by the coupling of two surface plasmons on metallic electrodes.

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Infrared spectroscopy of a single virus made possible by near-field microscopy

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Abstract

While infrared spectroscopy is a powerful tool for analysis of both chemical composition and structural properties of a sample, its application for microscopy is strongly limited by diffraction, because resolutions better than a few micrometers are generally needed.

Near-field techniques are known to overcome these problems. In fact the scattering type scanning near-field optical microscope has demonstrated resolutions of up to 20 nm even at mid-infrared wavelengths. It does so by exploiting the near-field coupling between a sharp metal tip and the sample, such confining the probed volume. [1-4]

We will describe infrared-spectroscopic mapping -in both amplitude and phase- of a single Tobacco Mosaic Virus, using the spectral range of the protein amide-I vibration band ($\sim 1600 - 1700 \text{ cm}^{-1}$). Clear spectral signature can be extracted, and good agreement is found with a simple model describing near-field contrast formation. The achieved resolution of about 50 nm exceeds that attainable by conventional infrared microscopy by a factor of ~ 100 .

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Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Organic Adsorbates

Talk

Noise Budget in very low current Scanning Tunneling/ElectroChemical Microscopy.

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Abstract

Electronic noise easily becomes the most serious limiting factor to measurements sensitivity or resolution in Scanning Probe Techniques. This is the case in the application of Scanning Tunneling Microscopy (STM) or Scanning ElectroChemical Microscopy (SECM) to organic materials, as a DNA strand adsorbed to a surface, which is possible only at a very low probe current, in the range from pA to fA. Moreover, accurate probe positioning along the three axis requires position sensors and amplifier electronics at the nanometer level, with their unavoidable noise contribute. A thorough evaluation of all electronic noise sources in a low current SECM/STM has been attempted, not only with respect to the probe head [1], but considering also the contributions from the scanning stage.

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Protein Structure by Mechanical Triangulation

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Abstract

Knowledge of protein structure is essential for understanding protein function. High resolution protein structure has so far been the domain of bulk methods. We demonstrate a simple single molecule mechanical method to measure Ångstrom-precise position of selected residues within a folded protein structure in solution. Construction of poly proteins linked covalently via two selected residues, permutation of linkage residues in protein sequence, and measurement of single poly protein extension response directly yields spatial position of linkage residues in the folded protein state. Mechanical triangulation can find many applications where bulk structural methods or current single-molecule techniques fail.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Tribology

Talk

AFM morphology studies on roughness gradient replicas

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Abstract

Surface morphology is an important factor influencing the biological response to surfaces, i.e. cell adhesion, proliferation and differentiation. For combinatorial studies of roughness dependence of cell response, we developed a fabrication method for roughness gradient substrates, on which the roughness parameters R_a and R_q vary about one order of magnitude from one end to the other. A replication of the gradients allows for the preparation of numerous identical substrates which can be coated with e.g. titanium oxide. The original gradient and replicas were characterized with an atomic force microscope. The replicas show a good congruence of the microstructure, whereas on the nanoscale there are some systematic differences due to crystalization processes and microcrack formation in the coating layer.

MAC Mode imaging and molecular recognition force microscopy of functionalized S-layer proteins under near physiological conditions

Authors

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Abstract

The growing field of nanobiotechnology requires functional arrays with lattice constants at the nanometer scale. Bacterial S-layer proteins form such highly ordered arrays with different symmetries and lattice constants. These S-layer proteins can be functionalized with capture sequences by genetic engineering. Here we used a chimeric protein, in which a streptavidin monomer was fused into the bacterial cell surface layer protein SbsB of *Geobacillus stearothermophilus*. Functional heterotetramers (HT) consisting of one molecule SbsB-streptavidin fusion protein and three molecules streptavidin were obtained by applying a rapid dilution procedure. Subsequently, the proteins were recrystallized on gold or mica substrates.

Topographical images of the S-layer crystals were obtained using dynamic force microscopy under physiological conditions. MAC Mode AFM, in which cantilever oscillations are magnetically driven, as a gentle imaging mode yielded detailed images of the S-layer lattice with the attached streptavidin molecules. The S-layer proteins assembled in a 2-D array with lattice constants of 10.2 nm and 8.0 nm, which is in good agreement to transmission electron microscopy data. Forty morphological units of an AFM image were averaged, revealing structural details of the lattice with high signal-to-noise ratio. The tetrameric streptavidin molecules protruding 0.4 nm out of the S-layer surface were clearly identified.

The functionality of the HT in the 2-D crystals was investigated using single-molecule force spectroscopy. Unbinding force measurements with biotin tethered tips yielded an unbinding force of 40 pN at a loading rate of 300 pN s⁻¹. The binding probability was ~20 % and blocking with free streptavidin decreased the binding probability down to 3 %. This proves the specificity of the streptavidin-biotin interaction.

In conclusion, streptavidin-tagged 2-D protein crystals promise broad applicability for biofunctional arrays, as virtually any biomolecule can easily be modified with biotin (or genetically tagged with a strep-tag) and then be bound to immobilized streptavidin.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Nanobiotechnology

Talk

Replicating Fischer-Patterns by Micro-Contact-Printing - An Analysis Based on Contact Mechanical Models and Frictional Behavior

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Abstract

Soft-Lithography is a common method to reproduce 2-dimensional structures on almost arbitrary substrates. Commonly, polydimethylsiloxane (PDMS) is used to form a stamp from a master structure which has previously been created by e-beam or optical lithography. Then thiols are diluted in a solvent and used as an ink for stamping the structures onto a substrate. This method allows to reproduce structures of lateral size down to some tens of nanometers. The obvious advantages are a true reproduction of the masters lateral structuring and the easy use of the stamping technique. However, the necessity of the creation of a master structure is still very expensive due to the need for an evaporation and lithography setup. The method that we are going to present is based on self-assembled colloidal-crystals of polystyrene (PS) spheres and allows the reproduction of structures which are commonly known as Fischer-Projection-Patterns (FPP). The colloidal crystal is used as the master structure for the creation of the stamp. The transfer of e.g. octadecanethiol (ODT) onto a gold layer atop a silicon substrate and a selective etching step allows the creation of triangular gold patterns, also known as FPPs. These gold particles can also be used as a template for the subsequent silanization. Etching away the remaining gold terminates the sample preparation process. The final structures are islands of silanes on top of an oxide surface with almost no topography, but lateral material contrast.

All samples have been characterized by means of Digital Pulsed Force Mode (DPFM) AFM and Lateral Force Microscopy (LFM). The DPFM measurements have been analyzed using a JKR-like model, which describes the interaction between an indenting structure and a sample surface. Furthermore, lateral force measurements have been conducted with the same setup, verifying the lubricating behaviour of silanes towards the sliding tip compared to the friction between a silicon tip on a silicon substrat.

Surface plasmons on metallic nanostructures fabricated by using colloidal crystals

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Abstract

The study of the interaction of light with surface plasmon polaritons (SPP) has acquired in the last 10 years an increasingly interest in both the understanding of fundamental properties of matter at the nanoscale and in the applications domain, namely, super-resolution, surface enhanced Raman spectroscopy (SERS) and second harmonic generation (SHG).

SPPs are charge quanta which can be localized or propagate along the interface between metallic nanostructures or thin films and dielectrics. Light can couple with SPPs by several mechanisms: by illuminating a metallic thin film deposited on glass (dielectric substrate) from the glass side for certain angles; by illuminating a metallic grating (Bragg mirror); and by SPP wave vector matching with the Fourier components of high spatial frequency describing the scattering of light at small nanostructures.

We have prepared 2D metallic structures and 2D gratings using arrays of colloidal polystyrene (PS) spheres coated by metallic thin films. The optical properties of these structures have been investigated in far- and near-field. The scattering patterns observed in far-field show a high sensitivity with the polarization and the direction of incidence of light. It seems possible to use the polarization of light and its direction of incidence to excite different SPP modes.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biology

Talk

The native photosynthetic apparatus of *Phaeospirillum molischianum*

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Abstract

Ubiquity and importance of photosynthetic organisms in nature has made the molecular mechanisms of photosynthesis a widely studied subject at structural and functional levels. A current challenge is to study the supramolecular assembly of the proteins involved in photosynthesis in native membranes. We have used atomic force microscopy (AFM) to study the native architecture of the photosynthetic apparatus and analyze structural aspects of single molecules in chromatophores of *Phaeospirillum molischianum*. Core-complexes are formed by the reaction center (RC) fully enclosed by an elliptical light harvesting complex 1(LH1). LH2 are octameric rings, assembled with cores and in hexagonal antenna domains. The symmetry mismatch of octameric LH2 packing in a hexagonal lattice suggests lipophobic effects rather than specific interactions to drive protein assembly. The LH2 show size variability most likely resulting from variations in subunit stoichiometry.

