8th Multifrequency AFM Conference

Book of Abstracts

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Force microscopy is one of the pillars that sustain the advances in nanoscience and nanotechnology. In the recent years, force microscopy has experienced a boom in its application range from academia to industry. However, the technique or more precisely the methodologies associated with AFM face numerous challenges to bring together molecular resolution, quantitative mapping and high speed imaging. To overcome those challenges will require developing new scientific approaches as well as novel engineering solutions. In this context, the AFM is experiencing the evolution from the single to the multifrequency excitation and detection schemes. This transition is also stimulated by the emergence of new topics such as energy storage and nanomedicine.

The Multifrequency AFM conference series started in September 2008, this is about ten years ago. The 8th meeting represents an excellent opportunity to gather some perspective about the evolution of the field. The overall goal of conference series has remained unchanged: to create the environment where experts and newcomers alike interact, exchange and share information, expertise and knowledge about the science and technology of the new generation of force microscopes.

The long term vision of the Multifrequency AFM conference series is to contribute to the expansion of nanomechanical tools and methodologies in academy and industry.

ORGANIZERS

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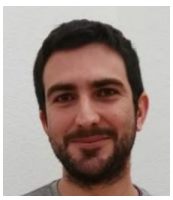

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http://www.icmm.csic.es/forcetool/

8th MULTIFREQUENCY AFM CONFERENCE SUMMARY

Session	Invited Speakers	Expert Speakers	Moderator		
Solid-Liquid Interfaces	Takeshi Fukuma	Kislon Voïtchovsky, Ing-Shouh Hwang	Ricardo Garcia	**Tuesday 27th October**	
Solid-Liquid Interfaces	Laura Fumagalli, Uri Sivan		Kislon Voïtchovsky		
Multifrequency methods	Roger Proksch		Julio Gómez	**Wednesday 28th October**	
Multifrequency methods		Andrew Fleming, David Haviland	Thilo Glatzel		**Parallel session**
Multifrequency methods		Miriam Jaafar	Pablo Ares		
Mechanical and Electrical Properties at the Nanoscale		Julio Gómez, Pablo Ares	David Haviland		
Novel Methods and Instrumentation	Olga S. Ovchinnikova		Agustina Asenjo		
Application talks			Manuel R. Uhlig		
Poster Session			Roland Bennewitz, Lorena Redondo-Morata, Christian Dietz		
High-Speed AFM Session	Peter Hinterdorfer	Noriyuki Kodera, Georg Fantner	Rubén Pérez	**Thursday 29th October**	
Machine Learning and Big Data		Matteo Chiesa	Manuel R. Uhlig		**Parallel session**
Nanomechanics			Paco Martínez		
Nanomechanics II			Manuel R. Uhlig		**Parallel session**
Mechanical and Electrical Properties at the Nanoscale			Agustina Asenjo		
Plenary Talk	Toshio Ando		Ricardo Garcia		
High-Speed AFM Session		James J. De Yoreo, Simon Scheuring,	Ricardo Garcia		
Nanomechanics		Hanna Cho, Jason Killgore	Roger Proksch		
Soft Matter and Cell Nanomechanics I		Tilman E. Schäffer, Rubén Pérez	Feliz Rico	**Friday 30th October**	**Parallel session**
			Maria C. Serrano		**Parallel session**
Soft Matter and Cell Nanomechanics II		Felix Rico, Christian Dietz	Tilman E. Schäffer		**Parallel session**
		Mingdong Dong	Lorena Redondo-Morata		**Parallel session**
Soft Matter and Cell Nanomechanics III	Arvind Raman	Robert Ros, Ozgur Sahin	Mingdong Dong		
Plenary Talk	Carlos J. Bustamante		Ricardo Garcia		

8th Multifrequency AFM Conference, October 27th-30th, 2020
Online Conference

Tuesday 27th October 2020

Time (Central European Time)	Duration (minutes)	Type	
Opening			
14:45-15:00	15	Welcome	**The Multifrequency AFM Conference Series** **Conference Chair:** Ricardo Garcia
Solid-Liquid Interfaces **Link:** to be announced			**Moderator:** Ricardo Garcia
15:00-15:25	25	Invited	Takeshi Fukuma, *Kanazawa University (Japan)*
15:25-15:50	25	Expert	Kislon Voïtchovsky, *Durham University (UK)*
15:50-16:15	25	Expert	Ing-Shouh Hwang, *Academia Sinica (Taiwan)*
16:15-16:35	20	Extended Oral	Roland Bennewitz, *INM Saarbrücken (Germany)*
Coffee Break: 16:35-16:55			
Solid-Liquid Interfaces II **Link:** to be announced			**Moderator:** Kislon Voïtchovsky
16:55-17:20	25	Invited	Laura Fumagalli, *University of Manchester (UK)*
17:20-17:45	25	Invited	Uri Sivan, *Technion (Israel)*
17:45-18:00	15	Oral	Manuel R. Uhlig, *ICMM, CSIC (Spain)*

Wednesday 28th October 2020

Multifrequency Methods

Link: to be announced **Moderator:** Julio Gómez

9:00-9:25	25	Invited	Roger Proksch, *Asylum Research (USA)*
9:25-9:45	20	Extended Oral	Daniel Ebeling, *JL University Gießen (Germany)*
9:45-10:00	15	Oral	Michael Ruppert, *University of Newcastle (Australia)*
10:00-10:15	15	Oral	Simone Benaglia, *ICMM, CSIC (Spain)*
10:15-10:30	15	Oral	Jonas Hafner, *TU Wien (Austria)*

Coffee Break: 10:30-11:00

Parallel Session: Multifrequency Methods

Link: to be announced **Moderator:** Thilo Glatzel				**Link:** to be announced **Moderator:** Pablo Ares			
11:00 - 11:25	25	Expert	Andrew Fleming *Univ. of Newcastle (Australia)*	11:00 - 11:20	20	Expert consolidator	Miriam Jaafar *UAM (Spain)*
11:25 - 11:50	25	Expert	David Haviland *KTH (Sweden)*	11:20 - 11:35	15	Oral	Daniel Platz *TU Wien (Austria)*
11:50 - 12:05	15	Oral	Eduardo Gil-Santos *IMN-CNM, CSIC (Spain)*				

15' break

Mechanical and Electrical Properties at the Nanoscale

Link: to be announced **Moderator:** David Haviland

12:20 - 12:45	25	Expert	Julio Gómez *Universidad Autónoma de Madrid (Spain)*
12:45 - 13:05	20	Expert consolidator	Pablo Ares *University of Manchester (UK)*

Lunch break: 13:05-15:00

Novel Methods and Instrumentation

Link: to be announced **Moderator:** Agustina Asenjo

15:00 - 15:25	25	Invited	Olga S. Ovchinnikova *Oak Ridge National Laboratory (USA)*
15:25 - 15:40	15	Oral	S. O. Reza Moheimani *University of Texas (USA)*
15.40 - 15:55	15	Oral	Lawrence Robins *NIST (USA)*

15' break

Application Talks			
Link: to be announced			**Moderator:** Manuel R. Uhlig
16:10 - 16:25	15	Oral	Andrea Cerreta *Park Systems Europe (Germany)*
16:25 - 16:40	15	Oral	Heiko Haschke *Bruker Nano (Germany)*
16.40 - 16:55	15	Oral	Daniel Forchheimer *Intermodulation Products (Sweden)*
16.55 - 17:10	15	Oral	Jonathan D. Adams *Nanosurf AG (Switzerland)*
20' break			
17:30 - 19:00	90	**Poster Session via Zoom** **Link:** Click Here **Poster Judges:** Roland Bennewitz, Lorena Redondo-Morata, Christian Dietz	

Thursday 29th October 2020

High-Speed AFM			
Link: to be announced			**Moderator:** Rubén Pérez
9:00 - 9:25	25	Invited	Peter Hinterdorfer *JKU Linz (Austria)*
9:25 - 9:50	25	Expert	Noriyuki Kodera *Kanazawa University (Japan)*
9:50 - 10:15	25	Expert	Georg Fantner *EPFL Lausanne (Switzerland)*
10:15 - 10:30	15	Oral	Víctor G. Gisbert, *ICMM CSIC (Spain)*
Coffee Break: 10:30-11:00			

Machine Learning and Big Data				Nanomechanics			
Link: to be announced		**Moderator:** Manuel R. Uhlig		**Link:** to be announced		**Moderator:** Paco Martínez	
11:00 - 11:25	25	Expert	Matteo Chiesa *Khalifa University (UAE)*	11:00 - 11:20	20	Extended Oral	Philippe Leclere *Université de Mons (Belgium)*
11:25 - 11:40	15	Oral	Jaime Carracedo Cosme, *UAM (Spain)*	11:20 - 11:35	15	Oral	Amir F. Payam *Ulster University (UK)*
				11:35 - 11:50	15	Oral	Guillaume Lamour *Université Paris-Saclay (France)*
				11:50 - 12:05	15	Oral	J.P. Cosas Fernandes *LIST (Luxembourg)*
10' break							

Nanomechanics II				Mechanical and Electrical Properties at the Nanoscale			
Link: to be announced		**Moderator:** Manuel R. Uhlig		**Link:** to be announced		**Moderator:** Agustina Asenjo	
12:15 - 12:35	20	Extended Oral	Thilo Glatzel *University of Basel (Switzerland)*	12:15 - 12:30	15	Oral	Neus Domingo *ICN2 Barcelona (Spain)*
12:35 - 12:50	15	Oral	Amelie Axt *MPI Mainz (Germany)*	12:30 - 12:45	15	Oral	Ilka M. Hermes

							Park Systems Europe (Germany)
12:50 - 13:05	15	Oral	Adrian Sanz-Jiménez *IMN CSIC (Spain)*	12:45 - 13:00	15	Oral	Sumit Agrawal *IIT Bombay (India)*
Lunch break: 13:05 – 14:00							
High-Speed AFM for Life Sciences **Link:** to be announced				**Moderator:** Ricardo Garcia			
14:00 - 14:45	45	Plenary	Toshio Ando *Kanazawa University (Japan)*				
15' break							

High-Speed AFM II **Link:** to be announced			**Moderator:** Ricardo Garcia
15:00 - 15:25	25	Expert	James J. De Yoreo *Pacific Northwest National Laboratory (USA)*
15:25 - 15:50	25	Expert	Simon Scheuring *Cornell University (USA)*
15:50 - 16:05	15	Oral	Shuai Zhang *University of Washington (USA)*
10' break			
Nanomechanics III **Link:** to be announced			**Moderator:** Roger Proksch
16:15 - 16:40	25	Expert	Hanna Cho *Ohio State University (USA)*
16:40 - 17:05	25	Expert	Jason Killgore *NIST (USA)*
17:05 - 17:20	15	Oral	Babak Eslami *Widener University (USA)*
17:20 - 17:35	15	Oral	Bahram Rajabifar *Purdue University (USA)*
10' break			
17:45 - 18:30	45	**Sponsor Event by Oxford Instruments: Webinar on High-speed AFM**	

Friday 30th October 2020
Symposium on Soft Matter and Cell Nanomechanics

Parallel Session: Soft Matter and Cell Nanomechanics I

Link: to be announced **Moderator:** Felix Rico				**Link:** to be announced **Moderator:** María C. Serrano			
9:00 - 9:25	25	Expert	Tilman E. Schäffer *University of Tübingen (Germany)*	9:00 - 9:15	15	Oral	Tanja Neumann *Bruker Nano (Germany)*
9:25 - 9:45	20	Extended Oral	Yuri M. Efremov *Sechenov University (Russia)*	9:15 - 9:30	15	Oral	Carmen Suay-Corredera *CNIC (Spain)*
				9:30 - 9:45	15	Oral	Thales F. D. Fernandes *INSERM, Université Montpellier (France)*
9:45 - 10:10	25	Expert	Rubén Pérez *UAM (Spain)*	9:45 - 10:00	15	Oral	Lorena Redondo-Morata *INSERM, Marseille (France)*

Coffee Break: 10:00-10:40

Parallel Session: Soft Matter and Cell Nanomechanics II

Link: to be announced **Moderator:** Tilman E. Schäffer				**Link:** to be announced **Moderator:** Lorena Redondo-Morata			
10:40 - 11:05	25	Expert	Felix Rico *Aix-Marseille Université, CNRS, INSERM (France)*	10:40 - 11:05	25	Expert	Mingdong Dong *INC, Aarhus University (Denmark)*
11:05 - 11:25	20	Expert Consolidator	Christian Dietz *TU Darmstadt (Germany)*	11:05 - 11:20	15	Oral	María C. Serrano *ICMM, CSIC (Spain)*
11:25 - 11:45	20	Extended oral	Francesco S. Ruggeri University of Cambridge (UK)	11:20 - 11:35	15	Oral	Marcos Penedo *Kanazawa University (Japan)*

Lunch break: 11:45– 13:45

Soft Matter and Cell Nanomechanics III

Link: to be announced **Moderator:** Mingdong Dong

13:45 - 14:10	25	Invited	Arvind Raman *Purdue University (USA)*
14:10 - 14:35	25	Expert	Robert Ros *Arizona State University (USA)*
14:35 - 15:00	25	Expert	Ozgur Sahin *Columbia University (USA)*
15:00 - 15:15	15	Oral	Clemens M. Franz *Kanazawa University (Japan)*
15:15 - 15:30	15	Oral	Alexander X. Cartagena-Rivera *National Institute of Health (USA)*

15' break

Biophysics – One Molecule at a Time

Link: to be announced **Moderator:** Ricardo Garcia

15:45 - 16:30	45	Plenary	Carlos J. Bustamante, *UC Berkeley (USA)*

End of Conference

8th Multifrequency AFM Conference Poster Session	
1	**Nonlinear Dynamics Perspectives on Intermodulation Atomic Force Microscopy** Arvind Raman *School of Mechanical Engineering, Purdue Univ., USA*
2	**Measurement of Surfaces at Cryostatic Temperatures with a Tuning Fork Cantilever Applying a Multifrequency Approach with Intermodulation Products** Marco Zutter *Department of Physics, University of Basel, Switzerland*
3	**Detector calibration using Intermodulation AFM** Daniel Forchheimer *Intermodulation Products AB, Landa Landavägen, Sweden* *Kungliga Tekniska Högskolan, Stockholm*
4	**Detecting non-linear forces on a cavity opto-mechanical sensor via multi-frequency lock-in measurement for AFM** Ermes Scarano *Nanostructure Physics, KTH Royal Institute of Technology, Sweden*
5	**Multi-frequency and Multidimensional Low Temperature UHV SFM** Hao Liu *Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland* *Department of Physics, University of Basel, Switzerland*
6	**Effects of Laser Spot Positioning with Optical Beam Deflection Method on Atomic Force Microscopes** Jesse Putnam *Mechanical and Aerospace Engineering Department, The George Washington University, USA*
7	**Variation of damping in non-smooth dynamic atomic force microscopy** Abhilash Chandrashekar *Department of precisions and microsystems engineering, TU Delft, Netherlands*
8	**Characterizing the Resolution Limits of Mechanical Resonators** Tomás Manzaneque *Department of Precision and Microsystems Engineering, Delft University of Technology, The Netherlands*
9	**Resonant mechanical force sensor based on cavity opto-mechanics** August Roos *KTH Royal Institute of Technology, Dept. of Applied Physics, Sweden*
10	**Metal AFM probes for measurements of friction** Michał Milczarek *Institute of Fundamental Technological Research, Poland*
11	**Customized MFM probes** Agustina Asenjo *Instituto de Ciencia de Materiales de Madrid, CSIC, Spain*
12	**MFM-KPFM characterization of magnetic nanocomposites for bioapplications** Agustina Asenjo *Instituto de Ciencia de Materiales de Madrid, CSIC, Spain*
13	**Nanoscale Analysis of Photocatalytic Reactions by In-liquid Local Potential Distribution Measurement Technique** Kaito Hirata *Kanazawa University, Japan*
14	**Study of the structural and conductivity properties of chemical delitiated LiCoO2 films by synchrotron XRD, SEM and C-AFM** Sara Quílez *Dpto. Física de la Materia Condensada, Universidad Autónoma de Madrid, Spain*
15	**Simultaneous viscosity and density measurement of small volumes of liquids using a vibrating microcantilever**

	Amir Farokh Payam *Department of Physics, Durham University, UK*
16	**Development of Fatigue Testing System for in-situ Observation of Micro/Nano scale Fatigue Mechanism by High Speed-AFM** Amir Farokh Payam *Department of Physics, Durham University, UK*
17	**Bimodal AM-FM nanomechanical mapping of Pentacene thin films** Sofia Drakopoulou *Departement of Life Sciences, University of Modena and Reggio Emilia, Italy*
18	**Effects of active screen plasma nitriding to the surface morphology** Dorina Kovács *Budapest University of Technology and Economics, Faculty of Mechanical Engineering, Department of Materials Science and Engineering, Hungary*
19	**Know your full potential: Quantitative Kelvin probe force microscopy on nanoscale electrical devices** Amelie Axt *Max-Planck-Institute for Polymer Research, Germany.* *Institute of Physics, Johannes Gutenberg University Mainz, Germany.*
20	**Mechanically soft domain walls in ferroelectrics** Christina Stefani *Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC* *Barcelona Institute of Science and Technology, Spain*
21	**Multi-scale characterisation of a semi-crystalline polymer reveals hidden ferroelectricity above the Curie transiton** Jonas Hafner[1], Simone Benaglia[2] *[1]Institute of Sensor and Actuator Systems, TU Wien, Austria* *[2]Instituto de Ciencia de Materiales de Madrid, CSIC, Spain*
22	**In-plane and out-of-plane nanomechanical characterization of HOPG at the atomic scale** Anna Lisa Eichhorn *Physics of Surfaces, Institute of Materials Science, Technische Universität Darmstadt, Germany*
23	**Nanomechanical Probing of Graphene-Liquid Interface using Dynamic Atomic Force Microscopy** Sanket Jugade *Centre for Nano Science and Engineering, Indian Institute of Science, India*
24	**Bottom-effect in atomic force microscopy nanomechanics** Stefano Chiodini *Instituto de Nanociencia de Aragón (INA), Campus Rio Ebro, Universidad de Zaragoza, Spain.* *Laboratorio de Microscopias Avanzadas (LMA), Campus Río Ebro, Universidad de Zaragoza, Spain* *Departamento de Química Física, Facultad de Ciencias, Universidad de Zaragoza Spain.*
25	**High-speed, Non-contact AFM Imaging of Nanoscale Silicon Structures** Solomon Davis *Technion – Israel Institute of Technology, Faculty of Physics, Technion City, Haifa, Israel*
26	**ESCRT-III spirals are loaded springs that govern spontaneous membrane deformation** Alma P. Perrino *Anesthesiology, Physiology and Biophysics, Weill Cornell Medicine, USA*
27	**Development of 3D-AFM for visualizing 3D structures of inside of chromosomes with nanometer-scale resolution** Keisuke Miyazawa *Kanazawa University, Japan* *WPI-NanoLSI, Kanazawa University, Japan*
28	**The mechanics of single cross-links which mediate cell attachment at a hydrogel surface** Roland Bennewitz *INM – Leibniz Institute for New Materials, Germany*