Determination of the elastic properties of Dictyostelium cells using the atomic force microscopy

Authors

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Abstract

In the current study we used the atomic force microscope (AFM) to investigate the elastic properties of the slime mould Dictyostelium. Live wild type Dictyostelium cells were compared in both the vegetative and the pre-aggregate state while adsorbed to a glass substrate. We use force volume imaging to simultaneously obtain topographic and elastic maps of single cells. Differences in the Young's modulus of polarized with respect to unpolarized cells were found, with a two fold increase in stiffness for the polarized AX3 wild type. Further, the Young's modulus of the front (pseudopod) and rear of polarized cells was found to be different. We also address the dependence of Young's moduli on sample thickness by performing measurements on a thin agarose gel.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Tribology

Talk

Investigation of the statistics of stick-slip friction on graphite

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Abstract

The stick-slip mechanism is believed to be one fundamental process of atomic friction. It is described by the Tomlinson model for $T=0K$ [1]. In this model a tip jumps between two equilibrium positions of a surface potential. The lateral forces, inducing the jumps are called jump heights. For finite Temperatures these jump heights follow a statistical distribution due to thermal excitation [2]. We measured stick-slip friction on the atomic scale with an AFM and we will introduce a new analysis method to extract the statistical information i. e. the corresponding jump height histograms from the experimental data, acquired with a Si-tip on a vacuum cleaved HOPG surface under UHV conditions. These histograms are in good quantitative agreement with the theory [3]. From the histograms we can extract quantitative values for the effective energy barrier and we find that the energy barrier depends strongly on the exact position of the tip within the surface potential [4].

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[4] Schirmeisen et al., PRB 71 (2005)

Piezo Technology for Scanning Applications

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Abstract

The presentation will give a short overview over the principles of piezo technology. The limitations, such as travel range, and the technology's strengths, such as stiffness and scan speed, will be discussed. Technical specifications will be presented, i.e. accuracy, repeatability and resonance frequency. The talk will supply typical order of magnitude of these specifications. Finally, products for scanning applications out of PI's product range will be shown.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biom mineralization

Talk

Hydrothermal Atomic Force Microscopy

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Abstract

High resolution microscopy of processes taking place on mineral surfaces can be performed by various methods. However, if the processes are taking place at the interfaces of (non-conducting) minerals and aqueous solutions, only atomic force microscopy AFM can accomplish an in-situ investigation in molecular resolution.

A considerable restriction in applying AFM to solid-liquid interfaces was the limitation to near room temperature conditions. This restriction has been overcome by the introduction of hydrothermal atomic force microscopy HAFM [1]. The capabilities of HAFM have been proven in various systems such as the barite-water interface [2] or the magnesite-water interface [3]. A second generation HAFM has now been constructed in our laboratories that allows the in-situ investigation of solid-liquid interfaces at pressures of up to 50 bars and temperatures of up to 180°C. Recent studies were focusing on the processes between phyllosilicates and aqueous solutions [4]. Studies of processes taking place at the interfaces of phyllosilicates and aqueous solutions as well as within the interlayers of phyllosilicates are of high importance since they can be used as a starting point to understand the interactions between phyllosilicates and organic matter. These interactions are crucial in many aspects ranging from local water quality to soil fertility and are even assumed to be relevant for the origin of life [5].

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Bimolecular Hydrogen-bond Networks - Mediated Coadsorption and Networks with In-situ Tunable Cavity Size

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Abstract

Self-assembled monolayers (SAMs) are an important grounding for future applications of long range ordered molecular structures in nanotechnology. Hence it is of general interest to understand the parameters determining the growth and stability of these systems. Bimolecular monolayers were grown by spontaneous self-assembly from solution at the liquid-solid interface and subsequently investigated by Scanning Tunneling Microscopy (STM).

Stable adsorption of one kind of molecule - TPT (1,3,5-tris(4-pyridyl)-2,4,6-triazine) - could never be observed. In the presence of another species, acting as a kind of "molecular glue", TPT molecules were stabilized on the surface and could be imaged by STM. As mediators both TMA (trimesic acid) and TPA (terephthalic acid), molecules equipped with carboxylic groups, were suitable. Common features of the various co-crystals are N...H-O H-bonds, rendering mixed molecular aggregates sufficiently large for stable adsorption of monolayers in equilibrium with the liquid phase above.

The co-adsorption of two different molecules, BTB (1,3,5-benzenetribenzoic acid) and TMA (trimesic acid) in open (loosely packed) networks was studied in two different solvents (heptanoic and nonanoic acid). Altering the absolute and relative concentrations of the two compounds in binary solutions resulted in phases with six different structures. All of these structures are stabilized by twofold intermolecular hydrogen bonding between the carboxylic acid head groups. Moreover, in-situ dilution of liquid mixtures induced phase transitions of the monolayer structures, accompanied by an alteration of the size and shape of cavity voids in the 2-dimensional molecular assembly.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biomineralization

Talk

A new AFM chemical dissection technique provides insights into the origins of bone fracture resistance

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Abstract

Loss in bone mineral density does not sufficiently explain the loss of fracture strength that accompanies osteoporosis. High resolution atomic force microscopy of bone fracture surfaces reveals collagen fibrils coated with a layer that may contain both mineral and non-fibrillar organic. In-situ chemical dissection with EDTA or NaF removes this layer and exposes the bare collagen fibrils, while treatment with H₂O shows no strong effect. NaF treatment of bone in vitro is known to decrease bone yield strength, without affecting bone mineral density. We propose that fibril-fibril separation is an important fracture mechanism for bone, and that adjacent fibrils are held together by a thin layer that functions as a “glue” which can be detached or degraded by NaF exposure.

Ultrafast Energy Transfer Processes in Highly Doped PMMA Films

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Abstract

Presently evolving nano devices and already implemented organic electronics structures rely on efficient charge and energy transport. For energy transfer within organic layers and to heterojunctions exciton migration is a key mechanism. Perylene dyes are promising materials for such applications due to their high quantum yield, their long term stability, and their large transition dipole moments. We investigate the applicability of perylene dyes in transport supporting matrices utilizing exciton migration. PMMA films of 1 μm thickness are doped with 0.3 M Perylene Orange and ten times less Perylene Red. This high concentration of Perylene Orange chromophores should lead to exciton migration by intermolecular energy transfer. Perylene Red exhibits a red shifted absorption and acts as energy collection unit. The dynamics in the electronically excited state and the ground state are simultaneously characterized over the whole visible spectral region by pump-probe absorption spectroscopy. A NOPA [1] pumped by a regenerative Ti:sapphire amplifier provides tunable 40 fs excitation pulses whereas for probing a whitelight continuum is used. The time resolved measurements and the deduced anisotropy show that an ultrafast Förster transfer on a time scale of 1 ps between the dye molecules can be achieved resulting in a high mobility of the optically generated excitons [2]. In addition we found that the excitons move to Perylene Orange dimers, which have formed in low concentration during the sample preparation. The observed energy transfer time is shorter than expected for a direct transfer and indicates that migration processes are important for the transport. Adding Perylene Red leads to efficient collection and trapping of the excitation energy at the added molecules with transfer times down to 0.6 ps. The results demonstrate that doping polymer matrices with perylene dyes is a promising strategy to design energy transport pathways based on exciton migration. In addition, the efficient transfer to long living collection units like the dimers shows that these systems are also interesting as artificial light harvesting complexes.

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Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biomaterials

Talk

Investigations of the structural and physical properties of synthetic amyloid fibrils

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Abstract

Amyloid fibrils are self-assembled, beta-sheet-rich superstructures of peptides or proteins. Although these aggregates have first been found in connection with protein-misfolding diseases, such as Alzheimer's or Parkinson's disease, there is evidence that the ability to form fibrils is a thermodynamic property of any polypeptide chain rather than a result of specific, disease-related amino-acid sequences. Fibrils can easily be formed in-vitro from non-disease-related proteins and even from artificially "bottom-up"-synthesized peptide chains which have no biological function at all. Furthermore, functional groups can be incorporated without significantly disturbing the fibril superstructure. This is why amyloid fibrils have recently attracted considerable interest as potentially useful, novel biomaterial.

Here, we present SPM-investigations of the structural and physical properties of the fibrillar system, TTR105-115, which forms well-defined nanorods of ca 10nm diameter. Fibrils could be chemically dissected into smaller protofilaments, which were studied at high resolution. Furthermore, it has been found that fibril adsorption on surfaces is influenced by electrostatic surface properties and can be controlled by appropriate choice of solvent conditions. A soft-lithographic method taking advantage of these findings has been developed to create hybrid peptide/metal, 2-d micronscale arrays for in-situ SPM-studies and which could be used in future protein-based biosensor devices.

Electrical AFM based definition of anchor points for layered biomolecular structures

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Abstract

Atomic force microscopy (AFM) based lithography has proven useful for local modification of surfaces at the nanoscale. Achieving control over localized positioning of particles and molecules is a key factor for nanosensor fabrication or for creating scaffolds for building up nanostructures. We previously reported on a method that uses electrostatic forces to guide particle deposition in liquid environments [1,2]. The electrostatic field is created by nanoscale charge patterns written into the sample with a conductive AFM tip. In this contribution, we will present how this general method defines anchor points with specific binding sites that allow for the docking of functionalized biomolecules and particles. By this means, larger structures, potentially serving as biosensors, are built up locally in a layer-by-layer procedure.

The guided assembly process consists of two parts: After defining the patterns via AFM-based charge writing, the sample is developed in a water-in-oil emulsion. Driven by electrostatic forces, the water droplets carry particles or molecules to the patterns. The basic characteristics of this deposition have been studied for a water-particle-oil model-system for different substrates [1]. Using the same process, biotin-labelled immunoglobulinG (IgG-biotin) was deposited with sub- μm resolution onto charge patterns on poly (methyl methacrylate) (PMMA) samples [2]. The IgG molecules at the same time help in stabilizing the emulsion [3].

As the samples are dried after developing them in the emulsion, re-wetting the structures is a necessary step prior to any further modifications made. To this end, the dry samples are immersed into a buffered blocking solution, which also prevents unspecific binding during the following reactions. As the emulsification and the drying steps might have caused conformational changes of the IgG, the activity and accessibility of the biotin groups on the IgG have to be proven. We verified the biotin activity by incubating the sample in a solution containing fluorescently labelled anti-biotin molecules, which are known to bind specifically to biotin.

For the layered structures, a streptavidin linker is used to attach 40 nm sized biotin-labelled polymer beads (biotin-beads) from aqueous solution. Fluorescently labelled biotin-beads bind to the free binding sites of previously attached streptavidin facing inside the solution. Fluorescence images reveal the high specificity of both reactions. The IgG-biotin still has enough functionality to detect the biotin-beads via a streptavidin linker.

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Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Tribology

Talk

Investigating lateral forces in supported lipid bilayers and protein-lipid interactions using nanomechanical cantilevers

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Abstract

The influence of the composition and mechanical properties of lipid membranes upon the structure and activity of membrane proteins has been observed in both model and natural systems. However, the direct measurement of lateral interactions between lipids and membrane proteins still poses experimental challenges, which need to be overcome. Thus far, such lateral interactions have been studied mainly at the air-water interface, using lipid monolayers as models for membranes and peptides or detergents as models for proteins.

We here report on the direct measurement of the lateral stress in supported lipid bilayers using a microfabricated cantilever array as a substrate. We will present our initial results on the formation of the supported lipid bilayers on the cantilevers and the interaction of surfactants and membrane proteins (OmpF and FhuA) with these bilayers. The corresponding mechanical effects in the bilayers will also be discussed.

Temperature Softening of a Protein in Single-Molecule Experiments

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Abstract

Mechanical flexibility is crucial for the function of proteins. However, such material properties are not easily accessible experimentally. We used single-molecule force spectroscopy to study the stiffness of a single domain of dictyostelium discoideum filamin (ddFLN4) in a temperature range from 5°C to 37°C. Analyzing the distributions of unfolding forces allowed us to extract transition barrier heights and positions of the underlying energy landscape. We found a marked narrowing of unfolding force distributions with increasing temperature. This narrowing reflects an increase in transition state position from 2.7 Å to 7.8 Å and thus a reduction of the molecular spring constant of the protein by a factor of 7. We suggest this temperature softening reflects a shift in the nature of the interactions responsible for mechanical stability from hydrogen bonds to hydrophobic interactions. This result has important consequences for all interpretations of protein mechanical studies if experimental results obtained at room temperature are to be transferred to physiological temperatures.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Industry Session

Talk

High-Resolution Imaging and Chemical Characterization of Heterogeneous Materials with the Confocal Raman AFM

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Abstract

A thorough knowledge of structural and chemical properties is essential for the fields of nanotechnology and materials science, leading to a growing demand for characterization methods for heterogeneous systems on the nanometer scale. However, certain properties are difficult to study with conventional characterization techniques due to either limited resolution or the inability to chemically differentiate materials without inflicting damage or using invasive techniques such as staining.

The Confocal Raman AFM combines Raman spectroscopy, a chemical analysis technique, with high-resolution imaging methods such as Confocal Microscopy and Atomic Force Microscopy (AFM). With this instrument it is possible to analyze heterogeneous materials with respect to their chemical composition and surface structure without laborious sample preparation. The materials can be analyzed under ambient conditions or in a liquid environment. With the Confocal Raman Microscope (CRM), it is possible to obtain Raman spectra from extremely small sample volumes (down to $0.02 \mu\text{m}^3$) and to collect high resolution Raman images. In the Raman spectral imaging mode, a complete Raman spectrum is acquired at every image pixel and the images are extracted by analyzing spectral features (sum, peak position, peak width, etc.). By simply rotating the microscope turret, the CRM is transformed into an Atomic Force Microscope (AFM). With this technique, a sharp tip is scanned over the sample, providing high resolution topographical images with sub-nanometer resolution. By investigating the tip-sample interaction, one can obtain not only the high resolution topographic structure of the surface but also information about the local mechanical properties of the sample components. The highly resolved topographic structures observed with the AFM can then be linked to the chemical information obtained by the CRM.

To demonstrate the capabilities of this unique combination of measuring techniques, examples from various fields of application such as biology, polymer, and semiconductor physics will be shown.

Scanning Force Microscopy and Spectroscopy in Dentistry

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Abstract

Biofilm formation plays an important role in many fields of medicine, especially in dentistry. However, there exist no standardized methods up to date to answer questions posed in this biofilm formation.

In dentistry, the adsorption of proteins and other macromolecules from the saliva onto the enamel leads to the formation of a biopolymer layer called pellicle. The understanding of this pellicle formation presents an important key to preventive dentistry and caries research.

Scanning force microscopy (SFM) and was used to investigate protein adsorption on different surfaces used in dentistry such as natural enamel and (artificial) filling and implant materials. In a further step, surface samples were fixed on an enamel brace and carried for a defined time in the oral cavity. This in-vivo formed biofilm shows a different morphology on the different substrates.

Furthermore, adhesion forces between different saliva proteins and these surfaces were measured by scanning force spectroscopy. Forces were resolved down to the piconewton regime while changing systematically the pH-values and exposure times. A kinetic model was developed to describe the time dependent changes in these adhesion force and a protein structure dependent model for the maximum adhesion force.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biomaterials

Talk

SFM imaging of single molecules of atactic poly(sodium 4-styrenesulfonate) adsorbed from ethanol-water solutions

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Abstract

We have reported recently the adsorption of polyelectrolyte molecules from aqueous solutions on monolayers of amphiphile molecules self assembled with their long axis parallel to the substrate surface (Severin et al, 2004). The amphiphilic monolayer creates a modulated surfaces potential which is sufficient to suppress surface diffusion of adsorbed polymers. The conformations of the polyelectrolytes on the surface depend on the molecule-substrate interaction. Here we report scanning force microscopy (SFM) imaging of poly(sodium 4-styrenesulfonate) (PSS) molecules on a preadsorbed octadecylamine layer on graphite deposited from ethanol-water solutions with different ethanol fractions. Conformations of the PSS molecules deposited from solutions with low ethanol fraction are extended, increase of the ethanol fraction leads to collapsed conformations of the PSS molecules. We attribute the conformational change on the surface to the coil-globule transition in solution induced by the variation of the ethanol fraction. We found a good correlation between the observed conformations of the molecules on the surface and the corresponding conformations in solution determined by dynamic light scattering.

Severin, N., Barner, J., Kalachev, A.A. and Rabe, J.P. (2004), *Manipulation and overstretching of genes on solid substrates*, Nano Letters 4, 577-579

AFM and Forensics

Authors

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Abstract

The exact determination of the date of crime is prevalent an unsolved problem in forensic science. However, this represents one of the most important forensic applications during crime scene investigation.

We are working on a new tool for the estimation of the date of crime. The atomic force microscopy (AFM) is used for high-resolution imaging and elasticity measurements of blood stains and in future for collagen fibrils in bone sections. Elasticity measurements of blood stains could help to date back the crime a few days or weeks. The force spectroscopy of collagen fibrils in bone sections will be developed to clarify crimes, which are several years ago where only parts of skeleton or bones are left.

To construe elasticity data received from bone sections an exact knowledge of the mechanical properties of single collagen fibrils is necessary. Structural and elasticity investigations of single collagen fibrils were done on in vitro assembled fibrils. Native and fibrous long spacing (FLS) collagen fibrils were formed by self-assembly, using a special dialysis setup. Depending on the assembly conditions, collagen forms a variety of different structures (native fibrils, FLS fibrils, cocoon-like fibrils). The received collagen fibrils of type I had a bending pattern of 67nm for the native fibrils, and 200 nm to 300 nm for the FLS fibrils. Collagen is a system that shows a large degree of polymorphism.

To determine the elastic properties of collagen fibrils the tip of the AFM was used as a nano-indentor by recording force displacement curves. The Youngs modulus can be calculated by the Hertzian theory. To confirm the collagen fibril assembly, the AFM was used as a nanodissection tool. Native fibrils were dissected and the structure of the cut area was determined.