29	**Carcinomas with occult metastasis potential: Diagnosis/prognosis accuracy improvement by means of force spectroscopy** Anahid Amiri *Physics of Surfaces, Institute of Materials Science, Technische Universität Darmstadt, Germany*
30	**Biomechanical properties of the human lens capsule assessed with AFM and nanoindenter** A.A.Frolova *Institute for Regenerative Medicine, Sechenov University, Russia*
31	**Bacterial cell wall mechanical damage studied by simultaneous nanoindentation and fluorescence microscopy** Adrián Del Valle *Madrid Institute for Advanced Studies in Nanoscience (IMDEA Nanoscience), Spain*
32	**Characterizing nanomechanical properties of comedones after treatment with sodium salicylate** Zeinab Al-Rekabi *National Physical Laboratory, Hampton Rd, TW11 0LW, Teddington*
33	**Correlation at the nanoscale of chemical, structural and conductivity properties of non-stoichiometry Li-ion battery cathodes** Jesus Díaz *Dpto Física Materia Condensada, Universidad Autónoma de Madrid, Spain*

INVITED SPEAKERS

MULTIFREQUENCY AFM			
	**Laura Fumagalli** The University of Manchester	Anomalously low dielectric constant of confined and interfacial water	**Tuesday 27th October**
	**Takeshi Fukuma** Kanazawa University	Visualizing Inside of 3D Self-Organizing Systems by 3D-AFM	
	**Uri Sivan** Russell Berrie Nanotechnology Institute	Can AFM teach us anything new about DNA?	
	**Roger Proksch** Asylum Research	Multifrequency results, challenges and opportunities in quantitative nanoelectromechanics	**Wednesday 28th October**
	**Olga S. Ovchinnikova** Oak Ridge National Laboratory	Unravelling the Origins of Functionality through Correlative Multimodal Chemical Imaging	
	**Peter Hinterdorfer** Johannes Kepler University	IgG walking and oligomerization on antigenic surfaces	**Thursday 29th October**
	**Toshio Ando** Kanazawa University	High-speed AFM for life sciences	**Thursday 29th October Plenary Talk**

SYMPOSIUM ON CELL AND SOFT MATTER NANOMECHANICS			
	**Arvind Raman** Purdue University	Recent advances in AFM tip-living cell nanomechanics	**Friday 30th October**
	**Carlos J. Bustamante** University of California	Biophysics, One Molecule at a Time	**Friday 30th October** **Plenary Talk**

ORAL PRESENTATIONS

Visualizing Inside of 3D Self-Organizing Systems by 3D-AFM

Takeshi Fukuma
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Recently, three-dimensional atomic force microscopy (3D-AFM) has been proven to be a powerful tool for investigating various structures and phenomena at solid-liquid interfaces [1,2]. In the method, a tip is scanned in the XY and Z directions in a 3D interfacial space. During the tip scan, the variations in the force applied to the tip is recorded to produce a 3D force image. At a solid-liquid interface, the tip interacts with surrounding solvent molecules during the tip scan. Thus, the obtained 3D image represents the distribution of solvent molecules. So far, the method has been used for visualizing 3D hydration structures on minerals, organic thin films, and biological systems with subnanometer-scale resolution. This emerging technology has attracted attention due to its potential applications in the research on interfacial control technologies for anti-fouling, lubrication, anti-freezing, colloidal dispersion, cosmetics and cleaning. In the meanwhile, here I would like to draw attention to another important implication of the success of the 3D hydration measurements. In the AFM community, it has been a common sense that we should fix atoms or molecules to a solid surface to visualize them with atomic or molecular resolution. However, 3D-AFM allows us to visualize subnanometer-scale distribution of mobile water molecules that are not fixed on a solid surface. This is a big surprise and may lead to the breakthrough for the aforementioned limitation of AFM. Then, the next question would be what is the requirements to be visualized by 3D-AFM. We believe that the answer is capability of self-organization. For example, in the case of 3D hydration measurements, the hydration structure is significantly disturbed during the vertical tip scan yet it is quickly recovered before starting the next vertical scan. Such a self-organization capability is essential for visualizing inside of 3D structures. One may think this is too severe condition yet we can find large number of important 3D self-organizing systems in both natural and artificial environments. Examples range include interfacial phenomena and devices (hydration, lubrication, electric double layer devices and liquid crystal devices) to biological systems (cells, nucleus, chromosomes and proteins). 3D-AFM may allow us to directly visualize inside of these various 3D self-organizing systems. Based on this idea, we have recently started to explore inside of various 3D self-organizing systems. At polymer-water interfaces, gel-phase polymer chains with a thickness of a few nanometers were visualized. At an ionic liquid - Au electrode interface, ordered ionic liquid distributions with ~5 nm thickness were visualized. Furthermore, a carbon nanotube (CNT) tip was developed and used for visualizing inside of chromosomes with a thickness higher than 500 nm. Finally, a focused ion milled Si needle probe was fabricated and used for visualizing inside of a live cell with a thickness of several microns. With these examples, here I would like to propose to apply 3D-AFM not only for visualizing hydration structures but also for imaging inside of various 3D self-organizing systems.

[1] T. Fukuma, Y. Ueda, S. Yoshioka, H. Asakawa, Phys. Rev. Lett. 104 (2010) 016101.
[2] T. Fukuma, R. Garcia, ACS Nano 12 (2018) 11785−11797.

Nanoscale mapping of the directional flow patterns at liquid-solid interfaces

L. Piantanida[1,2], A. F. Payam[1,3] and K. Voïtchovsky[1]

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The dynamics of liquid molecules and solutes along the interface with solids controls processes such as molecular exchanges, electrochemistry, nanofluidics, biomolecular function and lubrication. Depending on the nanoscale topography and chemistry of the solid, the liquid can locally adopt specific flow patterns when subjected to an external perturbation. Uncovering these flow patterns could provide unprecedented insights into the *dynamics* of interfacial processes and guide the design of functional interfaces.

Here we describe a novel approach based on atomic force microscopy to quantify the preferred flow direction naturally adopted by liquids at interfaces, locally and with nanometre precision. The approach combines a vertically oscillating tip with high frequency lateral oscillations to derive directional dissipation maps of the interfacial liquid. These dissipation maps can be converted into nanoscale flow charts parallel to the surface of solids [1]. By using small lateral oscillations, the high-resolution capabilities of modern AFM in liquid are preserved.

To illustrate the method's capabilities, we investigate the dynamics of aqueous solutions containing either KCl or $MgCl_2$ along the surface of a same graphene oxide flake. We show that that dissolved K^+ ions move evenly in all direction whereas Mg^{2+} ions favour specific directions that are in registry with the underlying graphene lattice. The results provide *in-situ* nanoscale insights into the ion-specific permeation through graphene oxide membranes [2, 3]. The approach is possible with commercial atomic force microscopes and can be applied across a variety of soft and hard interfaces.

[1] Ortiz-Young, D., Chiu, H. C., Kim, S., Voitchovsky, K., & Riedo, E. Nat. Commun., **4**, 2482 (2013).

[2] B. Mi, Science **343**, 740 (2014)

[3] J. Abraham, K. S. Vasu, C. D. Williams, K. Gopinadhan, Y. Su, C. T. Cherian, J. Dix, E. Prestat, S. J. Haigh, I. V. Grigorieva, P. Carbone, A. K. Geim, and R. R. Nair, Nat. Nanotechnol. **12**, 546 (2017)

Investigation of Row-Like Structures at the Interface between Water and Graphite

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With high-sensitivity atomic force microscopy (AFM), we identified a special type of row-like structures at the interface between water and highly oriented pyrolytic graphite (HOPG) at room temperature. These structures are difficult to be detected with conventional AFM techniques, such as the tapping mode or contact mode. It usually takes many minutes for nucleation of the ordered structures and the domains grow slowly over tens of minutes at the interface (Fig. 1). Our early AFM study suggested that formation of these structures might be related to N_2 molecules dissolved in water [1,2]. However, it is very unlikely that the weak interaction among N_2 molecules can form any ordered structure at room temperature. Our further study of nucleation of nanobubbles at the water-HOPG interface suggests that the row-like structures might be interfacial gas hydrates [3]. These ordered structures also play a crucial role in the formation of nanobubbles as well as in pinning of nanobubbles from movement in their lateral position [3,4]. Our study suggests that the row-like structures form through complicated bonding arrangement of dissolved N_2 and water molecules at the interface. We have been looking for methods to provide direct evidence to support this concept of interfacial gas hydrates. Recently, we find that the row-like structures survive after removal of water, and they even survive under ultra-high vacuum. We have conducted x-ray photoemission spectroscopy several times and consistently obtained strong intensity of oxygen and weak intensity of nitrogen. We have also performed thermal desorption experiments and obtain strong intensity of water (mass 18) and weak intensity of N_2 (mass 28). These experiments support the concept of interfacial gas hydrates for the row-like structures.

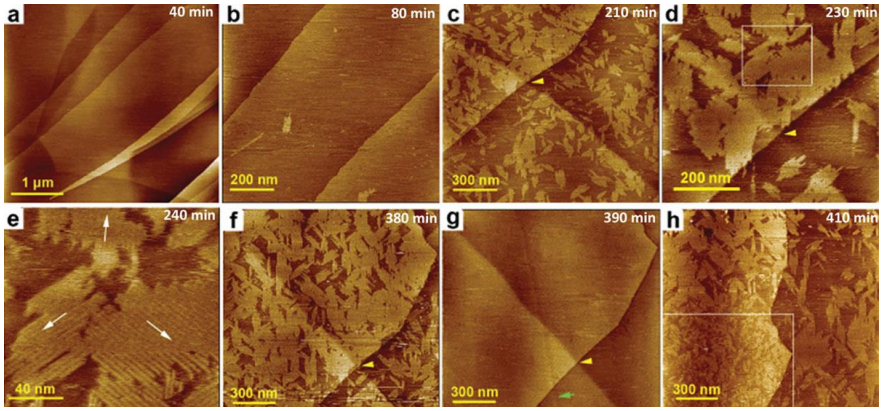

Fig. 1 AFM images of the evolution of the ordered structures at the water/graphite interface over time. All the images are acquired with the frequency-modulation AFM except for the one shown in (g), which is acquired with the tapping mode.

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[3] C.-K. Fang, H.-C. Ko, C.-W. Yang , Y.-H. Lu, I.-S. Hwang, Sci. Rep. **6**, 24651 (2016)
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Dynamic shear force microscopy of a nanometer-confined ionic liquid

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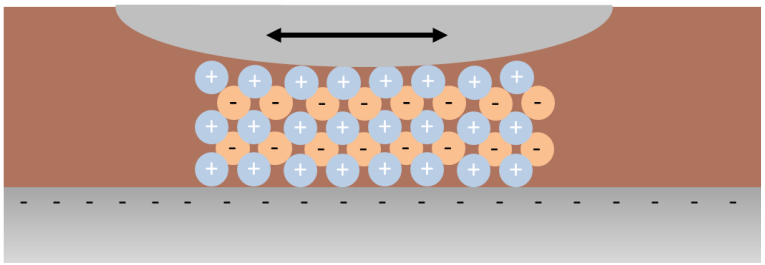
To study the shear properties of ionic liquid films, we implemented an AFM-based rheology with magnetically activated lateral tip oscillation. The ionic liquid [EMIM][NTf2] solidifies into a simple cubic structure upon confinement, where details of the shear properties depend on the electrochemical potential applied to the interface. The periodic potential of the solidified liquid can be extracted from frequency modulation signals using Giessibl's classical formula for Dynamic Force Microscopy. We confirm a generic mechanism of electrolubrication by ionic liquids, namely the shift of the slippage plane from the interface into the liquid with increasing surface charge [1].



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Anomalously low dielectric constant of confined and interfacial water

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In this talk, I will briefly review the scanning probe microscopy techniques [1] with which we measured the dielectric constant (or permittivity) of a variety of bio and non-bio materials in dry [2-5] and liquid [6] environment. I will then present our current work in which we addressed the case of the dielectric constant of confined and interfacial water [7], a scientific question that has vexed the scientific community for decades. Our experiments on water-filled nanochannels made of van der Waals crystals revealed the presence of an interfacial water layer with vanishingly small polarization. The electrically dead layer was found to be two-to-three molecules thick, in good agreement with molecular dynamics calculations. Our findings provide much needed feedback for theories describing water-mediated surface interactions and behaviour of interfacial water near surfaces, and show a way to measure the dielectric properties of other fluids and solids under extreme confinement.

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Can AFM teach us anything new about DNA?

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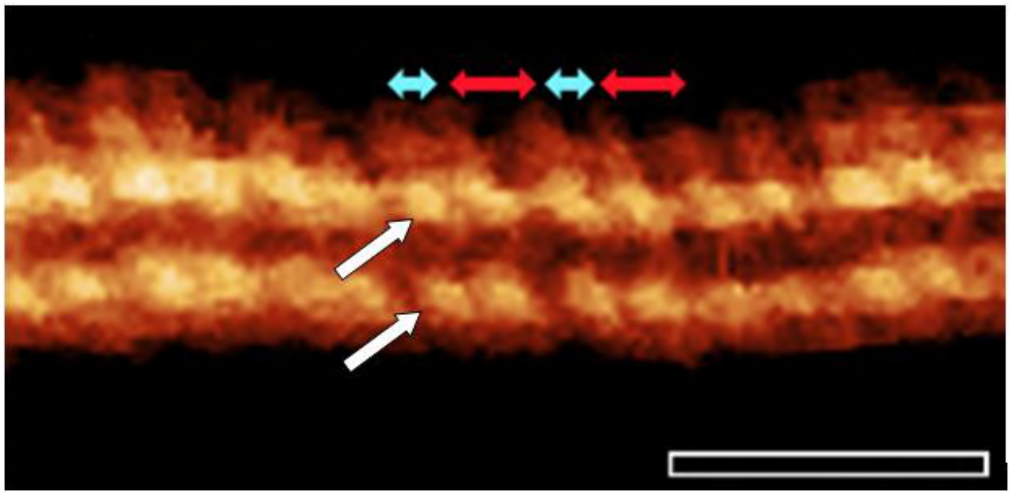
The current understanding of DNA and DNA−protein complexes' structure relies on crystallography and NMR spectroscopy, two bulk techniques that average out structural variations between individual molecules. These techniques are also restricted to a subset of short DNA sequences, typically, ∼10 base long, about a single turn of the helix. Furthermore, crystallography requires DNA crystallization in nonbiological solutions, and the crystallized molecules are exposed to significant packaging forces that may affect their native structure. The relevance of the resolved structures to long DNA molecules in their native environment is therefore debatable. Some of these limitations can be alleviated by atomic force microscopy of single molecules. Atomic resolution imaging and 3d mapping of hydration layers have been demonstrated in recent years with AFM probing atomically flat hard surfaces. This resolving power should in principle suffice to study hydration layers and molecular structure of long native DNA molecules, except imaging of soft molecules is far more challenging. Motivated by the lack of other tools, we have invested the recent years in developing AFM imaging of DNA to the point where valuable new information can be extracted. In the talk I will present two such examples. One resolving ordering of individual water molecules along DNA molecules and the other focusing on sequence-dependent deviation of constrained individual DNA molecules from the canonical B-form based on crystallography and NMR studies of bulk material. Both results rely on the development of an ultra-low noise FM-AFM, supplemented with a unique algorithm for tuning its feedback parameters.



This image showcases one of the highest resolutions currently attainable by AFM scanning of DNA in liquid at room temperature. Individual phosphates along the DNA backbone are revealed (white arrows), as well as minor and major grooves (cyan and red arrows, respectively). Scale bar, 10nm.

Atomic-scale mapping of hydrophobic layers on MoS2, MoSe2, WS2, WSe2 and graphene immersed in water

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Solid-liquid interfaces are highly relevant for a number of phenomena in nature and technology. However, when it comes to hydrophobic surfaces, the structure of water in close proximity to the surface and its role in mediating interactions are not well understood. Two dimensional materials provide a variety of large and atomically flat surfaces that are mildly hydrophobic. We exploited the angstrom resolution capabilities of three-dimensional AFM [1,2] to image the interfacial water organization on MoS2, MoSe2, WS2, WSe2 and graphene. We demonstrate that these interfaces are characterized by layers of oscillating density within 2 nm above the solid surface (Fig. 1a). The distances between adjacent layers for MoS2, MoSe2, WS2 and WSe2 and graphene are ≈ 0.50 nm (Fig. 1b). This value is larger than the one predicted and measured for water density oscillations (≈ 0.30 nm) [2,3]. The experiments indicate that on 2D material surfaces water molecules are expelled from the vicinity of the surface and replaced by several molecular-size hydrophobic solvation layers.

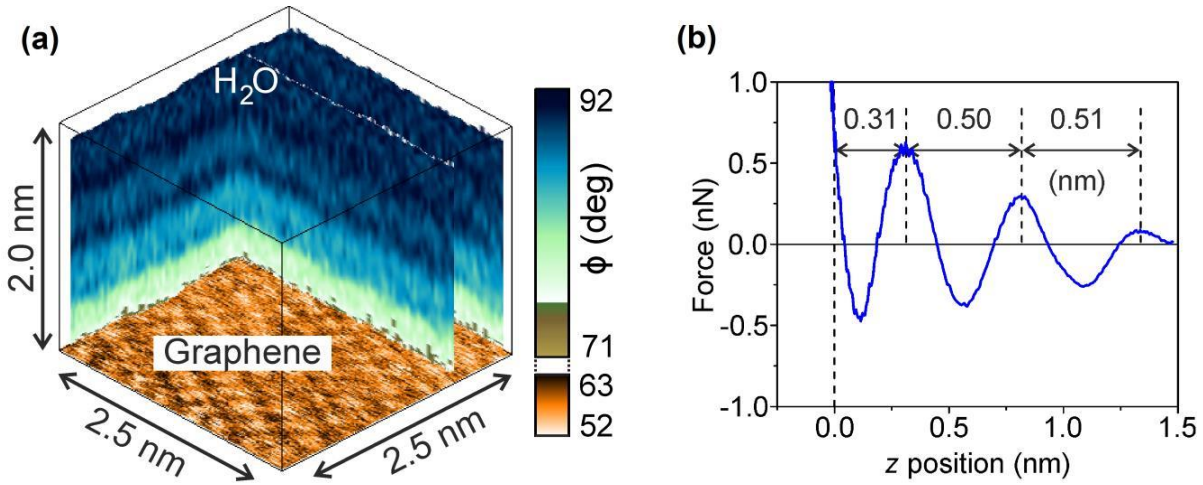

Fig 1: (a) Three-dimensional AFM image of the graphene-water interface. (b) Representative force curve obtained in close proximity to a two-dimensional material. Adapted from [4]

[1] T. Fukuma, Y. Ueda, S. Yoshioka, and H. Asakawa, Phys. Rev. Lett. **104**, 016101 (2010)
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Multifrequency results, challenges and opportunities in quantitative nanoelectromechanics

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PFM is a dynamic voltage-modulated electromechanical characterization technique where an oscillating potential is applied to a conductive tip in contact with an electromechanically active sample and the amplitude and phase observables of the resulting deformation change are measured. Information about electromechanical properties of the sample. In addition, DC voltages can be used to change the polarization strength and orientation of ferroelectric domains. PFM has been used to characterize a wide variety of electromechanically active materials including thin films, ceramics, polymers, energy storage and biological materials. Recent challenges in electromechanics involve thinner films and weaker materials. One well-known challenge for voltage modulated microscopy long-range electrostatic coupling between the sample and the body of the cantilever. It is nearly ubiquitous and is unfortunately synchronous with the PFM signal. Because of this, it becomes a background signal that is dependent on the tip-sample position, drive frequency, applied bias and AFM optical detector spot position. PFM measurements of piezo- and ferroelectrics with effective inverse piezoelectric sensitivities of $d_{eff} > 100$ *pm/V* tends to be relatively tolerant of this long-range electrostatic coupling. However, many of the materials now being studied and of relevance to semiconductors, photovoltaics and energy storage have sensitivities that are much smaller; $d_{eff} < 10$ *pm/V*. For many samples around that range of d_{eff} or lower, electrostatic coupling transitions from merely annoying to dominant, overwhelming the smaller signals from voltage modulated strain and effectively preventing quantitative and sometimes even qualitative PFM. Worse yet, if those long-range forces are time dependent or have some other source of hysteresis – as they are in many sample systems containing water and mobile ions – they can lead to measurement artifacts that can be mistaken for localized ferroelectricity. Recently,[1] we have shown how to both qualitatively evaluate the presence and order of magnitude of long-range electrostatic forces. In addition, by using a novel interferometric AFM,[2] we demonstrated that the false PFM signal can be effectively eliminated. An additional benefit of this approach is that allows truly quantitative limits on electromechanical strain. In this presentation, I will review the multifrequency dynamics and measurement pitfalls of strongly coupled, electrically driven cantilevers along with presenting a variety of new results on samples of both academic interest and practical, industrial relevance. These include the electromechanical response of photovoltaic methyl-ammonium lead iodide (MAPI),[3] AlN, PVDF and the piezo and ferroelectric response of thin film, Si-compatible Hf-based materials where we have quantified and unequivocally observed the onset of localized ferroelectricity with decreasing film thickness.