For the analytic procedure of the age determination of blood samples, a fresh spot was applied on a glass slide and the AFM-detection was started after drying the blood spot. After morphological investigations with high resolution AFM imaging, force distance curves were done on the blood sample. These measurements were repeated in determined intervals. The obtained elasticity pattern showed a decreasing elasticity over time, which is most probably influenced by the alteration of the blood spot during the drying and coagulation process. The preliminary data demonstrates the capacity of this method to use it for development of calibration curves, which can be used for estimation of blood stain ages during forensic investigations.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Organic Adsorbates

Talk

Structure and bonding of complex organic adsorbates investigated by STM and STS

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Abstract

The adsorption of complex organic molecules on solid surfaces is an issue of increasing importance in surface science. The challenge is not just the large size of these adsorbates, but also their anisotropy, flexibility, and chemical multi-functionality. Despite intense research efforts in many groups, the availability of atomic-scale structural data concerning adsorption sites and internal atomic coordinates for complex adsorbates is still the exception rather than the rule. The aim of our work is to explore ways of providing these data. To this end, we have studied the PTCDA/Ag(111) interface with Low Temperature Scanning Tunneling Microscope (LT-STM). PTCDA is a large organic π -conjugated molecule, which is often used as a model system in the context of organic epitaxial growth and complex chemisorption phenomena. On Ag(111) it forms a commensurate overlayer in which the molecules are arranged in a herringbone pattern. In our study we determine the adsorption site of PTCDA on Ag(111) using direct STM imaging. Images simultaneously showing the Ag lattice and the molecular overlayer reveal that the two non-equivalent PTCDA molecules of the commensurate overlayer (type A and B) both adsorb on bridge sites. Together with standing wave experiments in which the bond length and a molecular distortion of the adsorbate have been determined [1], and density functional calculations for the adsorbed monolayer, our STM data finally solve the atomic surface structure of PTCDA/Ag(111). These structural insights shed new light on the nature of the chemical bond between PTCDA and the substrate in which both the π -electrons of the perylene core and the carboxylic oxygens are implicated. High-resolution STS spectra indeed show site-specific differences in the electronic structures of type A and B molecules, in good agreement with simulated tunnelling spectra. Specifically, it is possible to identify both the direct influence of orbital mixing between PTCDA and the substrate and the indirect influence of intermolecular interactions on the electronic structure of the chemically bound adsorbate [2].

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Insights into the structural properties of collagen fibres using AFM

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Abstract

Collagen is the most abundant protein in mammals. It provides mechanical structure to our bodies, protecting and supporting the softer tissues and connecting them with the skeleton. Many of the most common skeletal diseases are related to either a loss of bone mass such in the case of osteoporosis or to a collagen disorder presents in diseases such as osteogenesis imperfecta, Marfan's syndrome. Investigations of the structural behaviour of collagen matrices down to the fibrillar and molecular level are essential to understand the implications of these diseases upon the architecture of our skeleton itself.

In this research, the mechanical properties of native collagen (rat tails) were studied as a precursor studies using atomic force microscopy based technique (nano-indentation; force volume). Mechanical properties and behaviours such as the evolution of Young's modulus, elasticity of collagen fibrils were characterised. Further studies lead to a controlled nano-dissection of the collagen fibres, allowing the outer layer of the fibre to be removed, hence exposing the internal structure of the fibre itself.

M. Wenger, P. Mesquida, L. Bozec, M. Horton, *Mechanical properties of Collagen on the Nanoscale*, - in preparation -

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Biology

Talk

A new way of SFM Manipulation of ds-DNA on Surfaces

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Abstract

It is well known that single polymer molecules can be manipulated by a scanning force microscope (SFM) tip on a surface [1, 2]. However, with this method only a point force can be exerted on the polymer. Here we demonstrate a new manipulation method based on the SFM tip generating a 2D-pressure in an ultra-thin liquid film, which then exerts a homogeneous force on a single polymer molecule embedded in that film. Under certain conditions, liquids can form quasi two-dimensional liquid layer on a solid substrate, which could be used to transmit forces isotropically across a flat surface. In particular, with this new manipulation method ds-DNA loops on surfaces have been moved, stretched, overstretched and finally torn apart in situ. Eventually, the force needed to break a single ds-DNA molecule is estimated.

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[2] Severin, N., Barner, J., Kalachev, A.A., & Rabe, J.P., (2004), *Manipulation and overstretching of genes on solid substrates*, Nano Letters 4, 577-57

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

Abstracts

Posters

Posters

(authors in alphabetical order)

Double-layers of Bis-Di(4-Methoxyphenyl)Amino-Substituted Hexa-Peri-Hexabenzocoronene studied by STM and STS at the liquid-solid Interface

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Abstract

Bis-di(4-methoxyphenyl)amino-substituted hexa-peri-hexabenzocoronene (HBC) has promising electronic properties; in particular it may be used as hole transport material in organic electronic devices [1]. In order to probe simultaneously structural and electronic properties of physisorbed multilayers on the nanoscale, scanning tunneling microscopy and -spectroscopy (STM/STS) is the method of choice. The study was performed at the interface between a solution of the HBC-derivative in 1,2,4-trichlorobenzene and the basal plane of highly oriented pyrolytic graphite. STM revealed double-layers, where the structure of the first layer was determined by the interaction with the substrate. The second layer, imaged at larger tip-sample-separation, exhibits the same lattice parameters as the first one, but some unoccupied lattice sites were observed. The electronic properties of the two layers have been investigated by STS, which reveals identical current-voltage-characteristics through mono- and bi-layers.

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Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

P8

Poster

High Resolution Scanning Electrochemical Microscopy of DNA single Molecules on Mica

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Abstract

A few years ago Guckenberger et al. reported very high resolution images of DNA molecules on mica, an insulator, obtained with a Scanning Tunneling Microscope [1]. In the following, Fan and Bard proposed an electrochemical (ionic) conduction mechanism in the thin water layer adsorbed on the mica surface [2].

We analyzed in detail the mechanism of conduction on the mica surface and speculated on the effect of electro active species codeposited with the DNA molecules. A scanning probe microscope was built, specifically conceived for these measurements [3]. Preliminary results will be presented.

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The CellHesion - quantifying adhesion forces between single cells

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Abstract

The CellHesion Development Kit has been designed to allow reproducible and quantitative analysis of cell-cell and cell-substrate binding forces. Using a combination of light microscopy and force spectroscopy, single cells can be selected, attached to a flexible cantilever and subsequently allowed to adhere to a second, specific cell or region of substrate. The instrument allows precise control of the force the cells are subjected to during a user-defined binding period. The bound cells can then be separated at a specific speed, to a distance of up to 100 μm . By monitoring the bending of the sensor during the retraction process a force-distance curve can be plotted. From this force-distance curve it is possible to calculate the amount of work and maximal force required to separate the two cells. Additionally, analysis of sub-features of the force-distance retraction curve allows the determination of the force required for the unbinding of single protein-protein interactions at the cell surface. The design of the CellHesion allows a combination of this AFM force spectroscopy with phase contrast, DIC and epifluorescent microscopy or confocal imaging. Such capabilities not only enable the selection of specific cells within a culture for binding measurements, but also the monitoring of multiple additional cellular processes that may occur on binding, such as changes to actin structure, calcium flushes, distribution of labelled proteins, or morphological changes.

Scanning Probe Microscopy & Organic Materials XIV

14 – 16 September 2005, Munich

P28

Poster

AFM measures of encapsulated *Spondylosium panduriforme* alga.

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Abstract

Many microalga species may accumulate extracellular polysaccharides, such as gelatinous mass enclosing its cells, which are called envelopes, sheaths or capsules. These structures are very common among the Desmidiaceae. However, studies concerning their functions, properties and structures are still incipient. *Spondylosium panduriforme* is a filamentous Desmidiaceae, whose filament is enclosed by a great and continuous capsule with well-defined borders firmly bond to the cell. The capsule of *S. panduriforme* could be considered as an essential part of the cell suggesting the importance of this structure to the appropriate functioning of the cell. The capsule structure is mainly composed by polysaccharides whose monosaccharide composition is already known and preliminary data using electron microscopy showed its delicate fibrillar structure. We used atomic force microscopy (AFM) to investigate the nanostructure of the algal capsule for a better understanding of its structure and to establish a methodology to image algal cells that are incipient in literature. Electron microscopy yields high resolution, but requires working in vacuum, staining and other special treatment. The AFM equipment resolution allows obtaining at nanometer resolution images in native conditions, without any destructive treatment. Besides the possibilities of carrying out experiments in liquid where both composition and temperature can mimic the natural environment of the algal cells. The cell immobilization is critical for imaging with AFM. Effective immobilization techniques must position the algal cells such that they are stable to tip forces in liquid environments. We used for immobilization a solution with 0.5 g of Agar-Agar and 10 mg ammonium sulfate and 10 mg de Chromium chloride in 100 ml of nanopure water (18.2 MW/cm). The freshly cleaved mica substrates were vertically dipped into the solution and allowed to air-dry overnight to obtain the treated mica. Samples of alga *S. panduriforme* were prepared for AFM imaging by simply planning a 10 mL droplet onto treated mica and allowed to air dry. After rehydration with algal growth medium the immobilized cells remained firmly attached to mica surfaces and the cell viability and natural surface properties were also maintained for AFM imaging. The images were obtained in a Park Bioprobe atomic force microscope. The analyzed samples were in partial dehydrated state. Topographic AFM images of *S. panduriforme* capsule appear to be smooth and continuous, but the roughness increase on the region that cover the algal cell can be due to the partial dehydrated algal cell. The measure of cell diameter, obtained from AFM images, was 23.1 nm. The capsule height was 0.13 μm. Further AFM measurements in liquid media are currently under investigation.