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High resolution imaging of organic molecules using Q-controlled amplitude modulation atomic force microscopy with CO-functionalized tips

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The so-called bond imaging atomic force microscopy (AFM) technique has become an invaluable tool for studying organic molecules on surfaces. The key feature of this technique is to functionalize the AFM-tip with a single molecule, e.g., CO. Hereby, the imaging capabilities of dynamic mode AFM are improved, which allows to determine the precise orientation and internal structure of adsorbed organic molecules. [1] Usually, these measurements are performed by operating tuning fork sensors in frequency modulation mode at low temperatures in ultrahigh vacuum conditions. The high quality factors of the tuning fork sensors under these conditions typically prohibit operation in amplitude modulation mode due to the slow response time caused by the low damping environment. Recently, it has been shown that amplitude modulation can be successfully applied under ultrahigh vacuum conditions to robustly image clean surfaces by tuning the effective quality factor of the tuning fork sensor. [2] Here, we use the *Q*-control technique for imaging organic molecules with CO-functionalized tips. By reducing the effective quality factor of the sensor from about 30000-20000 to 3000-1500 we are able to achieve lateral resolution and signal to noise performance comparable to frequency modulation imaging. In addition, we perform a systematic analysis of the imaging parameters, including oscillation amplitudes and tip-sample distances.

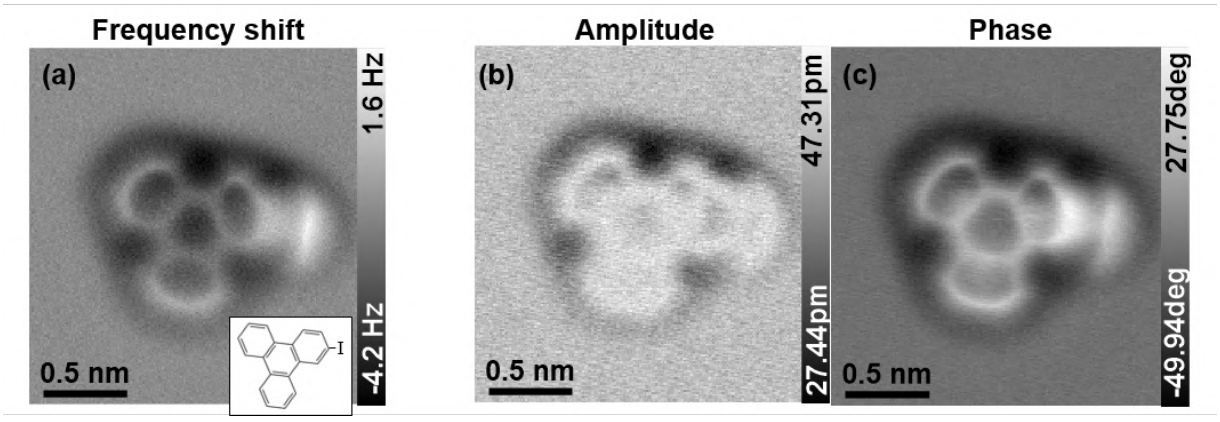

Figure 1: Constant height AFM images of 2-iodotriphenylene on Ag(111) imaged with a CO-functionalized tip in (a) frequency modulation and (b,c) *Q*-controlled amplitude modulation mode.

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Traditional and Novel Demodulators for Multifrequency Atomic Force Microscopy

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A number of multifrequency atomic force microscopy (MF-AFM) methods make use of the excitation and detection of higher harmonics of the fundamental frequency, higher flexural eigenmodes or intermodulation products generated by the non-linear tip-sample force [1]. Schematically, these methods are depicted in Figure 1(a) where the main difference is the resulting spacing and amplitude of the frequency components in the generated spectrum shown in Figure 1(b). Regardless of which particular MF-AFM method is employed, each requires a demodulator to obtain amplitude and phase to form observables for the characterization of nanomechanical sample information. Since high-speed non-synchronous demodulators such as the peak-hold method, peak detector and RMS-to-DC converter are incompatible with MF-AFM [2], there is a need for high-bandwidth demodulation techniques capable of estimating multiple frequencies at once while maintaining robustness against unwanted frequency components [3].

In this talk, the performance of traditional and recently proposed demodulators for multifrequency atomic force microscopy is assessed experimentally. The compared methods include the lock-in amplifier, coherent demodulator, Kalman filter, Lyapunov filter, and direct-design demodulator. Each method is implemented on a field-programmable gate array (FPGA) with a sampling rate of 1.5 MHz. The metrics for comparison include implementation complexity, the sensitivity to other frequency components and the magnitude of demodulation artifacts for a range of demodulator bandwidths. Performance differences are demonstrated through higher harmonic atomic force microscopy imaging.

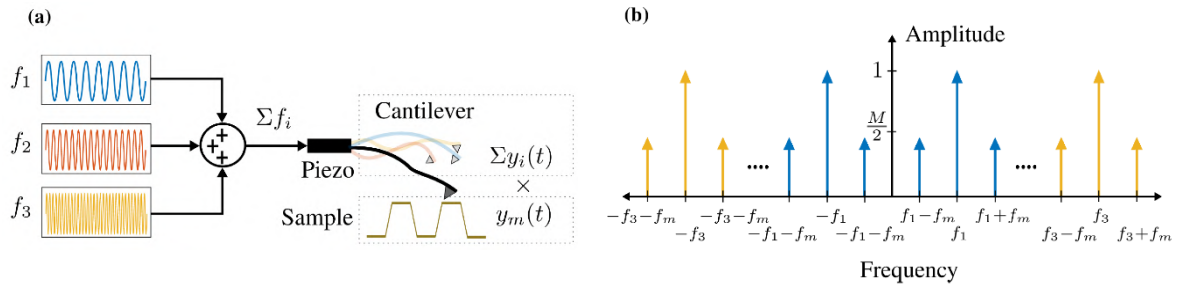

Figure 1: (a) Schematic diagram and (b) double-sided amplitude frequency spectrum of a cantilever oscillating at multiple frequencies amplitude modulated by a sample topography.

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[3] D. M. Harcombe, M. G. Ruppert and A. J. Fleming, Beilstein J. Nanotechnol. **11**, 76-91 (2020)

Fast, quantitative and high resolution mapping of viscoelastic properties with bimodal AFM

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Quantitative mapping of viscoelastic properties of soft matter with a nanoscale spatial resolution is an active and relevant research topic in atomic force microscopy (AFM) and nanoscale science characterization. Dissipative processes related to the viscoelasticity of soft materials have been thoroughly studied in AFM experiments, though models to convert these processes into sample's properties are not trivial [1]. Here we develop a theory to transform the energy dissipation values associated with viscoelastic interactions to material properties. We show how bimodal AM-FM [2] is applied to extract, using a Kelvin-Voigt model, several viscoelastic parameters such as the Young's modulus, the viscosity coefficient, the retardation time or loss tangent and the true topography of a poly(styrene-block-methylmethacrylate) (PS-b-PMMA) copolymer. We then develop a way to validate the accuracy of bimodal AFM experiment to determine the viscoelastic parameters through comparison with computer simulations [3]. Finally, we perform temperature dependence measurements of a semicrystalline polymer (PVDF-TrFE) which we compare with DMA experiments [4]. This allows us to directly relate the mechanical parameters extracted with bimodal AFM on the nanostructures of the polymer to the mechanical characteristics of the bulk sample.

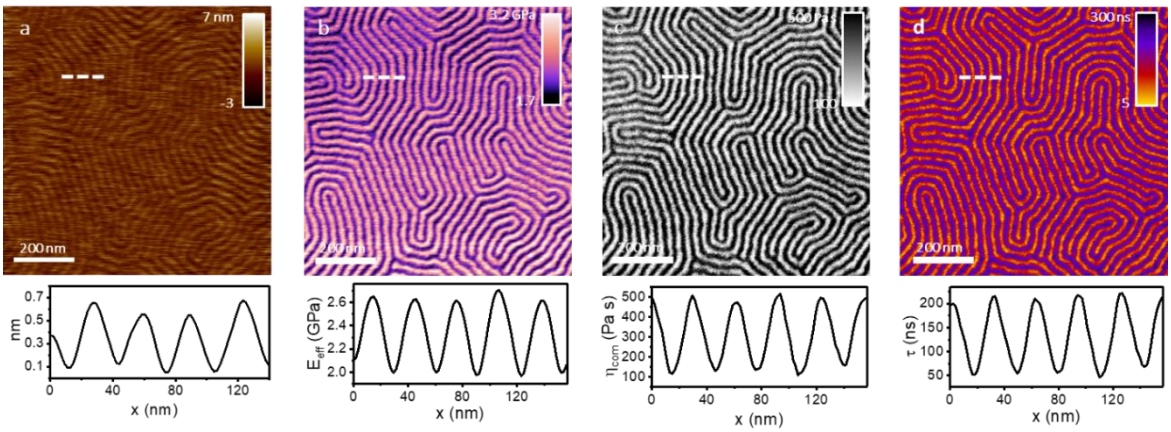

Fig.1 Apparent, deformation and true topography and bimodal nanomechanical maps of a PS-b-PMMA block co-polymer. (a) True topography. (b) Young's modulus. (c) Viscosity coefficient. (d) Retardation time.

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Scanning probes with high resonance frequency and low stiffness for high-speed AFM applications in liquid environments

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Conventional scanning probes for high-speed AFM applications are free-standing beams vibrating in one-dimensional modes, i.e. Euler Bernoulli modes. However, in these types of scanning probes high resonance frequencies and low spring constants are not compatible which limits their applicability to biological samples. Here, we present a novel concept for high-speed scanning probes, which combines both high resonance frequencies and low spring constants. Instead of a narrow beam oscillating in a one-dimensional Euler Bernoulli mode, we use a micromachined plate oscillating in a two-dimensional mode (see Fig. 1a,b). These non-conventional plate modes exhibit high resonance frequencies of more than 1 MHz in liquids[1,2]. The plate itself is not suitable as probe for high-speed AFM applications of soft or biological samples, since the spring constant k_p of two-dimensional plate modes can be in the order of 10^4 N/m. To circumvent this problem, we attach a short narrow beam at the centre of the free end of the plate. The beam consists of a soft material such as gold which leads to a low spring constant k_b of less than 0.1 N/m. Consequently, the effective stiffness k_{eff} of the two coupled microstructures in series is consistently lower than k_p or k_b. The beam equipped with a tip scans the surface. While scanning, the plate oscillates at a resonance frequency of a non-conventional mode and the attached short cantilever quasi-statically follows the movement of the plate. To demonstrate the performance of proposed probes, we performed high-speed AFM measurements of a biological sample in a buffer solution, i.e. in a liquid environment. We demonstrate for the first time high-speed AFM measurements using a scanning probe with a high resonance frequency of 1.02 MHz in the buffer solution. We imaged an area of 200 nm x 200 nm with 2 frames per second (see Fig. 1c). We anticipate that numerous AFM applications like high-speed AFM or high-speed force spectroscopy will greatly benefit from the properties of the novel probe design.

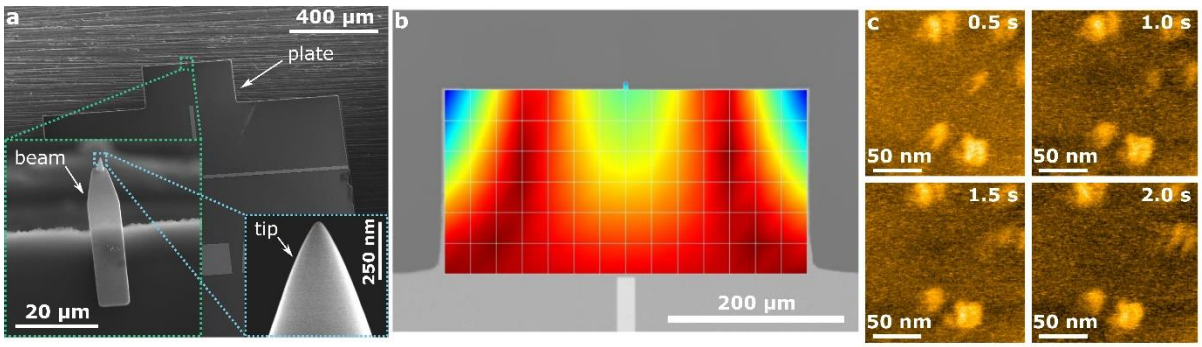

Fig. 1 *High-speed scanning probes with high resonance frequency and low stiffness.* **a** SEM image showing the two coupled microstructures. **b** Vibration mode measured with LDV in air. **c** Frames of a high-speed AFM video of protein complexes in a buffer solution.

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Overcoming the Limitations of Tip Enhanced Raman Spectroscopy with Intermittent Contact AFM

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Tip enhanced Raman spectroscopy (TERS) is a promising technique for mapping the chemical composition of surfaces with molecular scale. However, current TERS methods are limited by a number of issues including high tip-sample forces, high laser power, low topographical resolution, and short probe lifetime. As a result, TERS methods are best suited to robust samples that can tolerate high optical intensity. To overcome these issues and extend the application of TERS to delicate samples, a number of new probes and imaging modes are in development at the University of Newcastle. This talk will provide an overview of these methods and present preliminary results, including new methods for optical probe optimization and fabrication, and a new dynamic-mode AFM method to reduce contact forces and applied laser power.

Fast Multifrequency Measurement of Nonlinear Conductance

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The multifrequency measurement paradigm has proven very useful for Atomic Force Microscopy (AFM). The lock-in method has been extended to synchronous excitation and detection at many frequencies in band around a cantilever resonance, greatly improving the sensitivity, signal-to-noise ratio and information content of the measurement of the nonlinear tip-surface force. A key aspect of such measurements is the ability to coherently modulate and demodulate at many frequencies simultaneously, with one *single* phase reference for all data channels and without spectral leakage between channels. There now exists a commercially available lock-in amplifier optimized to perform this task for scanning probe microscopy (SPM). Here we demonstrate its application to the measurement of the current-voltage characteristics (IVC) at each image pixel in Conducting Atomic Force Microscopy (CAFM). IVC measurements in SPM are plagued by high source impedance and large stray capacitance. This combination causes a large parasitic displacement current when rapidly sweeping the voltage, as required for high resolution maps of electrical properties at reasonable scanning speed. We have developed a method to cancel this parasitic current using an active guard, driven by a second phase-coherent output of our multifrequency lock-in amplifier. The method avoids saturation of the current amplifier, allowing us to optimize its gain and bandwidth. We thus reconstruct the IVC at each pixel while scanning at a rate of up to about 1000 pixels/sec. The frequency domain data also contain information about the tip-sample capacitance at each pixel, allowing for a new type of image in SPM.

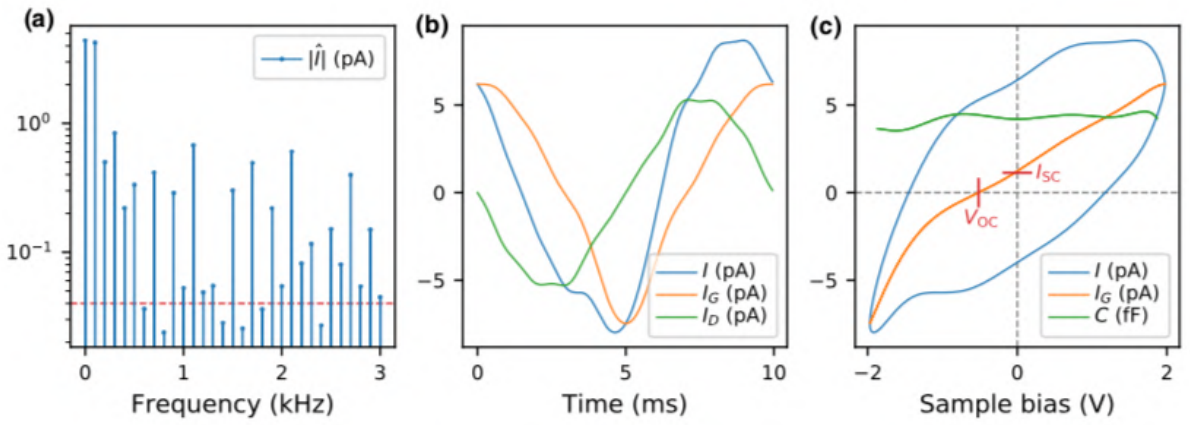

Figure 2: a) Discrete frequency spectrum of the tip-sample current measured in response to a pure cosine voltage waveform. Many harmonics at integer multiples of the excitation frequency are recorded. Only amplitude is shown but a coherent measurement of the phase is made at each frequency. b) Inverse Fourier transform gives the total current I and its two quadrature components corresponding to the galvanic current I_G and the displacement current I_D as a function of time. c) The total current and galvanic current are plotted versus voltage. From the displacement current we also reconstruct the tip-sample capacitance C versus voltage.

[1] R. Borgani, M. G. Kohan, A. Vomiero and D.B. Haviland, Phys. Rev. Applied, **11**, 044062 (2019).

Optomechanical detection of single bacterium mechanical modes

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Low-frequency phonon modes of biological particles such as proteins, viruses and bacteria involve coherent structural vibrations at frequencies in the THz and GHz domains. These vibration modes carry information on its structure and mechanical properties that play a pivotal role in many relevant biological processes. Despite the rapid advances of optical spectroscopy techniques, detection of low-frequency phonons of single bioparticles has remained elusive. Here we harness a particular regime in the physics of mechanical resonator sensing that serves for detecting them. By depositing single bacterium on the surface of ultra-high frequency optomechanical disk resonators in ambient conditions, we demonstrate that the vibration modes of the disk and bacterium hybridize when their associated frequencies are similar (Figure 1). A general theoretical framework is developed to describe the different regimes that can be found when an analyte adsorbs on a mechanical resonant sensor. The model recovers the classical inertial mass sensing regime as a limit case of a more general problem. Theory, numerical calculations and experiments show excellent agreement, allowing the use of inverse-problem algorithms for retrieving the eigenfrequencies and mechanical loss of the bacterium vibration modes. Our method is applied for analysis of the effect of hydration on the vibrational properties of a single bacterium. This work opens the door for a new class of vibrational spectrometry based on the use of high frequency mechanical resonators with the unique capability to obtain information on single biological entities [1].

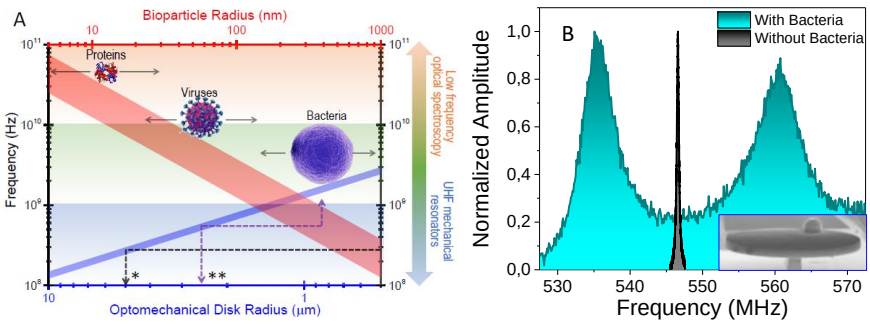

Figure 1. A. Frequency of the radial breathing mode of a 320 nm thick optomechanical disk (blue region) and of the fundamental mode of a quasi-spherical biological particle adsorbed on a rigid support (red region), as a function of the disk and bioparticle radii, respectively. B. Effect of bacterium adsorption on the radial breathing mode of an optomechanical disk (2.5 μm in radius and 320 nm in thickness). The inset shows a scanning electron microscopy image of the optomechanical disk with an attached *Staphylococcus epidermidis* cell.

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Advances in magnetic force microscopy

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Despite decades of advances in magnetic imaging, obtaining direct, quantitative information with high spatial resolution remains an outstanding challenge. The imaging technique most widely used for local characterization of magnetic nanostructures is the Magnetic Force Microscope (MFM), which is indeed a very active topic of investigation [1]. Advantages of MFM include relatively high spatial resolution, simplicity in operation as well as sample preparation, and the capability to applied in situ magnetic fields to study magnetization process [2]. Recently we have also demonstrate the possibility of operate in different environments including liquid media that allow us to investigate biological samples [3, 4]. In the present work, we try to approach some of the challenges of MFM, spatial resolution, sensitivity and quantitative measurements, by following different routes. One route is the development of high-performance MFM probes with sub-10 nm (sub-25 nm) topographic (magnetic) lateral resolution by following different easy and quick low-cost approaches [5]. This allows one to not only customize the tip stray field, avoiding tip-induced changes in the sample magnetization, but also to optimize MFM imaging in vacuum (or liquid media) by choosing tips mounted on hard (or soft) cantilevers, a technology that is currently not available on the market.

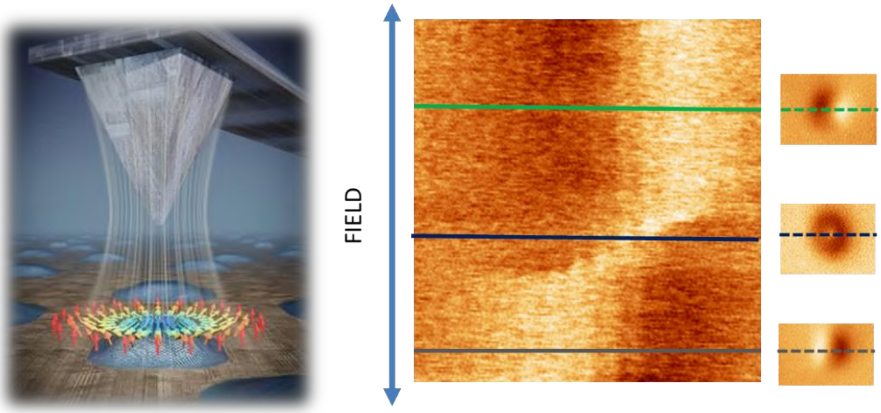

Figure1: Nonstandard MFM image of a Py nanodot (height ~30 nm). With this method, we can study the magnetization process of a single particle.

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Modelling Vibrational Modes Plates in Fluids for Applications in High-Speed Atomic Force Microscopy

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Novel probe designs played a vital role in the development of high-speed atomic force microscopy (AFM). Often new probe designs evolved from slender cantilever beam designs which are modeled with one-dimensional beam theory. Recently, it has been demonstrated that non-conventional vibrational modes in two-dimensional plate structures as depicted in figure 1 exhibit extraordinary high quality factors in liquids while their resonance frequency can easily be tuned by changing the plate width [1]. Due to these properties high-speed AFM methods for imaging biological samples in liquids would greatly benefit from plate-based probes. However, a theory for predicting quality factors of plate modes in liquids has been missing and theory for determining the fluid damping of slender beams in fluids is not applicable to plates. Here, we present a modelling approach for determining the dynamic response of plates immersed in fluids. The model comprises an elastic plate and a viscous fluid around the plate. For modelling the elastic plate, we use the Kirchhoff-Love plate equation which we solve with the method of finite elements. The finite element solution is obtained with a continuous/discontinuous Galerkin method which allows for the use of Lagrange-type elements while weakly imposing the physically required continuity conditions to the solution. We determine the fluid flow from an integral formulation of a Stokes flow and couple the resulting forces at the fluid-plate interface to the Kirchhoff Love equation. Using this method, we determine the spectral response of driven plate modes in gases and fluids as exemplified in figure 2 and compare the results with the spectral response of slender beam structures. Moreover, the resonance frequencies and quality factors of plate modes in different fluids are predicted. The presented method establishes a theoretical framework for the design of novel AFM which utilize vibrational modes in two-dimensional plate resonators and paves the way for practical methods for calibrating plate modes in AFM.

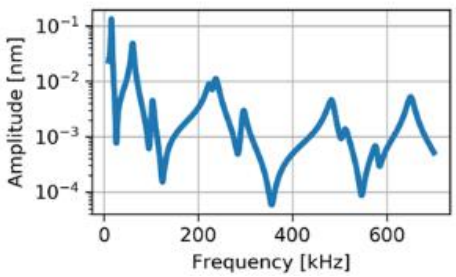

Figure 1: Simulated non-conventional mode shape of a silicon plate which oscillates in water and which is clamped at the left side. The plate has a length and a width of 300 µm and a thickness of 5 µm.

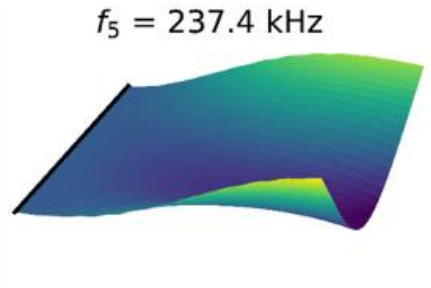

Figure 2: Simulated driven spectral response of the plate of figure 1 immersed in water.

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AFM manipulation of gold nanowires to build electrical circuits

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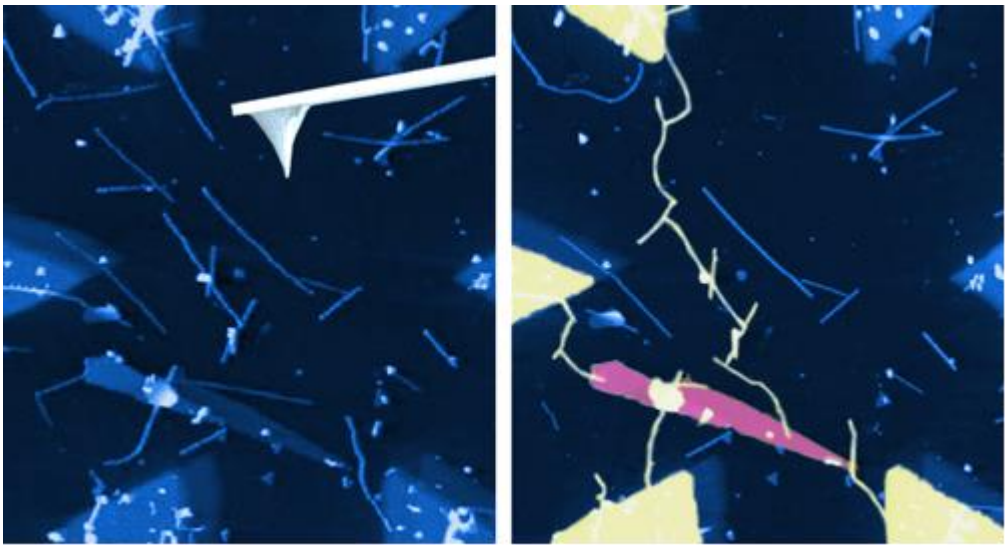
We introduce Scanning-Probe-Assisted Nanowire Circuitry (SPANC) [1] as a new method to fabricate electrodes for the characterization of electrical transport properties at the nanoscale. SPANC uses an atomic force microscope manipulating nanowires to create complex and highly conductive nanostructures (paths) that work as nanoelectrodes allowing connectivity and electrical characterization of other nanoobjects. The paths are formed by the spontaneous cold welding of gold nanowires upon mechanical contact leading to an excellent contact resistance of ~9 Ω/junction. SPANC is an easy to use and cost-effective technique that fabricates clean nanodevices. Hence, this new method can complement and/or be an alternative to other well-established methods to fabricate nanocircuits such as Electron Beam Lithography (EBL). The circuits made by SPANC are easily reconfigurable and their fabrication does not require the use of polymers and chemicals. In this work, we present a few examples that illustrate the capabilities of this method, allowing robust device fabrication and electrical characterization of several nanoobjects with sizes down to ~10 nm, well below the current smallest size able to be contacted in a device using the standard available technology (~30 nm). Importantly, we also provide the first experimental determination of the sheet resistance of thin antimonene flakes.



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Piezoelectricity in monolayer hexagonal boron nitride

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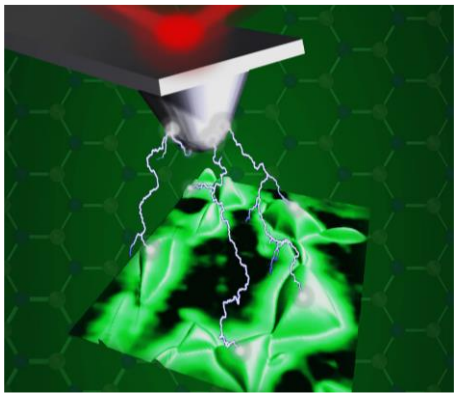
Two-dimensional (2D) hexagonal boron nitride (hBN) is a wide-bandgap van der Waals crystal with a unique combination of properties [1]. Furthermore, in recent years hBN crystals have become the material of choice for encapsulating other 2D crystals in a variety of technological applications [2]. Monolayer hBN was predicted to exhibit piezoelectric properties because it has no center of symmetry, however experimental evidence was lacking. In this work, we used AC Bias electrostatic force microscopy (EFM) to observe this effect [3] as a strain-induced change in the local electric field around bubbles and creases, in agreement with theoretical calculations. No piezoelectricity was found in bilayer and bulk hBN, where the center of symmetry is restored. Our results add piezoelectricity to the known properties of monolayer hBN, which makes it a desirable candidate for novel electromechanical and stretchable optoelectronic devices, and pave a way to control the local electric field and carrier concentration in van der Waals heterostructures via strain. The experimental approach used here also shows a way to investigate the piezoelectric properties of other materials on the nanoscale by using electrostatic scanning probe techniques.



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Unravelling the Origins of Functionality through Correlative Multimodal Chemical Imaging

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The key to advancing energy materials and biological systems is to understand and control the structure and chemistry at interfaces. While much of the dynamic chemistry can be studied on macro-scale systems, there is a lack of means to localize chemical measurements and correlate them to nanoscale structure of the material. Through a unique merger of advanced scanning probe and ion microscopy with mass spectrometry techniques rooted in innovative data processing and control algorithms, we are now able to understand the interplay between chemical and physical functionality at the fundamental length scales using multimodal chemical imaging. This multimodal imaging transcends existing techniques by providing nanoscale structural imaging with simultaneous chemical analysis. Here, I will discuss how we have developed and used this capability to visualize dynamic material transformations at interfaces, to correlate these changes with chemical composition, and to distil key performance-centric material parameters. One exciting capability is that the AFM can be used to drive materials away from equilibrium at the nanoscale with highly localized electric fields. This allows field confinement effects on localized chemistry in materials to be locally probed, especially at interfaces. This in turn yields direct information on key energy related questions such as electron and ion motion distribution and transport at and between interfaces. Overall, I will focus on ways to unlock the mystery of active interface formation through intertwining data analytics, nanoscale elemental and molecular characterization, with imaging; to better grasp the physical properties of materials and the mechanistic physics-chemistry interplay behind their properties

Q-Controlled Microcantilevers with A Collocated Piezoelectric Actuator-Sensor Pair for Multifrequency AFM

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A new class of AFM microcantilevers with a two-layer piezoelectric transducer is presented. The two-layer transducer is composed of a stack of top and bottom transducers acting as a collocated actuator-sensor pair for self-actuation and sensing of cantilever displacement at its resonance frequencies. As shown in Fig. 1, the top AlN layer is in-between top and middle metal electrodes as the top actuation transducer, and a bottom AlN layer, sandwiched between middle and bottom electrodes, behaves as the bottom readout transducer. This arrangement results in a minimal feedthrough capacitance from input actuation voltage to sensor output leading to high dynamic range displacement sensing. It also enables optimal use of the surface area available on the cantilever to maximize the total displacement-induced charges on the sensor. The microfabricated cantilever is characterized at the 1st and 2nd resonance modes using a lock-in-amplifier and a laser Doppler vibrometer (LDV). Fig. 2(a) and (b) show that the piezoelectric sensor follows the cantilever dynamics at the first two modes with a good dynamic range. AFM images of a standard grating with 110 nm height features are captured at these two modes. To achieve higher imaging rates and achieve a more robust AFM control loop at higher controller gains, quality factor of the cantilever at the 1st mode is reduced. For this purpose, we implement a positive position feedback controller using the modulated-demodulated control implementation technique [1]. AFM image of a calibration grating with *Q*-control is shown in Fig. 2(e). From height profiles of Fig. 2(f), one can observe the effectiveness of this *Q*-control on stability and faster response time of the proposed cantilever.

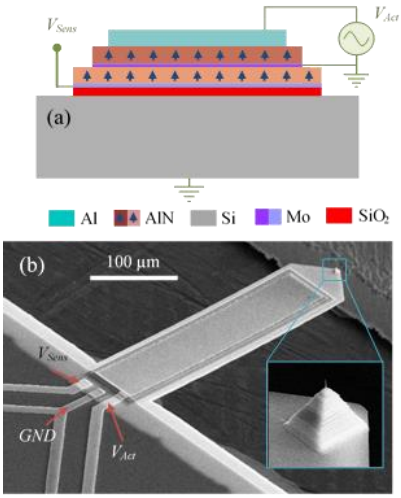

Figure 1. (a) Cross-sectional view and (b) SEM image of a microcantilever with a collocated piezoelectric actuator-sensor pair. The inset shows a Pt tip deposited with FIB/FEB.

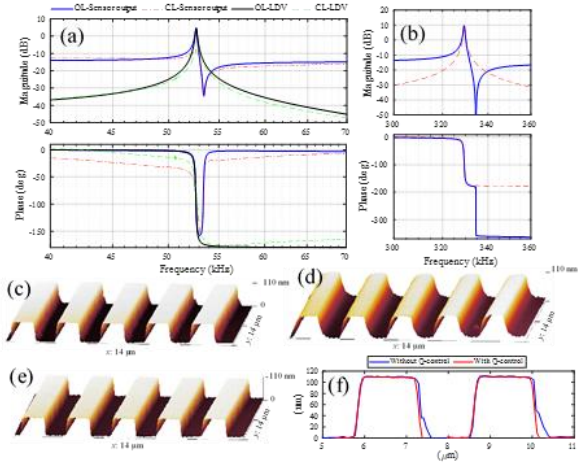

Figure 2. (a) Frequency response functions (FRF) of the cantilever at 1st mode measured with piezoelectric sensor and laser Doppler vibrometer with and without *Q*-control. (b) FRF at the 2nd mode. AFM images with uncontrolled cantilever at the (c) 1st and (d) 2nd mode. (e) AFM image with *Q*-control of the 1st mode. (f) Height profile with and without *Q*-control.

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Isomorphic contact resonance: demonstration of a new contact resonance force microscopy technique with simplified interpretation of image contrast

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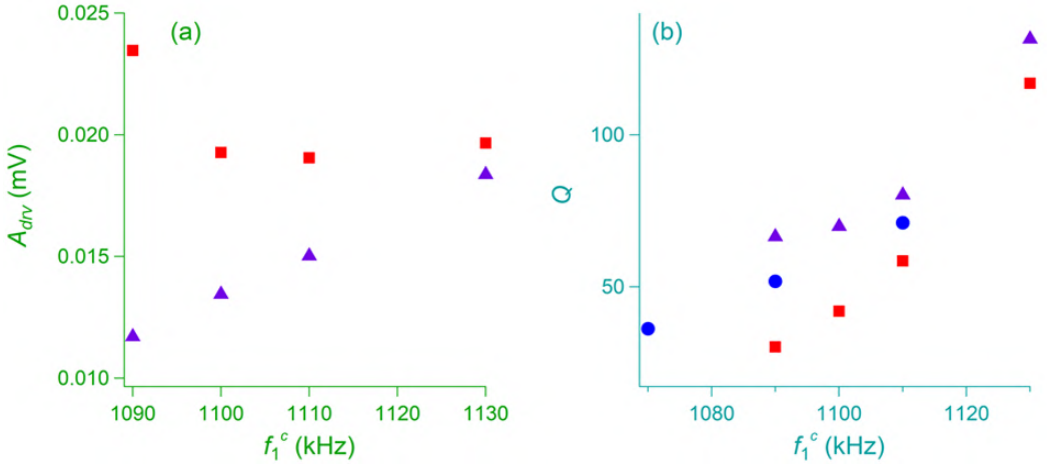
We introduce a new contact resonance force microscopy (CRFM) imaging technique, isomorphic contact resonance (iso-CR), that acquires data at constant CR frequency, and hence constant contact stiffness, throughout the scan area. Constant CR frequency is obtained by performing a force versus distance measurement at each pixel in the image, such that a preselected target frequency is reached at some point in the force-distance curve. (By comparison, in conventional CRFM, the force is constant throughout the scan area, while the CR frequency and contact stiffness vary between pixels.) As a consequence of the constant frequency and contact stiffness in iso-CR, the cantilever maintains an invariant vibrational shape and a constant environmental damping, thus simplifying interpretation of amplitude and quality factor image contrast. Advantages of iso-CR are demonstrated by presenting iso-CRFM (mechanically driven) and iso-CR piezoresponse force microscopy (iso-CR-PFM, electrically driven) images of a piezoelectric AlN thin film containing nanoscale Al-face or "up" domains, and N-face or "down" domains. The domain structure is revealed by iso-CR-PFM phase imaging, which shows nearly 180° contrast between "up" and "down" domains. The PFM amplitude and Q-factor images also show "up" versus "down" domain contrast, which decreases with increasing CR frequency. Further, the difference between the iso-CR-PFM and iso-CRFM Q-factors decreases with increasing frequency. These frequency-dependent effects, summarized in Figure 1(a) and 1(b), are ascribed to frequency-dependent electrostatic artifacts in the measured PFM signals. We conclude that the iso-CR capability to control the CR frequency across multiple excitation schemes helps elucidate the origin of the amplitude and Q-factor image contrast.