Probing of the Proteasome-Protein Interaction with Force Spectroscopy

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Abstract

Atomic force microscopy (AFM) is an established method to investigate biological samples in their physiological environment. In our group we are investigating the 20S proteasome from *Thermoplasma acidophilum*. Besides imaging we will focus on force measurements.

The proteasome is a barrel-shaped enzyme of 15 nm height and 11 nm width with a small opening at both ends. Through these two entrances unfolded proteins can access the inner part of the proteasome with the catalytic centers in order to be degraded. Our goal is to characterize the translocation mechanism and the forces involved, which are currently unknown. For this purpose unfolded substrate proteins will be bent to the tip. Via vertical approach of the tip to immobilized proteasomes the substrate proteins are offered to the proteasomes.

As in this stage the tip is not capable of imaging the surface anymore the first step is to immobilize the proteasomes in an upright standing manner in a very dense formation. This guarantees that the functionalized tip comes down over proteasomes.

One attempt is to immobilize the proteasomes on a lipid-layer. This approach turned out to be not practicable since the lipid-layer forms holes during incubation with proteasomes. New promising approaches will be shown on the poster.

Second step will be the investigation of the forces involved in the translocation mechanism. Therefore suitable proteins which are known to be degraded by the proteasome will be bound to the AFM-tip. The forces exerted on the substrate proteins by an interaction with the proteasomes are transmitted to the tip. While retracting the lever with the tip from the surface the deflection of the lever changes due to the forces. Similarly, when the lever is kept stationary it will be bent towards the sample when the protein is sucked into the proteasome. Such force distance curves are offering a promising route to a better understanding of the translocation mechanism.

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Dehydration damage on raft-exhibiting supported bilayers: effects of disaccharides and other stabilizing substances

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Abstract

Removal of water from biological membranes usually results in severe functional and structural damage. Nevertheless, many organisms exploit the properties of stabilizing molecules, like disaccharides, in order to survive complete dehydration.

Dehydration stress is indeed thought to cause both alterations of microdomains and macroscopical damage in biomembranes, leading to deleterious effects. These phenomena can be avoided if disaccharides are used during dehydration.

This study provided information about the effects of extreme environmental conditions on structural details of biomimetic membranes and, in particular, of lipidic microdomains. We used atomic force microscopy (AFM) to study hydrated sphingomyelin/dioleoyl-phosphatidylcholine/cholesterol supported bilayers, after dehydration either in the absence or in the presence of several stabilizing substances.

AFM allowed to directly visualize damage caused to supported lipid bilayers as a consequence of water removal. We compared the efficiency of dextran, dimethylsulphoxide, glucose, sucrose and trehalose in preserving the structural integrity of raft-exhibiting model membranes. Finally, our results shed some light on damage and alteration of the size and morphology of microdomains in membranes as a consequence of stressful drying conditions.

Towards Automatisisation of Nanotomography Imaging

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Abstract

Nanotomography is a layer-by-layer imaging technique based on scanning probe microscopy. We present our approach to automate the process for successive etching and imaging. Thermal drift and non-linearities of the piezo scanners make it difficult to image exactly the same spot of the specimen. We correct this problem by applying an appropriate offset to the piezo scanners which is calculated from the offset between two successive images using the cross correlation coefficient. As an example, we image a thin film of polypropylene with tapping mode scanning force microscopy and etch it successively with potassium permanganate. The etching and imaging is done in-situ in a liquid cell of a MultiMode SPM connected to reservoirs of the etchant and water for flushing after each etching step. The flow of the two liquids is controlled with solenoid valves which allow for an automated etching/flushing/imaging protocol. We will present first results and discuss our concepts for adjusting the imaging parameters to maintain a good imaging quality during the automated etching and imaging sequence.

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Poster

AFM Investigation of Tubular J-Aggregates Decorated with Ag Nanoparticles

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Abstract

J-aggregates of cyanine dyes are characterized by a strong absorption and fluorescence band that is significantly shifted towards the red compared to the monomer absorption. This property results from excitonic coupling of the transition dipoles of the dye molecules. It was shown by cryogenic transmission electron microscopy (cryo-TEM) for a special class of amphiphilic carbocyanine dyes, that they form tubular strands with a typical diameter of 10 to 20 nm and a length of micrometers [1]. These aggregates are decorated with silver (Ag) nanoparticles with a typical size of 5 - 20 nm. The metal particles act as fluorescence quencher that can be visualized by cryo-TEM or AFM. It is a challenging task to correlate the fluorescence intensity of single aggregates with the density and distribution of the quencher particles along the tubular aggregates.

We will present an AFM study to image the J-aggregates and the silver nanoparticles adsorbed at solid substrates. The particles can be identified by the material contrast in the phase images of intermittent contact images. A confocal microscopy setup is used additionally for local fluorescence spectroscopy of single aggregates.

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Glass tips completely coated by a thin metal layer - a powerful alternative to aperture SNOM probes

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Abstract

For biological applications, scanning near-field optical microscopy of single fluorescent dye molecules require probes which combine high near-field intensities with high optical and topographical resolution.

Such probes can be realised by glass tips completely covered by a thin metal layer. However, to achieve strong fields at the tip end, it is important to illuminate the tip under an inclined angle and with the polarisation parallel to the inclination plane. The optimal metal layer thickness and inclination angle are investigated by computer simulations. The achievable optical resolution is roughly given by the tip radius.

This kind of probes have been tested by imaging single fluorescent dye molecules. They show one or two peaked patterns, which can be explained by the electrical field distribution at the tip end.

This probe is especially suited for cantilever probes, where the inclined illumination easily can be realised.

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Poster

Lateral structuring of oxide surfaces by microcontact printing - A recipe for the reproducible generation of Fischer Projection Pattern like structures

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Abstract

High-resolution patterning is a major challenge in fabrication. Unfortunately, most methods aiming at structuring with nanometer dimensions are very expensive and time-consuming. Thus, it would be most valuable to use such a method only once to generate a master and consecutively reproduce this master with a less expensive method. An adequate replication technique is microcontact printing.

In this contribution, an alliance of microcontact printing with a self-assembled colloidal crystal is presented.

On the basis of a such a colloidal crystal, preferably a flat array of polystyrene spheres, structures commonly known as Fischer Projection Patterns (FPPs) can be produced by evaporating gold through the gaps between the spheres. Usually, for generating FPPs the employment of an evaporation system is needed for each sample. A much simpler way of manufacturing structures like this is to use a colloidal crystal as a master. In a first step, the master is replicated by pouring a liquid elastomer onto it and then curing the lot until copolymerisation is completed. The elastomer can then be lifted off and be used as a stamp for microcontact printing. Thereafter, it is easy to transfer the prominent parts of the stamp by inking it and subsequently pressing it onto a substrate, in this case gold. As this can be done many times, the merits of this method are obviously its swift and simple reproducibility, its applicability onto extensive areas and its abandonment of expensive and time-consuming equipment. Commonly, an alkanethiol, which forms a self-assembled monolayer on noble metal surfaces, is used as an ink. Such a monolayer is protective to certain etchants. Thus, a subsequent wet etch dissolves only bare gold areas, whereas areas protected by a thiol monolayer remain intact. What remains is a patterned surface resembling a Fischer Projection Pattern. Hence, the method presented in this contribution allows quick and reproducible lateral structuring of surfaces with FPP like structures.

By means of silanisation of the sample and a second etching step, the pattern can be inverted, providing a silane monolayer on silicon oxide. This structure barely has any differences in topography, but shows considerable differences in its mechanical and frictional behaviour. This was tested using atomic force microscopy.

High-speed AFM for imaging fast biological processes

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Abstract

We built a high-speed AFM using custom hard- and software. The scanning is controlled by a digital signal processor running custom code, allowing for fast digital feedback. Using this AFM, we acquired images of a compact disk surface at a rate of 5 images per second.

The aim of this high-speed AFM is to image fast biological processes in real-time. As one application, we investigate the two-dimensional motion of DNA on a mica surface. For this purpose, we optimized the imaging buffer so that the DNA adheres to the mica surface sufficiently for imaging, yet retains enough mobility for movements.

By using small cantilevers in conjunction with a special AFM head, the imaging rates can be increased even further.