Fig. 1. Median values of amplitude (A_{drv}) and Q-factor (Q) versus CR frequency: (a) A_{drv} (iso-



CR-PFM mode) over N-face (red squares) and Al-face (purple triangles) domains; (b) Q (iso-CR-PFM mode) over N-face (red squares) and Al-face (purple triangles) domains, and Q (iso-CRFM mode) over entire scan area (blue circles).

Capturing the full potential
Surface potential imaging of soft structures via sideband KPFM

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Semifluorinated alkanes consist of two chain segments – one $(CF_2)_xF$ and one $(CH_2)_yH$ block. F_xH_y are known to self-assemble in water and on solid substrates in various morphologies. Therefore, studying of semifluorinated alkanes like F14H20 adds to the general understanding of self-assembly.[1] For nanoscale characterization of soft, self-assembled F14H20 structures on solid substrates Kelvin probe force microscopy (KPFM) is ideally suited, since the electric dipole of F14H20 leads to a significant surface potential difference between F14H20 and the substrate.[2] In KPFM, a conductive cantilever scans the sample, while applying an AC and a DC voltage to detect changes in the electrostatic force between tip and sample caused by local deviations of the surface potential.[3] Here, we compared KPFM measurements of self-assembled F14H20 structures imaged by two techniques: Off-resonance and Park's newly implemented sideband KPFM. We found a significant improvement in the potential resolution for sideband KPFM.

KPFM on F14H20: For the resolution and accuracy of the surface potential in KPFM, the detection method of the electrostatic signal is decisive. In off-resonance KPFM, the AC voltage modulates the electrostatic force at a frequency far from the resonance of the cantilever (~17 kHz). The force is detected via the oscillation amplitude at the AC frequency, which is nullified by applying a DC bias matching the potential difference between tip and sample. However, the detection of the long-ranged force can lower the sensitivity.[3] For sideband KPFM, we apply a low-frequency AC voltage (2-5 kHz) to modulate the electrostatic force. The modulated electrostatic force *gradient* introduces frequency sidebands left and right of the cantilever resonance. Sideband KPFM nullifies these sidebands by applying a DC bias, which matches the potential difference. By detecting the force gradient instead of the force, long-range crosstalk decreases and the lateral resolution and potential sensitivity improve significantly.[3] Analyzing the sideband KPFM measurement on a F14H20 aggregate on silicon substrate, we observed a potential contrast of 700-750 mV between substrate and F14H20 as well as a defined lateral resolution, imaging even small gaps in the aggregate. Off-resonance KPFM on the other hand showed a potential difference below 300 mV and a lower lateral resolution.

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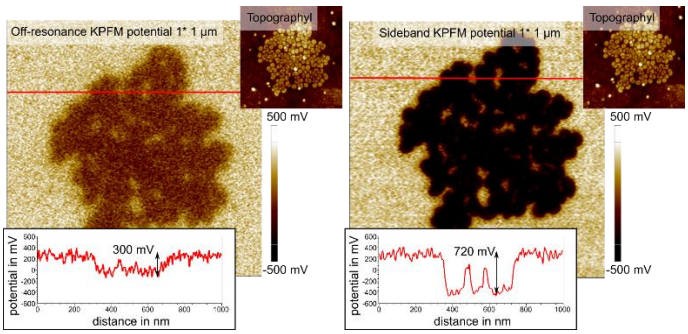

Figure 1: The same F14H20 aggregate was imaged using off-resonance and sideband KPFM. Cross sections (red) show improved lateral and potential resolution of sideband KPFM.

Investigating the Biotin-Streptavidin Binding Dynamics on DNA Origami Nanostructures with High Spatiotemporal Resolution Atomic Force Microscopy

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Atomic force microscopy (AFM) is a powerful tool, which allows the comprehensive study of mechanical properties and interactions with nanometer resolution. The ability of AFM to obtain three-dimensional topography images of biological molecules and complexes under near-physiological conditions together with the recently achieved high-temporal resolution makes high-speed AFM (HS-AFM) a perfect tool for investigating dynamic biological processes. We will present a newly developed High-Speed AFM (NanoRacer®) which enables scanning speeds of over 50 frames per second. In this way, the high-speed study of the time-resolved dynamics associated with cellular processes and the binding mechanisms of individual biomolecules is possible, e.g., the dynamics of individual protein binding behavior, two-dimensional protein assemblies, motor proteins and membrane trafficking. Here, we will discuss the binding dynamics of streptavidin to biotinylated DNA origami nanostructures, that are typically used for the study of early cell signaling cascades in epithelial cancer cells. [1]

DNA origami nanostructures (DONs) have emerged as excellent molecular pegboards for the immobilization of ligands on surfaces to study early signaling events in adherent cells. The bottom-up self-assembly of such supramolecular architecture can be harnessed to create bioinstructive materials, such as, nanocomposites for cell receptor stimulation [2] or biosensor surfaces for the investigation of nanoscale effects on early cell signaling [3]. These applications take advantage of the effective linkage between receptor ligands and the DONs through high-affinity biotin-streptavidin bridges. Here, we present high-speed AFM (HS-AFM) data obtained from DONs containing biotin binding sites, imaged in fluid in the presence of streptavidin at 20 ms per frame. The occupation of each binding site is analyzed revealing details on the binding properties and dynamics, which could be tailored by changing the chemical nature of the nanoscale binding sites.

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Multifrequency and intermodulation measurements with the MLA-3

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The MLA-3 is the third iteration multifrequency lockin amplifier from Intermodulation Products AB. It was from the ground up designed for multifrequency measurements, and specifically with AFM in mind. We will demonstrate how to setup and perform various custom multifrequency measurements. We will also show Intermodulation AFM, a specific multifrequency AFM mode which allows for fast tip-surface force reconstruction.

Optical guiding mechanism for the next generation of fully-motorized tip-scanning AFMs

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Tip scanning AFMs have several advantages, namely the ability to investigate samples of unrestricted size or weight as well as an unobstructed optical view from below. This is crucial for biological samples, where correlation with optical microscopy techniques is frequently needed. However, conventional optical beam deflection (OBD) is very challenging to effectively integrate into a tip-scanning architecture, limiting the number of existing tip-scanning AFM designs. The OBD adjustment mechanics are a crucial component affecting both ease of use and imaging performance. The trend towards fully automated instruments adds to the challenges for a tip-scanning design, since large and heavy motorized actuators cannot be placed onto the scanner without drastically decreasing the scanner resonance frequencies, and hence limiting imaging performance.

Here, we present a novel tip-scanning AFM architecture allowing for placing the light source and photodetector, along with the adjustment opto-mechanics, completely off the scanner.[1] Only a set of passive optical guiding mirrors, whose mass is ~1% of the total scanned mass, are placed onto the scanner. This approach enables the optimization of the scanning mechanics separate from the adjustment mechanics, resulting in a low-scanned-mass, stiff planar flexure scanner design coupled without compromising on a robust, fully-motorized adjustment. Such a design is also compatible with adding a 2nd light source for photothermal cantilever excitation.

In addition to describing the principles of our approach, we demonstrate the performance of our system through a variety of imaging applications.

[1] Adams, J.D. U.S. Patent 10,564,181B2 (2020)

IgG walking and oligomerization on antigenic surfaces

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Immunoglobulin G (IgG) antibodies play a central role in human health by alerting and activating components of the innate immune system upon infection or carcinogenesis. Molecular engineering of antibodies for therapeutic and diagnostic purposes emerges to be one of the major technologies in combating many human diseases. Despite its importance, a detailed description of the nanomechanical process of antibody-antigen binding and dissociation on the molecular level is lacking. Here, we utilize high-speed atomic force microscopy to examine the dynamics of antibody recognition and uncover a new principle. Contradicting the current textbook view, antibodies do not remain stationary on surfaces of regularly spaced epitopes; they rather exhibit "bipedal" random walking caused by mechanical strain due to imperfect binding[1]. Randomly walking antibodies gather in transient clusters that serve as docking sites for the complement system. Of special interest is the classical complement pathway, which is triggered by IgG-hexamer formation on cells[2,3]. The dynamic assembly of IgG hexamers on antigenic surfaces represents a recently recognized and yet underutilized effector function of IgGs. We employed high-speed atomic force microscopy to visualize the dynamic formation of IgG oligomers on antigenic lipid membranes[4,5]. With single-molecule force spectroscopy and quartz crystal microbalance we further characterized the molecular interactions by determining chemical rate constants and energies[5]. The low affinity of Fc-Fc interactions prevents IgG oligomerization and thus unwanted complement activation at physiological concentrations in solution. Upon surface-epitope binding, however, oligomerization may be initiated via two different pathways: recruitment from solution, or diffusion-driven lateral collisions. Our findings will inspire the rational design of antibodies and antibody formats to exploit/inhibit steric strain-induced dynamic effects and hexamerization.

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Improving the temporal resolution of high-speed AFM

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High-speed AFM (HS-AFM) allowed us to directly visualize the dynamic behaviors of biological molecules in action at nanometer spatial and sub-second temporal resolution [1]. The power of this microscopy has been demonstrated by increasing number of HS-AFM imaging studies not only on purified protein systems, but also on dynamic processes occurring on the surfaces of bacteria, eukaryotic cells and isolated intracellular organelles [2]. However, there are still a huge number of biological processes that cannot be visualized with the current HS-AFM system. One of the reasons for this incapability is insufficient temporal resolution. In the current system, the temporal resolution is mainly limited by the Z-scanner and the amplitude detector for measuring the oscillation amplitude of cantilever. Here, we present the recent progress of the development of these devices.

Using a tiny piezo and a new holding method of piezo (See the inset shown in Fig. 1), the resonant frequency of the Z-scanner was greatly improved from ~0.2 MHz to ~1.1 MHz (the highest one was ~1.3 MHz as shown in Fig. 1). The Z-scanning range is ~150 nm with 50 V, which is sufficient for imaging of purified protein systems. Importantly, this new Z-scanner can be attached to any conventional Z-scanners with a wide scanning range, without remarkable decrease of the resonance frequency of the conventional Z-scanner. Thus, this configuration will enable us to perform high-speed and wide-range imaging of biological specimens. In addition, we are now developing a new amplitude detector (to be precise, square amplitude detector), by employing a calculation method based on the trigonometry [3]. At the oscillation frequency of 500 kHz, our new detector responses with a time delay of 0.29 μs compared with the time delay of 1.0 μs achieved by the S/H (sample & hold) detection method, when exposed to an abrupt amplitude change. After installing the two devices into the HS-AFM system, actin filaments, fragile protein complexes, were successfully imaged without damaging even at a scanning speed of 75 ms/frame for a scanning area of 400 × 200 nm^2, demonstrating that the temporal resolution of HS-AFM was greatly improved.

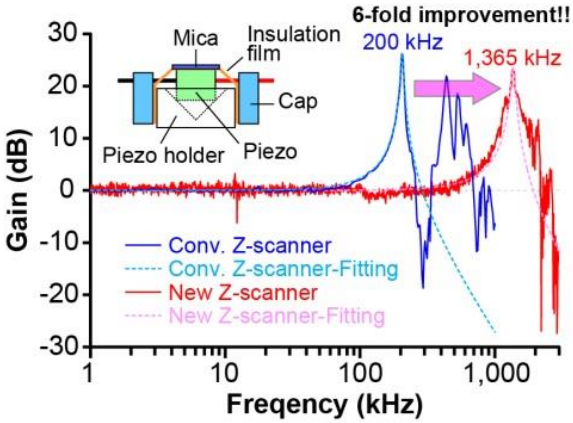

Fig. 1: Comparison of frequency response between the conventional Z-scanner and the new

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Recent developments in cantilever technology for high-speed AFM using photothermal off-resonance tapping (PORT)

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Force distance curve based AFM imaging modes record the full-force distance curve and extract from this curve the parameter for the feedback loop (primarily the peak interaction force) as well as other parameters of the sample such as mechanical properties [1]. These imaging modes exist under different trade names (Pulsed force mode, PeakForce tapping, HybriD mode etc.). These modes have in common that they perform the oscillation motion well below the resonance frequency, which is why we refer to them as off-resonance modes. Off resonance modes have become very popular in conventional speed AFM applications, because they allow for easier feedback control, lower interaction forces [2], and the direct extraction of materials properties. For HS-AFM in liquid, however, the gold standard remains small amplitude tapping [3]. In our previous work, we have introduced photothermal off-resonance tapping (PORT) to bring the benefits of force-distance based imaging to HS-AFM. By using photothermal direct cantilever actuation we were able to perform PORT measurements at two orders of magnitude faster than conventional ORT [4]. However, because the cantilevers needed to be operated away from the resonance frequency, this method was slower than small amplitude tapping mode HS-AFM. We are working to overcome this limitation by increasing the PORT frequency to be above the resonance frequency. This requires design of custom small cantilevers optimized for high photothermal actuation bandwidth. In this presentation I will show preliminary results and discuss remaining challenges.

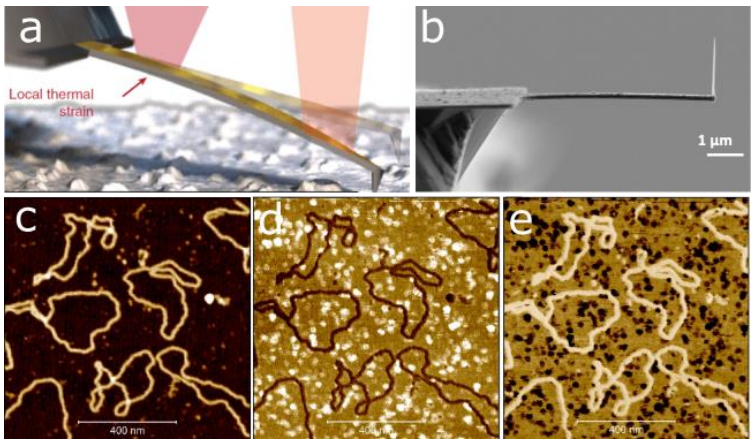

Figure 3: High frequency photothermal off-resonance tapping.a) PORT concept. b) prototype cantilever for MHz PORT. c,d,e) HS-PORT images of plasmid DNA (height, dissipation, adhesion)

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High-Speed Bimodal AFM nanomechanical mapping of collagen self-assembly

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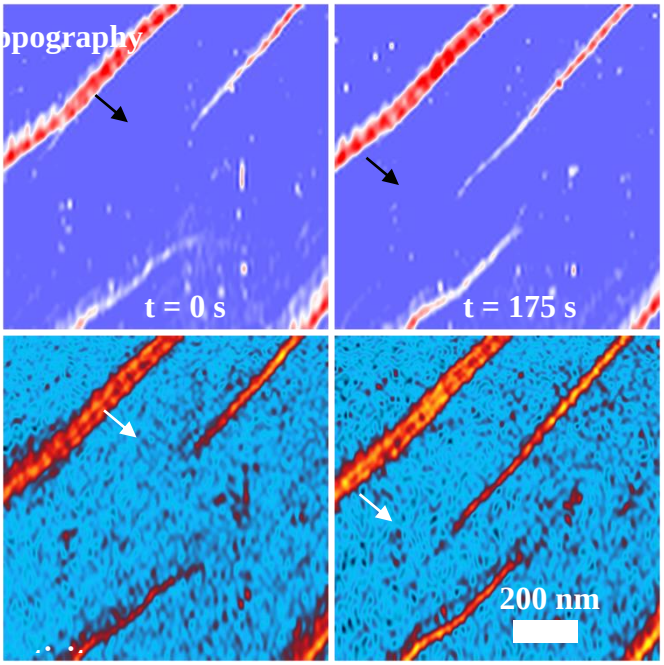
Collagen is the most abundant structural protein of the extracellular matrix. The assembly of collagen fibrils play relevant roles in a variety biological processes. The formation fibrils during the self-assembly process of collagen I have been studied by AFM [1,2]. Those studies lacked the time and mechanical properties resolution to clarify the mechanism of the earlier stages of collagen assembly and fibril structure formation. We have developed a high-speed bimodal AFM that combines the *ms* time resolution of high-speed AFM [3] with the nanomechanical force sensitivity of bimodal AFM [4,5]. High-speed bimodal AFM characterizes the earliest stages of the self-assembly of the collagen fibrils by proving time-resolved and high-spatial resolution maps of the evolution of the elasticity of the fibrils during the growth.



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Rapid discrimination of surface terminations in self-assembled 2D heterostructures by direct van der Waals identification

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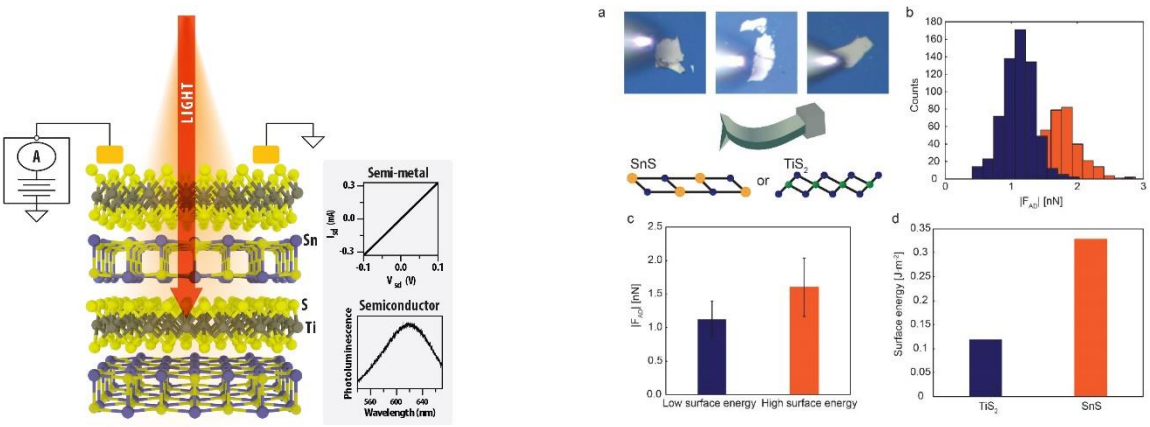
We demonstrate that surfaces presenting heterogeneous and atomically flat domains can be directly and rapidly discriminated via robust intensive quantifiables by exploiting one-pass noninvasive methods in standard AFM, single ~ 2 minute passes, or direct force reconstruction, i.e. ~10^3 force profiles (~ 10 minute collection time), allowing data collection, interpretation and presentation in under 20 minutes, including experimental AFM preparation and excluding only sample fabrication, in situ and without extra experimental or time load. We employ a misfit a $SnTiS_3$ compound as a model system. Such heterostructures can be exploited as multifunctional surface-systems providing multiple support sites with distinguishable chemical, mechanical or optico-electronic distinct properties, thus acting as an ideal model system to exemplify how current AFM methods can significantly support material discovery across fields.