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Study of the rehydration process of metaphase chromosomes by high time resolution AFM tracking

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Abstract

We investigated height and stiffness changes of human metaphase chromosomes during the process of rehydration in electrolyte solutions. To allow for high time resolution measurements over large time spans, we developed a tracking algorithm in which the AFM tip “locks on” to a chromosome and acquires a force curve every 3 s. This tracking algorithm compensates for both lateral and vertical drift, which are especially large after exchanging fluid. It compensates for the lateral drift by continuously locating the highest point of the chromosome, and for the vertical drift by continuously comparing the current force curve with the previous one, thereby keeping the maximum tip-sample force constant.

We observed that chromosomes increase their height and decrease their stiffness in form of a saturation curve during the rehydration process. The time until saturation is different for each chromosome and can last from 15 min for initially soft chromosomes to 7 h for initially very stiff chromosomes.

Imaging Structural Discontinuities in Myelinated Axons: An Approach to the Properties of Cajal Bands

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Abstract

Different types of discontinuous structures have been observed in myelinated axons since the initial studies of S. Ramón y Cajal and Pío del Río Hortega 100 years ago. Among those are the well-known nodes of Ranvier and the less well-known Cajal bands. Here, we investigate the Cajal bands with the purpose of elucidating their internal structure. We have imaged the micromechanical properties of isolated peripheral nerve fibrils from mouse using the AFM in the force volume mode. This type of measurement allows drawing conclusions about functional properties of the fibrils. Our preliminary results suggest that there are elastic variations within the Cajal bands, in agreement with the hypothesis that these structures supply flexibility for the growth and development of the axons.

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Scanning Probe Microscope (SPM) for the International Space Station ISS

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Abstract

The importance of nanotechnology in the disciplines of life and physical sciences has grown enormously since the first experiments two decades ago. Both the number of nanotechnological publications and the interest of the general public in these fields have steadily increased, and more and more industrial applications are being discovered and implemented each year. Consequently, orbital research of life and physical sciences on the ISS would greatly profit from the incorporation of experiments in the field of nanotechnology.

For this reason, in cooperation with Kayser-Threde GmbH and financed by ESA, we have developed a scanning probe microscope (SPM) capable of atomic and molecular scale imaging for the special requirements onboard the International Space Station ISS. In addition to a small and light stand-alone control electronics unit, it is equipped with a custom-designed suspension and locking system for use in a microgravity environment. Based on a proven instrument, the microscope was constructed with emphasis on compact dimensions, low weight and a straightforward design, whilst incorporating remote operating capabilities. Simple operation and maintenance of the SPM was also a major design factor.

With this instrument we plan to investigate biological samples and materials in-situ, as well as fundamental nanoscale self-assembly mechanisms, thus furthering both basic knowledge and application aspects of nanotechnology in microgravity. We are also interested in initiating new research projects as well as developing products which apply nanotechnology in the microgravity environment of space.

Frequency dependence of mechanical properties of thin polymer films

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Abstract

It is well known from measurements on bulk polymer samples that their mechanical properties reveal a certain frequency dependence. These materials have also been investigated on the nanometer scale with AFM techniques such as force-distance-curves at low frequencies (~ 1 Hz) and Pulsed-Force Mode at higher frequencies (~ 1 kHz).

The aim of our work has been the investigation of the frequency-dependent behaviour in the intermediate range. SFM force-distance-curves have been measured on thin films of PMMA (Polymethylmethacrylate) spin-coated on a silicon substrate at different frequencies (0.25 - 512 Hz). Both force-distance-curves and the subsequently scanned AFM images of the remaining indentations have been evaluated quantitatively in order to reveal the mechanical properties of the polymer. The experimental setup and the results of the measurements will be presented.

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Tapered pipettes as AFM probes for use in SICM

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Abstract

Scanning ion conductance microscopy (SICM) is an SPM-technique which allows measuring the local ion current over a sample. Usually, the ion current is used as feedback signal for imaging the sample topography. But for measuring the ion current independently of the sample topography, a complementary distance control mechanism is needed. Here we pursue an approach based on using tapered pipettes as AFM probes for use in SICM. We use both bent and straight pipettes, replacing the cantilever and the tip of an AFM. We managed to bend pipettes with a total taper length of 5 mm to form tips with about 100 μm length using a heating wire. In a first step, we imaged the topography of a CD surface with bent and straight pipettes with a resolution of 100 nm. We also plan to use these pipettes for scanning lithography.

Structural studies of oligonucleotides containing G-quadruplex motifs using molecular modeling

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Abstract

Studies of synthetic oligodeoxynucleotides (ODN) expressing unmethylated CpG motifs show that these molecules have immunoregulatory effects, being recognized as a pathogen-associated molecular pattern triggering a rapid immune response[1]. Structural analysis of interaction between CpG motifs and Toll-like receptors leads to a better understanding of how the mammalian immune system has evolved to selectively recognize pathogens, giving insights for the rationale use of DNA technology in DNA vaccines, gene therapy and in anti-cancer drug design [2].

Different kind of structural analysis can be complementary to elucidate this recognition mechanism. Atomic force microscopy (AFM) revealed that the tertiary structures formed by a novel type of CpG ODN are nucleic acid-based nanoparticles in the size range of viruses [3,4]. Based on atomic force microscopy (AFM) analysis it was proposed that monomeric CpG-A ODN form a duplex Watson-Crick base pairing of their palindrome sequences. Subsequently, four poly G ends of the two duplexes are linked through G-tetrads and further polymerization of quadruplexes takes place. [3,4].

In this project we use molecular modeling techniques to understand the structural behavior of synthetic CpG ODN in solution. Using model building and molecular mechanics studies we were able to construct the tertiary structure of the synthetic CpG ODNs based on a bi-dimensional model [4]. Four different types of units were constructed and dynamics simulations were performed to investigate stability and dynamic behaviour of these structures.

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Controlled Positioning of Biomolecules with AFM

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Abstract

We endeavor several methods to position single molecules and nanoparticles. As a basis for automated positioning on the nanoscale it is necessary to develop molecular handles that are switchable and allow the grabbing and dropping of nanometer size particles. Combined use of Atomic Force Microscopy and fluorescence methods allows a controlled exploration of several model handles. As a positioning grid we explore functionalized surface layer proteins that form a monolayer by self assembly, or DNA-functionalized glass.

Modification and imaging of surface charges on polymers using atomic force microscopy

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Abstract

The atomic force microscope (AFM) is widely used to investigate and modify mechanical properties of thin polymer films. Different static and dynamic modes like force curve measurements, Pulsed Force Mode or intermittent contact techniques apply certain amounts of normal and lateral forces to the sample. This implies the possibility to create surface charges, similar to the macroscopic contact electrification of insulators. These charges can be imaged in the 'Surface Potential' or 'Kelvin Probe Force' mode of the AFM.

The quantity of charge depends on parameters like contact time, force or velocity. By an adjustable tip voltage during contact, the amount and polarity can be controlled and charges can also be erased. The long-term stability of these surface charges, measured on different polymers at various temperatures, will be also in this work. In addition, some applications as high density data storage or creation of templates for molecule deposition on surfaces will be described.

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AFM investigations on bioengineered membrane channels

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Abstract

The membrane proteins FhuA and OmpF are promising candidates for controllable channels in artificial membranes. Mutants of these proteins have been designed, expressed and purified to create universal and switchable channels. The wild type proteins and their mutants have already been characterised from a biochemical point of view.

Atomic force microscopy will now be used to investigate structure and functionality of the proteins. Preliminary results of AFM on reconstituted proteins in buffer on a mica surface will be presented.

Combined optical and force microscopy

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Abstract

Atomic force (AFM) and optical microscopy are powerful tools for gaining information about structure and function of biomolecules and living cells. AFM is a technique capable of resolving molecular details of biological surfaces and performing force spectroscopy on receptor/ligand complexes, whereas fluorescence microscopy allows selective and specific visualization of labelled molecules, in particular in complex biological samples. In order to perform selective force microscopy or spectroscopy on specific cellular structures, we developed a combination of both methodologies: Fluorescently labelled features are identified in the optical microscope and subsequently characterized using AFM.

Here we present a first application of this instrument to study the lateral distribution of the Scavenger Receptor class B type I (SRBI) on Chinese Hamster Ovarian cells (CHO). This receptor has an important role in the uptake process of High Density Lipoprotein particles (HDL). A fusion construct with a green fluorescent protein (eGFP) was used to visualize the receptor in the fluorescence microscope. An antibody against the HDL receptor (anti-SRBI) was coupled to the AFM tip via a PEG-crosslinker. The fluorescence microscope allowed positioning of the functionalized AFM tips above the receptor clusters, resulting in a high binding probability of the antibody on the tip to the SRBI receptors at the plasma membrane. Control measurements in regions of the plasma membrane without positive eGFP signal yielded no specific binding events, which demonstrates the specificity of this assay.