Superposition of Semiconductor and Semi-metal properties of Self-Assembled 2D $SnTiS_3$ Heterostructures. AFM characterization and discrimination process between SnS or TiS_2 atomic/molecular terminations. a) Optical image obtained by the standard optics of the AFM to locate a sample of a $SnTiS_3$ flake resting on a silicon support. b) Histogram detailing the results obtained from 1000 AFM tip−sample force profiles in terms of (absolute value) adhesion force reconstructed for 5 randomly picked flakes, i.e. 200 profiles for each flake. c) Bar plot showing the difference between the two distributions in terms of mean and standard deviation. d) Predictions in terms of surface energy for the two terminations.

Molecular identification based on AFM with CO tips and Deep Learning

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Non-contact atomic force microscopy (NC-AFM) with CO-functionalized metal tips images the internal structure of molecules with unprecedented resolution, resolving intermolecular features and bond orders in aromatic compounds [1]. Its capability to address individual molecules has paved the way for the identification of natural compounds (where conventional methods failed) and of intermediates and final products generated in on-surface reactions, and to resolve a hundred different types of molecules in complex mixtures such as asphaltenes [1]. However, the unambiguous identification of the structure and composition of individual molecules, without any prior information, remains a formidable challenge.

Here, we present our strategy to transform these potentialities into a robust characterization technology, combining high-resolution imaging and machine learning in order to achieve molecular identification. We focus on a large set of quasi-planar molecules that spans all the relevant structural and compositional moieties. We describe how, from each of these molecules, we build the training dataset of 2D theoretical images, striking the right balance to incorporate enough variation and to prevent overfitting. These simulations are based on a range of theoretical approaches, from the original probe-particle (PP) model [2] to recent DFT-based models that incorporate the electronic charge density [3,4].

Firstly, we show the limited performance of several well-established deep learning models [5,6] to identify AFM theoretical images by analysing the transfer of relevant information across the different layers. Thus, based on this analysis, we develop a specific deep learning architecture for molecular identification that shows an excellent performance in its application to theoretical images. Finally, we test the model with experimental images, trying to understand the differences between experimental and simulated AFM images that hamper a proper classification. We solve this problem with a (VAE) Variational Autoencoder [7] that increases the amount of image features of the dataset leading to an optimal identification.

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On the Mapping of the Viscoelastic Properties of Polymer Blends by Multifrequency AFM

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Université de Montpellier, Montpellier (France).
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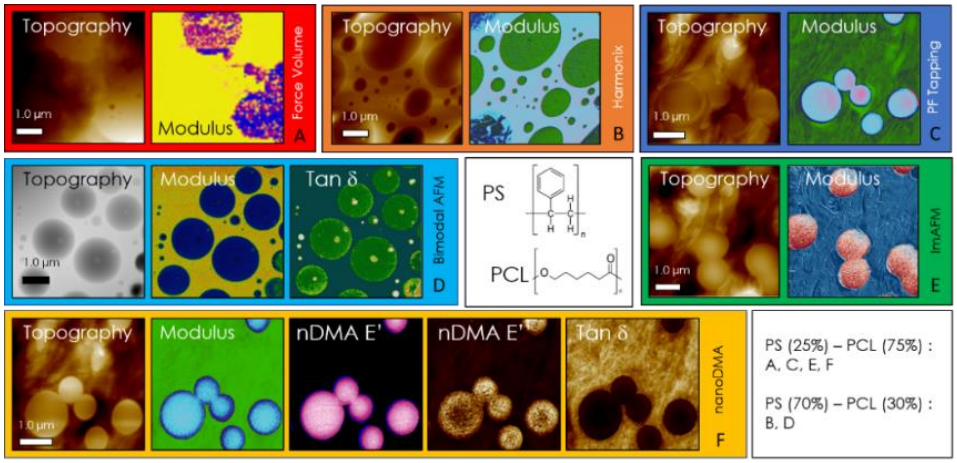
Over the past few decades (nano)composites and functional polymer blends have replaced metals in many applications from aerospace to sports gear, from automobiles to wind turbines, and from circuit boards to civil structures such as bridges and buildings. With these novel materials impacting every part of our lives, they have become ubiquitous. Mechanical property mapping can provide critical insights into the fundamental processes at the local scale that lead to deformation phenomena in these materials. The relatively recent development of dynamic mechanical scanning probe microscopies allows measuring mechanical properties of materials, providing well-adapted, fast, and versatile methods for mapping these properties. However, there is a lack of quantitative flexibility measurements, such as the elastic modulus or the viscoelastic properties (storage and loss modulus, loss tangent). In this work, we systematically compare the performances (accuracy, resolution, acquisition time, …) of the most promising (recent) methodologies based on multifrequency SPM (namely HarmoniX, Bimodal AFM, Contact Resonance AFM, Intermodulation AFM, and nanoDynamic Mechanical Analysis AFM) to more classical approaches. For instance, by considering, as model systems poly(styrene) - poly(ε-caprolactone) polymer blends, we propose adapted protocols for the data analysis, expecting to help the scientific community to better understand the key parameters in the optimization of the behaviour of materials not only for fundamental aspects but also for industrial applications.

Figure 1. Typical maps of the mechanical properties of poly(styrene) - poly(ε-caprolactone) polymer blends



obtained by some dynamic AFM modes.

Non-destructive methods to calibrate the flexural/torsional spring constants of atomic force microscope cantilevers in viscous environments

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Accurate calibration of the flexural and torsional spring constant of microcantilevers is crucial for sensing devices, microactuators, and atomic force microscopy (AFM). Existing calibration methods are either time consuming and destructive (ex situ static approaches), rely on models using the frequency and quality factor (Q-factor) of the cantilever resonance as input parameters (in situ dynamical approaches) or precise knowledge of cantilever geometry with significant simplifications [1]-[4]. Here, we develop simple equations to calculate the flexural and torsional spring constants of arbitrarily shaped cantilevers in fluid that does not depend on the cantilever flexural/torsional Q-factor. Our approach, verified here with AFM, only requires the measurement of one and/or two resonance frequencies of the cantilever in air and in a liquid, with no need for additional input or knowledge about the system. We validate the method with cantilevers of different shapes and compare its predictions with existing models (Fig.1). Significantly, the developed equations can be extended to calculate the spring constants of the cantilever's higher eigenmodes.

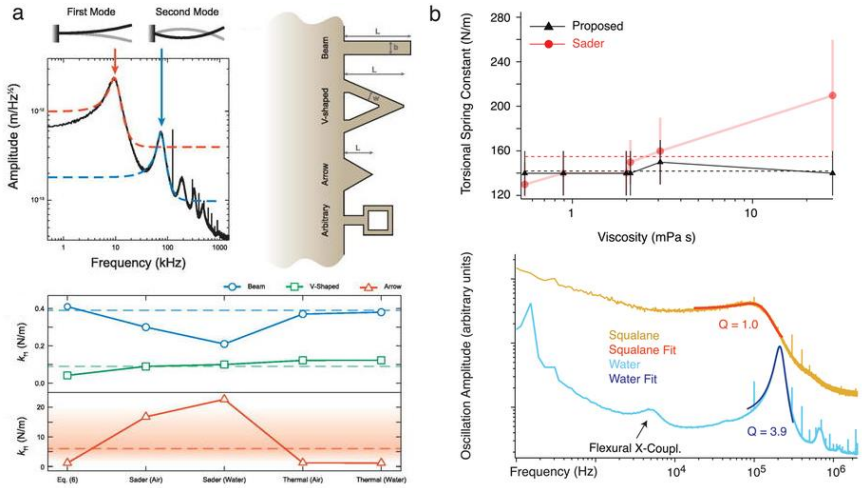

Fig 1: a) Calculation and comparison of the flexural spring constants from cantilever dynamics b) Comparison of torsional spring constant predictions derived with the proposed method and the Sader method in fluids of varying viscosity

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Nanomechanics of soft lipid nanotubes

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Lipid nanotubes serve in intra and extracellular transport processes, for instance by generating vesicles in endocytosis [1]. While these processes crucially involve the ill-understood local mechanics of the nanotube [2], existing micromanipulation assays (optical tweezers, micropipette) only give access to its global, averaged mechanical properties. Moreover, micromanipulation assays can analyze only one tube at a time. Here we develop a new platform to study nanotube local mechanics using atomic force microscopy (AFM) [3]. On a single coverslip we generate millions of substrate-bound nanotubes, out of which dozens can be imaged by AFM in a single experiment. AFM provides not only the fine morphology of the nanotube, but also a map of the local rigidity with spatial resolution in the order of nanometers (**Fig. 1**). A full theoretical description of the AFM tip-membrane interaction allows us to accurately relate AFM measurements of the nanotubes' heights, widths, and stiffnesses to the membrane bending stiffness and tension, thus demonstrating our assay as an accurate probe of nanotube mechanics. We reveal a universal relationship between nanotube height and stiffness, which is unaffected by the specific conditions of attachment to the substrate. Moreover, we show that the parabolic shape of force-displacement curves results from thermal fluctuations of the membrane that collides intermittently with the AFM tip. We also show that membrane nanotubes can exhibit high resilience against extreme lateral compression. Finally, we mimic in vivo actin polymerization on nanotubes, and use AFM to assess the induced changes in nanotube physical properties. Our assay thus provides access to high-throughput nanomechanical mapping of bare and protein-coated lipid nanotubes. It may help unraveling the local mechanics of membrane-protein interactions, including membrane remodeling in nanotube scission and vesicle formation.

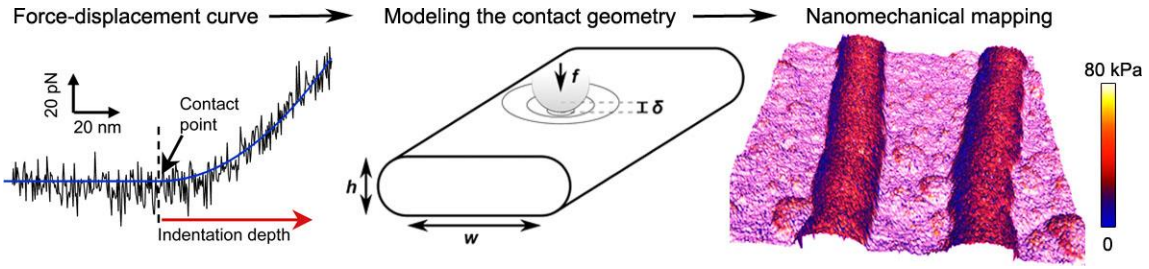

Fig. 1: AFM force mapping and modeling of soft nanotubes gives access to the mechanical properties (tension and bending stiffness) of lipid membranes with nanometer resolution.

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Phase-specific viscoelastic properties and sulfur distribution at nanoscale in binary elastomeric blends

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Blends of elastomers are frequently the basis of tire compound formulations; matrices such as Natural Rubber (NR), Butadiene Rubber (BR) and Styrene-Butadiene Rubber (SBR) are used to provide products for industrial applications with good mechanical properties, abrasion resistance and dynamic properties. The fundamental technological requirements associated with producing elastomeric blends comprise the creation of the desired phase morphology through mixing and processing steps, and the development of curing packages for proper covulcanization of the two phases. Rubber curatives may have different degrees of solubility in each elastomer, which consume vulcanizing agents at different rates due to their different degrees of unsaturation.[1] Therefore, in immiscible binary blends, it is important that the selected curing system leads to optimum compound properties upon vulcanization of the two phases. The substantial developments of the past years in characterization techniques have enabled the characterization of properties with nanoscale resolution. Atomic Force Microscopy (AFM) allows probing the mechanical properties of the material's surface with high spatial resolution. Viscoelastic properties measurements such as modulus and loss tangent (tan δ = E″/E′) can provide valuable information towards quantitative interpretation of the effects of curatives in the properties of each elastomeric phase in the blend. [2] Aiming at an improved understanding of phase-specific network structure in rubber blends, in this communication we will present an innovative strategy for the quantitative characterization of the phase-specific viscoelastic properties on immiscible rubber blends by combining rheometry and AFM measurements. We will show that quantitative nanomechanical characterization by AFM of unfilled NR matrices with different degrees of cross-link densities, as determined by tensile tests, may allow the estimation of phase-specific cross-link densities in NR/BR elastomeric blends with varying curing packages. In addition, the phase-specific AFM results were complemented with nanochemical analyses to correlate the viscoelastic properties with the distribution of sulfur and its relative concentration in each elastomeric phase. NanoSIMS, a high-resolution nanoscale chemical mapping by Second Ion Mass Spectrometry, was able to reveal differences in sulfur contents in each phase with a lateral resolution comparable to the AFM images. An interpretation of the complementary nanomechanical and nanochemical characterization is based on the solubility preferences of the curing additives.

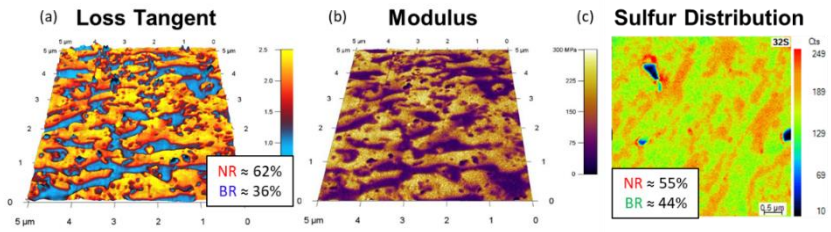

Figure 4. AFM nanomechanical analysis of a NR/BR blend showing (a) Loss Tangent image and (b) Modulus image. (c) NanoSIMS analysis of sulfur distribution in the blend. Images represent an area of 5x5µm².

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Molecular structure of a two-dimensional polymer synthesized at the air/water interface measured by advanced AFM

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A trifunctional, partially fluorinated anthracene-substituted triptycene monomer is spread at the air/water interface into a monolayer, which is transformed into a long-range ordered 2D polymer by irradiation with a standard ultraviolet lamp using 365nm light [1]. The polymer is analyzed by multimode non-contact atomic force microscopy (nc-AFM) in ultra-high vacuum after the transfer from the air/water interface onto highly oriented pyrolytic graphite. For the measurements we used a beam deflection AFM capable of combining high stability topography control on the fist resonance and high lateral resolution at the second by using oscillation amplitudes of a view 100pm [2]. The measured structure confirms a network structure, the lattice parameters of which are virtually identical to a structural model network based on X-ray diffractometry of a closely related 2D polymer unequivocally established in a single crystal. The nc-AFM images prove long-range order (see Fig. 1) over areas of at least 300×300nm^2. As required for a 2D polymer, the pore sizes are monodisperse, except for the regions, where the network is somewhat stretched because it spans over protrusions. Together with a previous report on the nature of the cross-links in this network, the structural information provided here leaves no doubt that a 2D polymer has been synthesized under ambient conditions at an air/water interface [3].

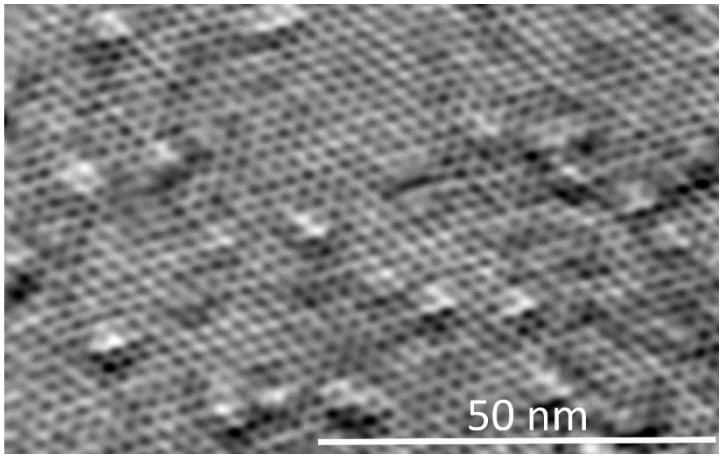

Fig. 1: nc-AFM frequency shift image of a 120x120nm^2 area of a 2D polymer. Parameters: f_{2nd} = 1MHz, A = 400 pm, Δf_{1st} = −27 Hz.

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Membrane protection and membrane fusion: contradicting functions of the IM30 protein visualized by AFM

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The inner membrane-associated protein of 30kDa(IM30) is a protein native to chloroplasts and cyanobacteria and essential to oxygenic photosynthesis. While the activity of IM30 is linked to the biogenesis / maintenance of thylakoid membranes, its exact physiological function still is unclear. In our work, we show that IM30 is not only involved in thylakoid membrane fusion [1], but can also protect the membrane against stress. Membrane fusion and stabilization are two contradicting mechanisms, since fusion involves partial destabilization of the membrane. We visualize how Mg2+-binding switches the function of IM30 from a membrane chaperone to a membrane fusion protein with atomic force microscopy (AFM). We also image the local structural change IM30 supercomplexes undergo upon membrane binding. IM30 supercomplexes bind to negatively charged solid-supported bilayers and forms defects in the presence of Mg2+ [2]. In the absence of Mg2+ IM30 binds to the bilayer but disassembles and forms membrane protective plaques that span several hundred square nanometer [3]. Our results help to improve the understanding of thylakoid membrane fusion and biogenesis and the switching of protein functions. In a broader sense, our findings contradict the widely distributed notion that plaques on membranes always have detrimental effects, as observed in Alzheimer´s or Parkinson's disease.