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The microstructure of the lingulid brachiopods *Discradisca*, *Discinisca* and *Lingula*

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Abstract

The microstructure of the lingulid brachiopods *Discradisca*, *Discinisca* and *Lingula* were examined with SEM, TEM, Vickers microhardness indentation and EDX-analysis. The shell can be described as an organic/inorganic composite structure with varying organic content. The inorganic material is a hexagonal or at least pseudo-hexagonal calciumphosphate, most likely a carbonate substituted Apatite with an average size of 20 nm in diameter (usually slightly elongated). A texture can be seen which corresponds to the growth direction of the shell. Microhardness indentations show large variations in microhardness throughout the shell, but no anisotropic behavior occurs.

Frequency Response of magnetically driven cantilever in liquids

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Abstract

Our aim is to screen the possibility of using the second normal mode of a cantilever to improve the performance of existing MAC mode AFM in liquids. This work is inspired by a theoretical study (Garcia et. al, Appl. Phys. Lett., 84, 449, 2004) which describes compositional mapping of surfaces in air by excitation of the second normal mode. The nonlinear dynamics of the tip motion, the coupling of its first two modes, and the sensitivity of the second mode to long-range attractive forces make it possible to use this mode for probing compositional changes while the signal from the first mode is used to image the sample surface. We demonstrated that the second normal mode is applicable for imaging biological samples (Lysozyme, Human Rhino Virus II) in liquids, resulting in topography images comparable to those obtained with the first normal mode. The second normal mode was also used for TREC (=Simultaneous Topography and RECOgnition) imaging, (C.M. Stroh et. al, Biophys. J., 87, 1981, 2004; A. Ebner et. al, ChemPhysChem, 6, 897, 2005), using avidin with a biotin-functionalized tip.

For a quantitative analysis, the frequency response of a cantilever in liquids using MAC mode was investigated and compared to a theoretical model (Sader, J. Appl. Phys., 84, 64, 1998), in which the hydrodynamic problem was reduced to an infinitely thin rectangular beam oscillating in liquid far away from a surface. The model shows good agreement with the experimental data for large distances ($>40\ \mu\text{m}$) between tip and sample.

In addition, the dependence of Amplitude-frequency (tuning) curves on the tip-sample separation was experimentally determined. The tuning curves show a significant frequency shift to lower values for the first resonance peak with decreasing tip-sample separation, whereas the peak of the second normal mode is not affected. A theoretical model (Rankl et. al, Ultramicroscopy, 100, 301, 2004), which takes into account the interaction force due to the liquid that is squeezed out between the cantilever and sample surface describes well the distance-dependent value of the first normal mode, but fails in predicting the behaviour of the cantilever at the second normal mode. The theoretical description will be improved by combining the two models.

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Poster

Lithography of polymer thin films by acoustic force microscopy

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Abstract

Lithography of polymer thin films with increased lateral resolution is an area of intensive research due to its impact in technological and scientific applications. Actual feature size produced by standard fabrication methods applied in the industry is limited by the light diffraction limit. In the last years new methods for high resolution surface lithography has been developed, such as electron beam lithography or nanoimprint lithography. The most versatile approach to this problem is the use of a force microscope (Rubio-Sierra et al. 2005). By this method, feature size is only limited by the physical dimensions of the AFM tip, overcoming light diffraction limit. In this work we present a new technique to use the force microscope for surface lithography. We have used ultrasonic waves transmitted to the sample surface by an ultrasonic transducer to enhance the vibrations of a force microscope cantilever while the microscope imaging feedback is disconnected. By this method, the tip is intermittently indented into the polymer surface producing controlled plastic surface deformation. First results, together with the influence of process parameters in the size of the produced features, are presented in this work.

Rubio-Sierra, F.J., Heckl, W.M., Stark, R.W. (2005) *Nanomanipulation by Atomic Force Microscopy*, *Advanced Engineering Materials*, 7 (4).

Intermixed Patterns of Perylene Derivates on Ag(111)

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Abstract

Self-assembly of molecules on surfaces directed by different supramolecular interactions has been widely explored. There are striking examples of molecular surface structures, whose formation is driven by metal co-ordination [1], dipolar coupling [2] or hydrogen bonding [3]. In our approach we also made use of H-bonding to form well-ordered patterns of two different perylene derivates. These are the well known PTCDA (3,4,9,10-perylene-tetracarboxylic-dianhydride) and recently synthesized DPDI (4,9-diaminoperylenequinone-3,10-diimine) [4].

In a UHV setup, thin films of DPDI and PTCDA were prepared on Ag(111) by evaporation and were investigated with a room temperature STM. For a ratio of 1:1 and a total coverage of about one monolayer, an ordered intermixed pattern was observed. Annealing the sample up to 580 K resulted into an improved ordering, however induced no changes. Furthermore, different intermixed patterns were observed depending on the ratio of the molecules and on the total coverage. We assume that the hydrogen bond is formed between oxygen atoms of PTCDA and the hydrogen atoms of DPDI.

To confirm and enhance our results, measuring over a broader range of temperatures and scanning tunneling spectroscopy (point spectroscopy, dI/dV maps) measurements are of high interest.

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Scanning Ion Conductance Microscopy with Shear-Force Distance Control

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Abstract

We have built an improved scanning ion conductance microscope (SICM) for imaging the ion conductance over surfaces. The employed probe is a tapered micropipette with an opening diameter of less than 70 nm that is filled with an electrolyte. To obtain images of ion conductance independently of sample topography, we use a complementary shear-force distance control. Hereby the micropipette is mechanically oscillated in the liquid parallel to the sample surface at a fixed frequency (10-70 kHz). Shear forces are detected optically with the help of a focused laser beam. Samples as soft as cells can be imaged this way. We also demonstrated complementary imaging of ion current and topography by using porous polycarbonate membranes. The holes were clearly resolved in both ion current and topography. Optionally, we used active Q-control for minimizing the tip-sample forces. Furthermore, we applied advanced imaging modes such as the force mapping mode and recorded the ion current in all three spatial dimensions over the sample.

Structural and functional investigation of nuclear pore complex transport mechanisms with atomic force microscopy

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Abstract

Bi-directional transport of macromolecules between the cytoplasm and the nucleus proceeds through nuclear pore complexes (NPCs) of the nuclear envelope and is regulated by multiple families of soluble transport receptors and effectors. Most of this traffic is controlled by the small GTPase Ran, which regulates cargo-receptor complex formation. The transport is suggested to involve sequential binding of this complexes to different locations of the NPC. The detailed structure of the NPC is still under investigation and transport mechanics, dynamics, and selectivity are still under debate.

In this work we address some of these issues by using atomic force microscopy imaging and molecular recognition force microscopy (MRFM) measurements. Nuclear envelopes from *Xenopus laevis* oocytes were adsorbed to modified surfaces under physiological conditions. Topographical images of the NPC from the cytosolic side were acquired in air and in buffer solution, showing the eightfold symmetry of the NPC.

Molecular recognition force microscopy measurements of receptors bound to the AFM-tip using distensible poly(ethylene glycol) crosslinkers revealed binding sites on the NPC. The force-distance cycles between tip-bound receptors (e.g. Ran) and intact NPCs under physiological condition showed molecular recognition at the single molecule level.

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Pulling adsorbed polymers from surfaces with the AFM: Influence of polymer-surface friction

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Abstract

We consider the structure of an adsorbed polymer that is pulled by an AFM within a simple geometric framework. The three cases of i) fixed polymer-surface contact point, ii) sticky case where the polymer is peeled off from the substrate, and iii) slippery case where the polymer glides over the surface are treated. The resultant behavior depends on the value of the surface friction coefficient and the adsorption strength. The force profiles in principle allow to extract these two parameters from non-equilibrium force-spectroscopic data obtained by pulling the polymer either vertically or horizontally.

Functional Nanostructures for Electronic Devices with Macrocyclic Oligothiophenes

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Abstract

The aim of this project is to produce nanostructured surfaces on structured substrates adapted to molecular structures. Furthermore we want to study the local electric and optical properties of those surfaces.

Various series of self-assembly-structures of macrocycles Cyclo(terthiophen-diine), Cyclo(quinque-thiophen-diine) and Cyclo[n]thiophenes C[n]T were studied with STM. Different patterns and nanostructures for different molecule-symmetries and diameters (1-5nm) of the cycles were found.

The characterization of the devices will be done with a combined AFM/STM. A combined AFM/STM distance-control can avoid the damage of the tip in a nonconductive region of a sample. The concept of such an AFM/STM-combination is described. Combined with SNOM-techniques and/or confocal fluorescence microscopy an extensive electro-optical characterization will be possible.

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Nanoantennas and ultrafast spectroscopy: Towards time-resolved near-field optical microscopy

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Abstract

In the project presented, we aim to combine two concepts: Ultrafast spectroscopy, and local probe microscopy.

Ultrafast laser-spectroscopy gives insight into the (structural-) dynamics of molecules and on chemical reactions. Typically, such measurements are performed averaging an ensemble of objects. Scanning near-field optical microscopy allows specifically addressing single particles, to probe their optical response, and to manipulate them. From the combination, we expect to access the spectral response of single particles, and to track down dynamical channels usually blurred in ensemble averaging techniques.

For this purpose, we want use triangular silver nanoparticles as local optical probes taking advantage of their remarkable properties. These nanoantennas can be tailored to match a desired spectral response.

Results on the ultrafast dynamics of these particles probe by femtosecond transient absorption will be presented and discussed in the context of this project.

Solid-wetting self-assembly

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Abstract

Supramolecular self-assembly of uniform components usually produces structures with a high degree of symmetry. This excludes applications which require controllable structural complexity. In order to extend the range of applications for supramolecular self-assembly to complex systems, the controlled generation of asymmetrical patterns must be possible, which requires the ability to locally guide self-assembly processes on the molecular scale. This has so far been very challenging.

We describe a novel, locally guidable self-assembly process under ambient conditions, which enables dynamic features (reversibility, self-healing, self-contacting) with virtually insoluble molecules. We term this process “supramolecular solid-wetting”: similar to liquid droplets, solid nanocrystals of insoluble organic semiconductors wet a substrate surface, which results in the growth of surface-supported nanostructures under ambient conditions.

We show that this approach allows for the first time the guidance of supramolecular self-assembly locally at the nanoscale. No template is needed. In addition, the dynamic potential of supramolecular chemistry such as reversibility or self-contacting of surface supported nanostructures can be tapped with insoluble molecules such as organic semiconductors. This opens up the way to self-assembled nanodevices with predefined complexity and structural dynamics (e.g. reconfigurable, self-repairing and self-contacting devices).

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Hydrophobic recovery of SU-8 after O₂-plasma treatment

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Abstract

Micrometer structures for fluidic systems are often manufactured in polymers like SU-8 or PDMS, which both are highly hydrophobic. With decreasing dimension the role of the capillary forces become important. Thus, controlling the surface free energy and the wetting behaviour of the system becomes crucial [1]. To render polymeric surfaces hydrophilic an O₂ plasma treatment is commonly employed [2]. The influence of various gases in a RF discharge to the wetting behaviour and the surface structure of several polymers such as PMMA and polystyrene have been reported previously [3,4]. However, the effects on epoxy-based photo resists like SU-8 have not yet been examined in detail. Especially the evolution of the surface energy over time due to ageing effects is important for the calculation of the capillarity in bio-MEMS applications. To gain a better understanding of the hydrophobic recovery we carried out contact angle goniometry and surface energy measurements over time. Topographic imaging by atomic force microscopy (AFM) allowed the quantification of changes of the surface roughness induced by the plasma process.

The AFM measurements on the surfaces showed a drastic increase of the surface roughness by the plasma treatment. Granular nano-aggregates developed with a size depending on the treatment time. The effect of plasma treatment can be clearly seen by comparing AFM images of a SU-8 surface before and after plasma treatment. For all experiments the plasma power was kept constant at 150 W and 13,56 MHz. By the analysis of the height histogram the surface roughening can be demonstrated. Evaluating the maxima, a shift from 1 nm (before treatment) most frequently populated height to 10 nm (60 s plasma) and 20 nm (120 s plasma) is evident; saturation appears at 120nm after 4min exposure and more. The observation of the contact angle over several weeks indicated that after the O₂ plasma activation the hydrophobicity recovered within several days. Depending on the time the surface is exposed to the plasma, the contact angle remained super-hydrophilic (< 20) for a time span from a few hours to one week. The contact measurements indicate that the contact angle for water on SU-8 changed from over 70/90 (GBL / cyclopentanone solvent) to less than 4 after plasma treatment. Within several weeks after the activation the contact angle increased to reach a saturation value of about 60. Longer exposition time effected a slower hydrophobic recovery of the surface. To account for varying production conditions, different types of SU-8 (solvent: GBL or cyclopentanone), with or without hardbake and HF- or LF Plasma were investigated. The effect on the contact angle and surface topology appeared to be independent on changes in these production conditions.

We also determined the surface energy over time by measuring the contact angle not only for pure water (18.2 mN/m), but also for benzyl alcohol, glycerol and ethylene glycol. Before plasma activation, the surface energy of SU-8 was 35 mN/m and 30 mN/m for GBL and cyclopentanone SU-8, respectively. The initial surface energy after plasma activation (80 mN/m) decreased corresponding to the contact angles increase with a saturation value of 48 mN/m. For shorter plasma treatment time (60 s), the trend of the surface energy decay within the first days is faster than for longer treatment (120 s) approving the results obtained by contact angle goniometry.

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One Step ahead - Combining AFM and Optical Spectroscopy

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Abstract

Atomic Force Microscopy has developed into a potent tool for both imaging of surfaces with atomic or molecular resolution and single molecule force spectroscopy. It enables measurements in ambient condition and aqueous solution.

Optical spectroscopy techniques like Fluorescence Resonant Energy Transfer (FRET) can be used as a sensitive optical ruler in the nanometer range as the dependence of the distance between donor and acceptor is proportional to R^{-6} .

Simultaneous fluorescence and AFM measurements open up various new opportunities. The correlation of fluorescence and force data in single molecule force spectroscopy can bear supplementary information of (un-) binding events of guest-host-complexes or folding dynamics of proteins.

We present a new experimental setup capable of combining, optical and force spectroscopy or topographical measurements.

Probing the interaction between vesicular stomatitis virus and phosphatidylserine

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Abstract

The entry of enveloped animal viruses into their host cells always depends on membrane fusion triggered by conformational changes in viral envelope glycoproteins. Vesicular stomatitis virus (VSV) infection is mediated by virus spike glycoprotein G, which induces membrane fusion between the viral envelope and the endosomal membrane at the acidic environment of this compartment. In this work, we evaluated VSV interactions with membranes of different phospholipid compositions, at neutral and acidic pH, using atomic force microscopy (AFM) operating in the force spectroscopy mode, isothermal calorimetry (ITC) and molecular dynamics simulation. We found that the binding forces differed dramatically depending on the membrane phospholipid composition, revealing a high specificity of G protein binding to membranes containing phosphatidylserine (PS). In a previous work, we showed that the sequence corresponding amino acid 145-165 of VSV G protein was as efficient as the virus in catalyzing membrane fusion at pH 6.0. Here, we used this sequence to explore VSV-PS interaction using ITC. We found that peptide binding to membranes was exothermic, suggesting the participation of electrostatic interactions. Peptide-membrane interaction at pH 7.5 was shown to be specific to PS and dependent on the presence of His residues in the fusion peptide. The application of the simplified continuum Gouy-Chapman theory to our system predicted a pH of 5.0 at membrane surface, suggesting that the His residues should be protonated when located close to the membrane. Molecular dynamics simulations suggested that the peptide interacts with the lipid bilayer through its N-terminal residues, especially Val145 and His148.

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Stand-alone viscosimeter based on a microcantilever and an array detector

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Abstract

We developed a new stand-alone device for measuring the viscosity of transparent liquids. In this device, a column of liquid is vertically oscillated at 20Hz by using a piezoelectric actuator. A microcantilever, as it is usually used in atomic force microscopes, is positioned in the liquid and acts as a flow sensor. The viscosity of the liquid is derived from the oscillatory deflection of the cantilever. The deflection is measured optically by using an array detector that consists of a linear arrangement of 16 individual photodiode segments. This array detector allows measuring the intensity profile of the optical beam that is reflected from the cantilever. The sensitivity of the detection is therefore independent of the position of the optical beam on the detector. This characteristic is important for continuous measurements at different temperatures, where large thermal deflections can be compensated for automatically, without having to manually readjust the detector position. The shift of the beam on the detector in our setup amounts to approx. 0.18 mm/C.

We have measured the viscosity of aqueous glycerol solutions within a range of approximately 1-60 cP.

Advantages over commercially available viscosimeters (e.g., falling ball, air bubble, rotational viscosimeter) are a small required liquid volume (approx. 4 ml) and the possibility of viscosity measurement at small length scales.

Dynamic Imaging of single DNA-protein interaction using the Torsional Resonance Mode AFM

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Abstract

The Torsional Resonance mode (TR modeTM)¹ is a recently introduced technique similar to Shear Force Microscopy (ShFM) for Atomic Force Microscopy (AFM). In the TR mode, a cantilever tip vibrates in lateral direction comparable to the vertical vibration in tapping mode. The tip in the TR mode remains at a constant height and interacts very stiffly with the surface. Thus the phase signal changes with topography variation very sensitively and is a good tool for the measurement of the material properties. In this report, we measure the DNA-Protein interaction by the TR mode AFM. Atomic force microscopy (AFM) imaging of static DNA-protein complexes, in air, can be used directly to obtain quantitative and qualitative information on the structure of the different complexes. For instance, the location of the protein binding and bending of DNA as a result of the complex formation can all be measured. Our measurement shows that the improved dynamics of the TR mode AFM achieve better correlation between the topography and the phase image and higher phase contrast compared to tapping mode AFM.

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