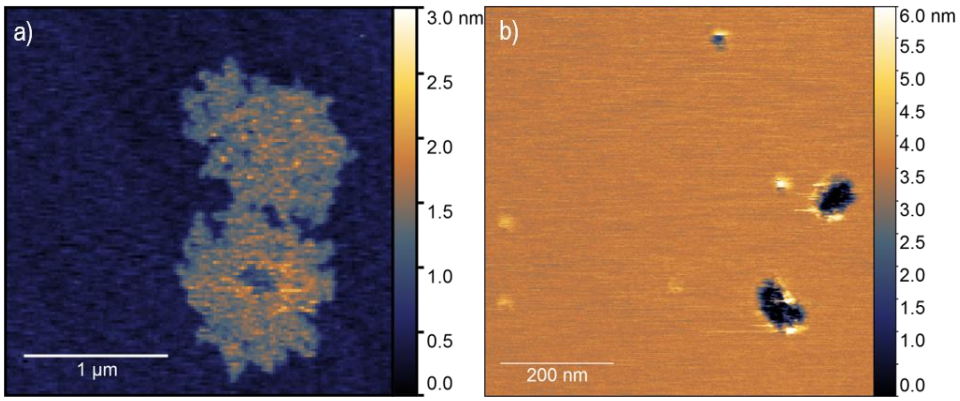

Figure 1. **Interaction between supported lipid bilayer (SLB) and IM30 protein**
Topography image of **a)** IM30 protein on SLB forms carpet-like structures in the absence of Mg2+. **b)** IM30 protein on SLB forms defects in the presence of Mg2+.Both imaged via AFM (the false-color ruler indicates the heights in the images). [2][3]

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[2] Junglas, B. Axt, A. Siebenhaller, C. Mg2+ triggers formation of IM30-induced bilayer defects (in preparation)
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Nanomechanical spectrometry of *E. coli* by multifrequency tracking

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Nanomechanical spectrometry can identify species by measuring the relative frequency shift of nanoelectromechanical resonators (NEMS) when an analyte lands on its surface. This approach was first exploited to measure the mass of IgM antibodies [1]. Furthermore, not only the mass of the analyte but also its stiffness was measured [2]. Recently, the mass of the bacteriophage T5 has been determined making use of the same technique [3]. The latter works tracked several resonance modes of a NEMS by a phase-locked loop (PLL) system. In the present work, we developed a method that eludes the use of PLL systems and still accomplishes the same precision in mass and stiffness than previous work [2]. The method is based in the continuous fitting of the resonance peak and the phase (Fig. 1(a)). Our nanomechanical mass and stiffness spectrometer mounts a µ-electrospray ionization system to nebulize the analytes. The laser beam deflection technique is used to measure up to four resonance frequencies of NEMS. Between them, a heated capillary ensures the desolvation and an aerolens decelerates the adsorbates. The mass of *Escherichia coli* bacteria has been measured by tracking the first four vibration modes of two NEMS: a commercial rectangular cantilever (Bruker MLCT-O10) and a commercial silicon nitride membrane (Norcada Inc) with size 250x250x0.05 µm. The square membrane provides a very high sensing area so that the efficiency is largely increased. Fig. 1(b) shows the real-time relative frequency shift of the first four flexural modes of the cantilever during *E. coli* adsorption. As shown in Fig. 1(c), the calculated mass for *E. coli* is identical for the two NEMS under a ~<10% of tolerance. The figure also shows the underestimation of the mass when stiffness is not considered.

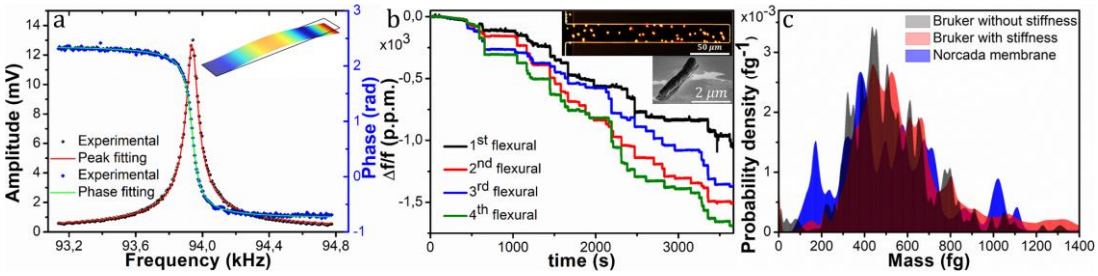

Fig. 1. (a) Fitting of the peak resonance and the phase of the second flexural mode (inset: FEM simulation) of the rectangular cantilever. (b) Relative frequency shift of the first four flexural modes of a microcantilever during the deposition of *E. coli* bacterial cells (inset: dark-field optical microscope image of the cantilever after the experiment with *E. coli* (top) and SEM image of a single bacterium (bottom)). (c) Probability density function of the *E. coli* mass for the NEMS used in this work.

This work was supported by Horizon 2020 FET project, No. 731868 VIRUSCAN.

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Dual simultaneous BE for multifrequency contact resonance mode measurements on ferroelectrics

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Amplitude Modulation – Frequency Modulation (AM-FM) multifrequency measurements are well known to allow viscoelastic characterization in contact mode by sensing the energy dissipation between the tip and the sample and the energy transfer between different excitation modes. While the theoretical background describing the movement and interactions of an AFM tip in this dynamic mode is already in a mature state, their counterpart in contact mode, that is, the corresponding multifrequency approach when a cantilever is in contact with the surface is still to be developed. Resonant contact modes, such as Contact Resonance Frequency (CRF) and DART Piezoresponse Force Microscopy (DART-PFM), have been widely and successfully applied to measure mechanical and electromechanical properties at the nanoscale on hard surfaces. Still, quantification of piezoelectric and ferroelectric properties in most cases has been hindered by different effects that can become dominant at the nanoscale: a) the strong perturbation of electrostatic interactions due to the electric field emanated by the tip, b) the coupling of classical phenomena to gradient based electromechanical responses such as direct and converse flexoelectricity, and c) electrochemistry and ionic conductivity on the surface and the bulk of ferroelectrics. To address these, we have applied Dual simultaneous BE at two resonance contact modes to measure the coupling of the different electromechanical modes among them and to the known physical phenomena happening in PFM. In this talk, we will show the correlation between the fundamental and first excited eigenmodes as a function of the polarization state of different ferroelectric materials and their mechanical stiffness. We expect that our results will serve to encourage the community to bring the multifrequency approach a step forward into the contact force microscopy realm to promote a likewise transformational impact.

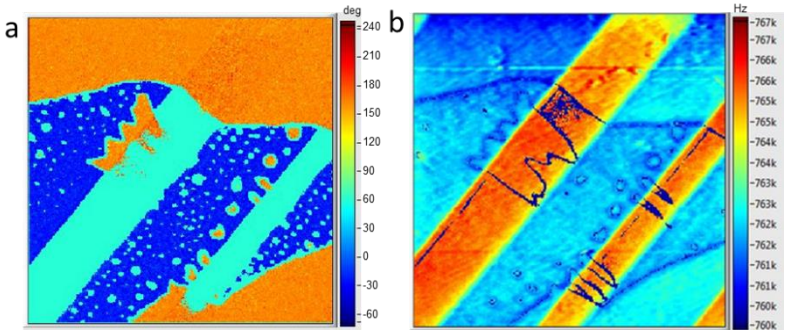

Figure 1 – a. Band Excitation PFM phase image and b. Band Excitation thermally excited CRF image of the a/c ferroelectric domains of a BTO single crystal

Simultaneous vertical and lateral resonance tracking PFM on ferroelectric thin films

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Even prior to the rise of perovskite solar cells, functional ferroelectric thin films have been investigated for their potential application in electronic and optoelectronic devices. For instance, the bulk photovoltaic effect based on ferroelectricity promises above band gap photovoltages for photovoltaics. Furthermore, conductive domain walls between ferroelectric domains could act as charge carrier pathways lowering recombination rates and, thus, increase the charge collection in electronic devices.[1–3] To correlate ferroelectric effects in domains on electronic and optoelectronic properties in ferroelectric functional materials vertical and lateral domains should be visualized with a high spatial resolution. The local piezoelectric information becomes available via piezoresponse force microscopy (PFM). For thin films, the weak piezoresponse can be enhanced by driving the electrical excitation of PFM close to the vertical (deflection) or lateral (torsion) contact resonance. Since the contact resonance depends on a consistent tip-sample contact a high surface roughness often introduces topographic crosstalk.[4] Dual frequency resonance tracking (DFRT) improves the stability of the resonance enhancement.[5] Here, we demonstrate our capabilities to capture the out-of-plane and in-plane DFRT piezoresponse simultaneously, by driving the electrical excitation of the cantilever at the contact resonance of the vertical deflection, as well as the torsional resonance on ferroelectric thin films, including bismuth ferrite and lead zirconium titanate.

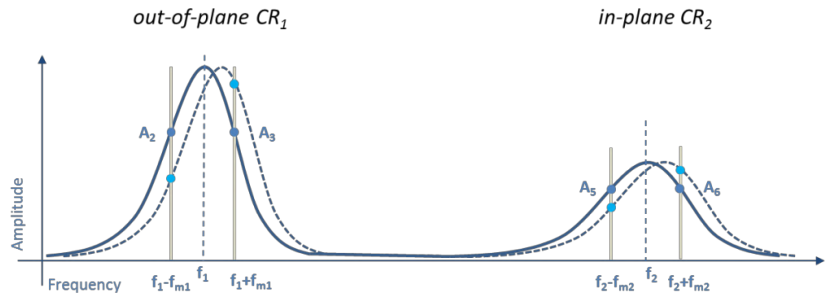

Figure 5: Frequency spectrum of cantilever in contact including the out-of-plane vertical resonance CR_1 and the in-plane lateral resonance CR_2. The sidebands used for the resonance tracking (A_2 and A_3 for the vertical resonance and A_5 and A_6 for the lateral resonance) are visualized in grey.

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Symmetric-Asymmetric Cantilever Responses in Tapping Mode SPM

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Tapping mode scanning probe microscopy is typically used to measure surface topography of biological samples, as it can also be employed in liquid, is able to resolve sub-nanometer features, and is less likely to damage the sample. The dynamics of the cantilever during tapping mode imaging in air and in liquid, are very different and exhibit interesting dynamics. Symmetrical motion of the cantilever about its equilibrium position in tapping mode imaging in air has been observed in experiments and numerical simulations. Symmetrical motion here is defined as an oscillation where, the cantilever travels the same distance from its equilibrium position to the sample's surface as it does from the equilibrium position to its maximum height above. This is non-intuitive as the part of the oscillation that is above the equilibrium position is unconstrained unlike the motion below, which is constrained by the sample. In some cases of imaging in liquid, the part of the oscillation above equilibrium position remains the same while the motion below varies with the surface profile. Higher harmonics are also seen during imaging in liquid. Although in a high damping environment the higher harmonics would have decayed faster, but in this case, the higher harmonics are more prevalent than those seen in air. Motion of the cantilever in tapping mode AFM imaging in air and liquid is investigated here by analysis of an equivalent impact oscillator (Figure 1); the cantilever is modelled as a single degree of freedom oscillator and the tip-sample interaction forces as forces arising due to impact on a hard surface. Transient analysis of the response of the oscillator is used to explain the behavior of the probe's motion for imaging in air as well as in liquid (Figure 2). The region of operating frequency to obtain better imaging is delivered. Also, for high speed SPM in air, the optimal quality factor is estimated to achieve faster decay of transients while preserving the properties of symmetricity of the cantilever's motion.

Figure 1. Schematic of impact oscillator model for tapping mode scanning probe microscopy operation. 'd' is the separation distance between the equilibrium position of the oscillator and the sample's surface.

Figure 2. Response with variation in surface profile, while operating in a high damping environment (such as liquid) and at the damped natural frequency. As the surface height varies the part of the oscillation below equilibrium varies but motion above does not change visibly. Also, the dominance of higher harmonics increases as the surface height increases.

High-speed AFM for life sciences

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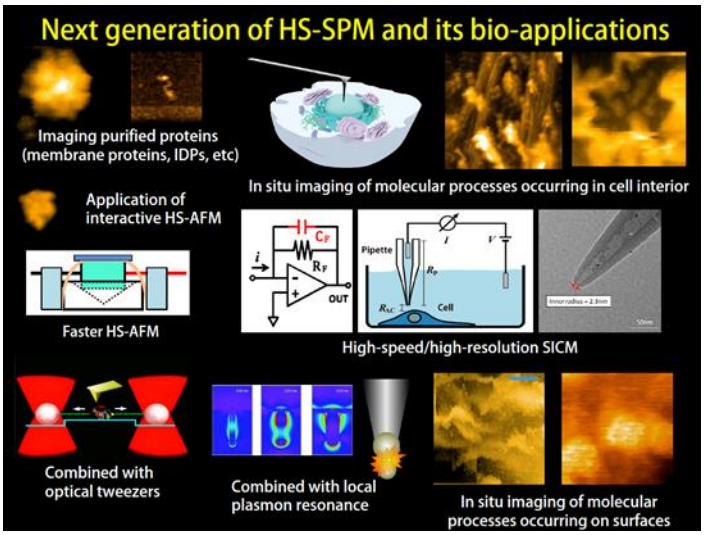
High-speed AFM (HS-AFM) was established in 2008 through long-term efforts to develop all underlying techniques[1,2]. This microscopy made it possible for the first time to directly observe individual protein molecules in dynamic action at high spatiotemporal resolution, without disturbing their function, thus transforming our way of understanding how proteins function. In fact, HS-AFM movies of various proteins captured in the last decade have provided mechanistic insights into their molecular processes inaccessible with other approaches[3,4]. There is no doubt that this line of studies will continue to be performed more actively on more diverse biomolecules, as seen in a recent clear trend of increasing number of biological studies with HS-AFM. What will further happen to HS-AFM techniques and its biological applications in the next decade? First, the speed performance will be increased from ~15 fps to ~100 fps by the improvement of current rate-limiting components, Z-scanner (~200 kHz), amplitude detector, and small cantilevers (~1 MHz). Second, the low-invasive performance will be further improved by the detection of elastic tip-sample collision or by the development of a higher sensitive sensor for detecting tip-sample interactions. Third, the functionality will be expanded, such as visualization of biomolecules under an external force and simultaneous super-resolution fluorescence and HS-AFM observation. Fourth, the targets of HS-AFM imaging will be extended from purified molecules to molecules on cell and organelle surfaces and in the interior of de-rooted cells. However, HS-AFM relying on the cantilever has limitation in imaging extremely soft and fragile targets, like the membranes of organelles and cells. Scanning ion conductance microscopy (SICM) is probably most fit for such targets. Nonetheless, its spatiotemporal resolution is far lower than the level desired in biological studies. Of note, this low resolution is not given theoretically but practically. There is a lot of room for improvement[5]. Thus, I believe that in a decade or so we will be able to reach much better understanding of biomolecular processes, if we have young talents who dedicate themselves to the development of these techniques.



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Using high-speed, molecularly-resolved AFM to investigate nucleation from solution at crystal surfaces

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Investigating nucleation from solutions is challenging, because it is a consequence of unstable density fluctuations, making the structures and events that must be probed both transient in nature and small in spatial extent. Moreover, processes like nucleation are inherently linked to the structure and dynamics of the interfacial region between the solution and crystal interface, particularly when nucleation is heterogeneous. Thus high-speed, molecularly resolved AFM provides a powerful and unrivalled method to investigate nucleation from solution onto crystal surfaces. Here I illustrate the deep level of fundamental insight into the pathways and energetics of nucleation made possible by this method using results from two contrasting systems: gibbsite (Al(OH)3) films grown epitaxially on muscovite mica (001) and 2D ordered arrays of peptides selected via phage display for their ability to bind to MoS2 (0001).

In the case of gibbsite (Fig. 1), we combine AFM observations of individual molecular adsorbates, transient clusters, and stable islands with surface potential measurements, density functional theory (DFT), and Monte Carlo simulations to put together a coherent picture of surface speciation and nucleation. The results reveal a surface population of ions that is dominated by hydrolyzed species (Al(OH)2+ and Al(OH)2+) even though Al3+ vastly dominates the bulk solution. DFT shows the source of this inversion is the charged state of the mica surface, which acts as a proton sink and creates a region with an effective pH higher than that of the bulk, thus driving formation and adsorption of the hydrolyzed species. These adsorbed ions evolve into subcritical clusters with increasing saturation state and temperature, constituting a population that decreases exponentially with size and exhibits dynamic fluctuations consistent with classical predictions. However, severe discrepancies with classical theory emerge when the values of key thermodynamic parameters are extracted from the AFM data. These discrepancies are resolved when the impact adsorbate charge on the capacitance of the mica-solution system is taken into account in calculating the work of cluster formation.

In the case of peptides assembly on MoS2 (0001), we combined AFM observations of nucleation dynamics and molecular packing with MD simulations of structure and binding. We find the peptide arrays form 2D crystalline arrays that exhibit an epitaxial relationship to the underlying hexagonal lattice, but assemble row-by-row from dimeric growth units. The nuclei are ordered from the earliest time of observation and, although the final crystals are 2D, due to the 1D nature of the constituent rows many of the signature features of nucleation in 2D and 3D are absent: there is no critical size, the nucleation rate varies linearly with concentration and that rate is finite for all concentrations above the solubility limit. These findings verify long-standing but unproven predictions of classical nucleation theory while revealing the key interactions responsible for ordered assembly.

Our results show that high speed AFM provides a direct molecular view of nucleation at surfaces that reveals both consistencies and deviations from the simple classical picture and highlights the inherent role that surface charge and dimensionality play in the process.

High-Speed Atomic Force Microscopy: A forceful Tool for Molecular Biophysics

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High-speed atomic force microscopy (HS-AFM) is a powerful technique that provides dynamic movies of biomolecules at work. HS-AFM has the notable advantage that it permits to subject the proteins under investigation to environmental cues such as changes of pH, ions, ligands, temperature, light and force (1). This is particularly advantageous for the study of ion channels that have evolved to respond to a wide range of stimuli. To break current temporal limitations to characterize molecular dynamics using HS-AFM, we developed HS-AFM height spectroscopy (HS-AFM-HS), a technique whereby we oscillate the HS-AFM tip at a fixed position and detect the motions of the molecules under the tip. This gives sub-nanometer spatial resolution combined with microseconds temporal resolution of molecular fluctuations (2).

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[2] Heath et al. Nature Communications, 2018, 9(1):4983, High-Speed AFM Height Spectroscopy (HS-AFM-HS): Microsecond dynamics of unlabeled biomolecules

Quantifying the Dynamics of Proteins at Solid-Liquid Interfaces by High-Speed AFM and Deep Learning

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In the last few decades, inspired by nature, various approaches have been developed for building novel supramolecular nanomaterials, using proteins as versatile building blocks assembled into hierarchical structures. However, creating 2D protein architectures at solid-liquid interfaces in a pre-designed manner is still extremely challenging. Both the dynamics and final organization of proteins at solid-liquid interfaces is determined by the energy-landscape arising from the interplay between protein-protein and protein-surface interactions. Yet how that energy landscape responds to solution conditions and environmental stimuli remains unclear. To explore this mystery, *in-situ* characterization with high temporal and spatial resolution, in combination with statistical analysis, is required.

Recently, our team developed an approach to direct protein nanorod self-assembly at inorganic interfaces through programmed protein-protein and protein-substrate interfaces.[1] To determine the energy landscape of the proteins on the surface and the dependence on electrolyte type and strength, as well as the size of the protein-substrate interface, we used high-speed AFM to record the in-plain dynamics of the protein nanorods on muscovite mica as a function of cation type and nanorod length. We then statistically analyzed the data using a deep learning approach adapted for AFM data on rod-shaped objects. We quantitatively described the in-plain angular dynamics of the protein nanorods, including the probabilities of lying along any orientation of the mica lattice and of transitioning between orientations exhibiting high probabilities of occupation. In addition to determining the thermodynamically most stable state, several meta-stable states in the energy landscape were identified, the shape of the energy minima were determined, and the barriers to transitions between states were extracted. The findings provide insight into the remarkable diversity of self-assembled architectures adopted by these protein nanorods that extend well beyond what is expected from the basic design. More generally, this study lays out a methodology for elucidating how cations and programmed protein interfaces control the in-plain dynamics of macromolecules at solid-liquid interfaces.

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A Micromechanical Cantilever with Multi-Frequency Transduction Capability for Multi-Modal Atomic Force Microscopy

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Since its introduction in 1986, AFM has been studied in depth to extend its capability as an advanced metrology tool at the atomic scale. Compared to more commonplace microscopy techniques (e.g., optical microscope, scanning electron microscope), AFM is unique in that it employs a mechanical transducer (i.e., a micro-cantilever with a nanoscale tip) to 'feel' a surface and then transduce its 'feeling.' Recent research efforts have focused on expanding the capability of AFM for various materials characterizations by interpreting this feeling encoded in the cantilever's dynamic motion. We developed a new AFM cantilever design that allows unambiguous delivery of the sample information through the cantilever's dynamic motion. This new cantilever system, named the inner-paddled cantilever, is a two-field design which consists of a base-cantilever incorporating an inner-paddle in the form of a silicon nanomembrane. The structurally dissimilar inner-paddle, which is free to vibrate over a middle-cavity, enables dual-frequency transduction capability in the cantilever system (as opposed to single-frequency transduction in a conventional cantilever), thereby, providing an additional, independent pathway to respond to variations in the sample's properties during dynamic AFM operation. In certain variations of dynamic AFM imaging (e.g., piezoresponse force microscopy and AFM-based infrared spectroscopy), the two-transduction channels can resolve the long-time issue of crosstalk between surface topography and material functionality from which a conventional AFM cantilever has inherently suffered. Moreover, when this new cantilever system is tested in tapping mode AFM, the inner-paddle can amplify a higher harmonic that coincides precisely with a higher vibration mode, resulting in multi-frequency AFM for compositional mapping. This is the result of internal resonance between the fundamental bending beam mode and a higher mode. The advantage of this approach over other multi-frequency techniques is that, only a single excitation frequency is used, yet multiple harmonics with strong signal-to-noise ratios are excited due to the reduced force constant of the inner-paddle.

Due to its advanced capabilities and ease of use, the inner-paddled cantilever has attracted the attention of many researchers across various areas of material science. We are currently working toward developing a batch fabrication process to mass produce the inner-paddled cantilever so that it can be tested in customer settings. The two-field inner-paddled cantilever marks a milestone for the advancement of AFM and promises to bring about a paradigm shift to the design of an AFM cantilever. As an important tool in nano-science and technology, its advancement can ultimately lead to further scientific discoveries and new technologies.

Mapping the Cure Kinetics of Voxel-scale Photopolymerization

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Photopolymerization is a critical synthesis technique used in thin film coatings, biomaterials and additive manufacturing. For many emerging applications, polymerization takes place in dimensionally constrained volumes. However, characterization of the polymerization process still relies on bulk scale tools such as rheometry which are blind to the fundamental phenomena such as species diffusion and light absorption which heavily influence heterogeneity in thin films and additively manufactured parts. Here, we introduce a hybrid atomic force microscope + stereolithographic 3d printer projection instrument that enables micro and nanoscale characterization of the 3d printing process in-situ. Building off the recently introduced Sample-Coupled-Resonance-Photorheology measurement [1], by monitoring the resonance frequency and quality factor of an AFM cantilever with a needle-like tip immersed in a thiolene photopolymer resin, the reaction rate of the local polymerization can be inferred. Specifically, the quality factor is correlated with the resin viscosity, which has a predictable monotonic dependence on molecular weight. Hence, fast measurement of changing quality factor can infer polymerization rate. Here, we adjust the location, intensity and size of the polymerization-initiating 405 nm patterned light source to spatially map the localization of the polymerization process. In figure 1, a line of light 5 pixels (~3.5 µm) wide is positioned some controlled distance away from the oscillating tip. The purple bar indicates the width and duration of the illumination. Intensity is varied from 1 mW/cm^2 to 4 mW/cm^2, while total dose (J/cm^2) is kept constant by proportionally adjusting exposure time. The position of the illumination is varied ~70 µm relative to the tip. Of first note is the considerable conversion that is detected even 35 µm from the illumination in Figure 1a. Such conversion is attributed to oligomer diffusion during the reaction process. Also of note, maintaining constant exposure dose does not result in equivalent spatial cure characteristics, contradicting a common assumption in photopolymerization processes. Finally, we find that changing illumination size, but maintaining area-normalized exposure intensity, results in radically different reaction rate inside and outside the illuminated region. Overall, the new characterization can provide data to optimize polymerization conditions for better part homogeneity and improved mechanical performance in additive manufacturing and thin films.

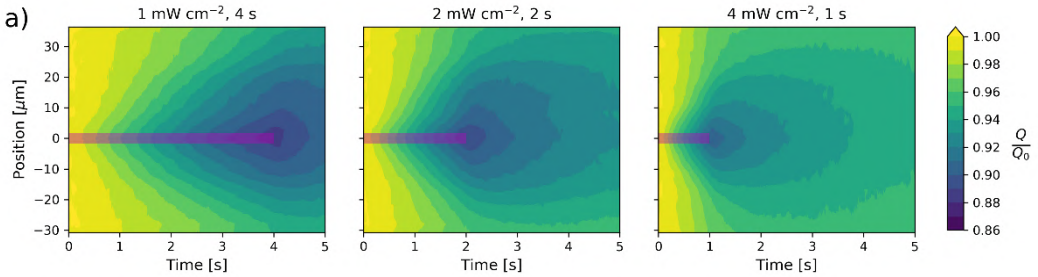

Figure 1: Thiolene conversion (represented as change in cantilever damping *Q*) as a function of position relative to illumination and also duration of exposure. Reaction is found to occur tens of microns from the light source, and depends strongly on polymerization conditions and pattern size.

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Effect of Excitation Frequency on Multifrequency AFM on Soft Matter Characterization: Air and Liquid Environment

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By emergence of multifrequency atomic force microscopy (AFM), there have been many studies focused on optimizing the imaging parameters. Specifically, by having higher number of eigenmodes, selection of amplitude and frequency for sensitive imaging becomes challenging during experiments. In this talk, a set of studies is presented with different guidelines for selection of excitation frequency of the first and higher eigenmode in both air [1] and liquid [2] environment. It is found that depending on the desired observable (i.e., 2nd eigenmode phase, virial or dissipated power, or loss-tangent), the procedure for selection of excitation frequency would be different. Based on these studies, it is found the conventional method of selecting the excitation frequency to be *at* or *near* eigenmode frequency is not necessarily the best method. Additionally, a new imaging method called *biharmonic AFM* that provides promising advantages compared to bimodal AFM is presented [3]. In this study, it is found that the ratio of second to first eigenmode frequency heavily dictates the sensitivity of the cantilever to tip-sample force interactions. Numerical and experimental studies are done on polymer blend samples. Results show the highest 2nd eigenmode phase contrast is observed with a cantilever that has the lowest second to first eigenmode frequency and is excited with its first eigenmode and 6th harmonic (i.e., the nearest harmonic to the second eigenmode).

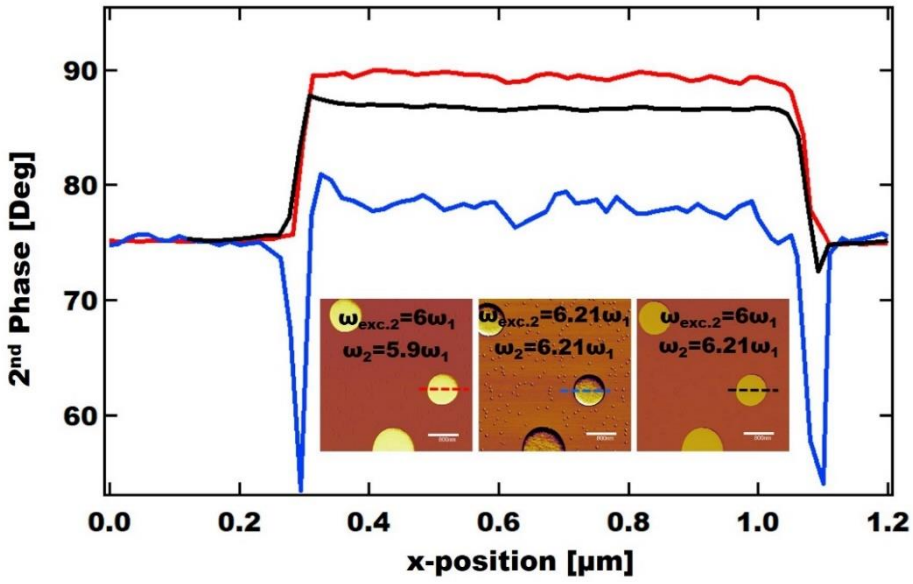

Figure 6 Phase Contrast Comparison for Different Excitation Frequencies

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Multifrequency AFM on Viscoelastic Polymer Samples with Surface Forces

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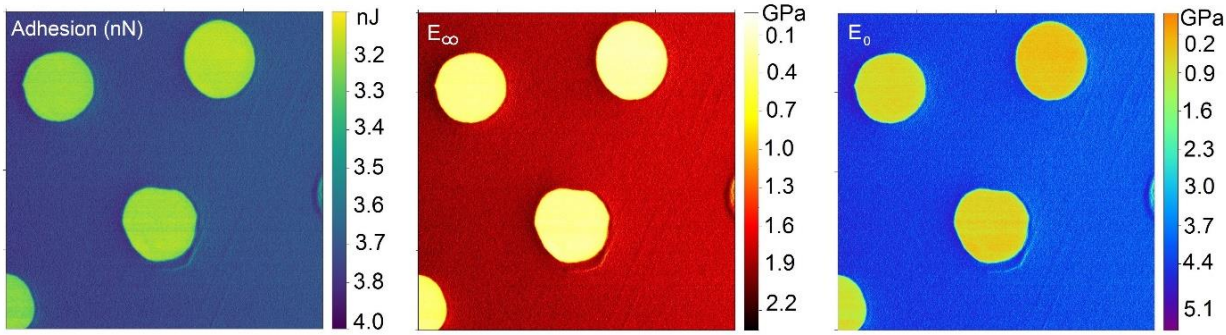
Quantitative mapping using Multi-frequency AFM requires a rigorous approach to link the multi-frequency observables and the local physical properties of the polymer such as local relaxation, short/long-time moduli, adhesion, etc. [1-3]. This link can be established with the use of computational models that allow the simulation of oscillating tips on polymer samples within a rigorous mathematical model framework. We have recently developed a code which allows for the simulation of surface deformation and interaction force history of a rigid oscillating axisymmetric tip interacting with a polymer with arbitrary surface force models (including Lennard Jones pressure) and linear viscoelastic constitutive relations within the context of full three-dimensional viscoelasticity. The code is based on the method introduced by Attard and co-workers [4, 5]. In a collaboration with Dow Analytical Division, we have recently [6] used this approach together with the analytical theory of dynamic AFM to predict the surface deformation and force deformation history of polymers in tapping mode AFM and compared with experimental results. Then, we extended this approach to bimodal AFM in which augmented observables are provided by the instrument. The developed model successfully connects the physical properties of polymers and AFM observables and was experimentally verified.

Algorithm's spatially resolved viscoelastic and surface properties predictions for a PS-LDPE



sample based on the acquired bimodal observables

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Filming Living Cells with High-Speed Scanning Ion Conductance Microscopy

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Scanning ion conductance microscopy (SICM) is a pipette-based, high-resolution imaging technique with an increasing number of applications in chemistry, physics, and biology. A drawback of conventional SICM setups, however, is their relatively slow imaging rate of 4-100 s/frame. We present a high-speed SICM (HS-SICM) setup with a rate of 0.6 s per frame. In combination with a "turn step" protocol for rapid pipette retraction, we imaged fast morphodynamics of live epithelial cells and human platelets.

For ultrahigh-speed imaging of cardiomyocyte contractions, we combined SICM with a microelectrode array (MEA). By synchronizing SICM with the simultaneously recorded action potential by the MEA device, we achieved an imaging rate of 5000 frames per second. This allowed us to reconstruct the time-resolved 3D morphology of cardiomyocytes during a full contraction-relaxation cycle. We used the combined MEA-SICM setup to visualize the effect of blebbistatin, a myosin II inhibitor, on the morphodynamics of contractions.

Viscoelastic mapping with AFM force volume mode

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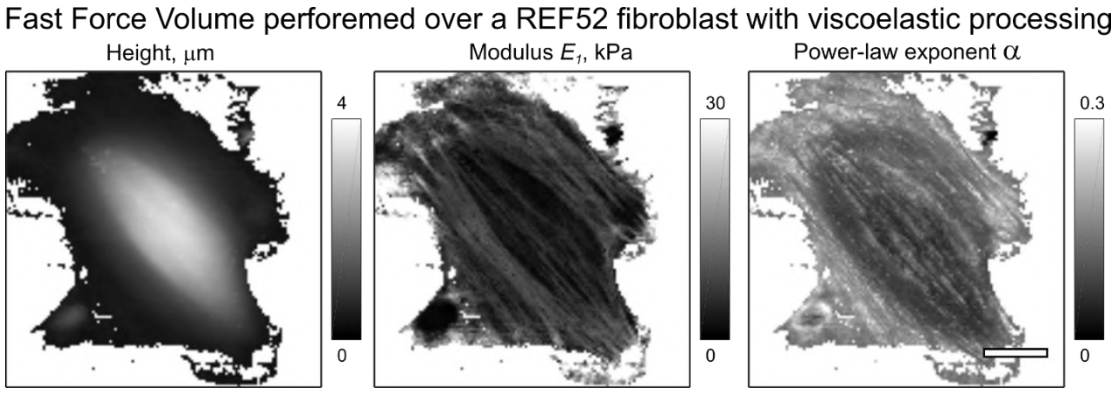
Force Volume (FV) technique remains the most commonly used AFM mode to measure and map the elastic properties of living cells and hydrogels. The recent developments in processing algorithms now also allow extraction of viscoelastic properties directly from the conventional FV experiments data [1]. Here, we used the numerical solution of the Ting's viscoelastic model for a general contact problem with an arbitrary history of the contact area. A significant benefit of the numerical solution is the possibility to use different models describing the viscoelastic behavior of the sample (e.g., Kelvin-Voight model, Standard linear solid model, power-law rheology model). We have compared the numerical solution with available analytical solutions of the Ting's equations for specific viscoelastic models and have found perfect agreement. Important experimental factors were also considered. The finite thickness of the sample was accounted for via the bottom-effect correction factor [2] implemented directly into the calculation process. The hydrodynamic drag forces occurring in a liquid environment were calculated and excluded for the complete indentation cycle. Experiments were performed at different indentation frequencies to select the best viscoelastic model. Using the acquired maps of the viscoelastic properties over the different types of cells we analyzed the characteristic features of different cellular elements, including the nucleus, actin stress fibers, and others. This work was supported by the Russian Foundation for Basic Research, grant 19-79-00354 (development of numerical algorithms), by grant of the President of the Russian Federation for young scientists MK-1613.2020.7 (AFM experiments), and Russian academic excellence project '5-100' (cell experiments).



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Sequence-dependent regulation of the mechanical properties of double-stranded DNA and RNA at short length scales

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Sequence-dependent DNA conformation and flexibility play a fundamental role in specificity of DNA-protein interactions. Here we quantify the DNA crookedness: a sequence-dependent deformation of DNA that consists on periodic bends of the base pair centers chain. Using extensive 100 microsecond-long all-atom constant-force molecular dynamics (MD) simulations [1], we found that DNA crookedness and its associated flexibility are bijective: unveiling a one-to-one relation between DNA structure and dynamics [2]. This allowed us to build a predictive model to compute DNA stretch modulus from solely its structure. Sequences with very little crookedness show extremely high stiffness and have been previously shown to form unstable nucleosomes and promote gene expression. Interestingly, the crookedness can be tailored by epigenetic modifications, known to affect gene expression. Our results rationalize the idea that the DNA sequence is not only a chemical code, but also a physical one that allows to finely regulate its mechanical properties and, possibly, its 3D arrangement inside the cell.

Mechanical properties also play a key role in many biological functions of double-stranded (dsRNA) --like the interaction with proteins that regulate gene silencing--, but how sequence affects the global mechanical response has so-far remained unexplored. Using the same MD protocol, we find that the nucleotide sequence affects in a strikingly different manner the overall stretching and twisting flexibility of RNA and DNA duplexes [3]. For instance, poly-CG sequences soften the stretching response in dsRNA but in contrast they make the dsDNA duplex stiffer. Our extensive simulations unveil how similar local base-pair motions can lead to divergent sequence effects in the global mechanical properties of DNA and RNA duplexes.

Finally, motivated by our simulations, we use a combination of single-molecule experiments (including AFM and optical and magnetic tweezers) to prove these sequence effects. In dsDNA, we rationalize the mechanical properties of A-tracts across multiple length scales, shedding light on the cryptic character of these sequences [4]. For dsRNA, we show that a sequence of alternating adenines and uracils, which we named AU-tract, can induce bending of these molecules by locally compressing the major groove between the two strands of the double helix [5].

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Determination of topography and viscoelastic properties of hydrogel, cells and tissues by Atomic Force microscopy

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Over the past few decades, it has become increasingly clear that the functions of many biological systems are closely related to their mechanical properties and their response to the mechanical environment around them. A crucial aspect of investigating mechanobiology is to go beyond purely elastic models, which do not reflect the complex composition of most biological samples. Atomic force microscopy (AFM) uniquely enables the nanoscale imaging of the topography of living biological systems and soft matter while simultaneously characterising biomechanical properties[(1)] and gaining valuable insights into molecular and cellular dynamics. Rheological measurements can be performed to characterize sample response at different time scales and measure viscoelastic properties via relaxation[(2,3)] or modulation experiments[(4–6)]. Here, AFM was used to combine rheological measurements with optical microscopy. This is vital for identifying regions of interest and enables the correlation of results with optical measurements. The sine modulation segments can be integrated with Force Mapping measurements to provide a clear spatial resolution at a specific probing frequency.

The flexible design of the approach is characterized by user-defined contact and relaxation phases, defined amplitude, frequency, and periods, and linear or log spacing of frequency values. A choice of loop logic through positions and frequencies as well as closed-loop control during sine oscillation are also possible. These measurements derive rheological properties e.g. the elastic storage modulus E′, viscoelastic loss modulus E″ or the ratio of the loss tangent [(6)].

We will demonstrate rheology experiments on living mammalian cells and spheroids over large ranges (0.5 - 500 Hz) and discuss the benefit of using a colloid-like spherical tip with a high tip-height to reduce hydrodynamic effects.

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Samples: CEAT and Dr L. Powell (SKOV-3 spheroids); Prof. A. Hermann, Humboldt University, Berlin, Germany (Vero cells).

A test of statistical significance to compare distributions of protein unfolding forces obtained by Atomic Force Spectroscopy

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Single-molecule Atomic Force Spectroscopy (AFS) is the preferred technique to measure the mechanical properties of proteins [1]. The determination of the distribution of unfolding forces of a particular protein relies on the calibration of the AFS cantilever [2]. This calibration is affected by an inherent uncertainty that can be up to 25% [3], which hampers comparison of mechanical properties of proteins (e.g. wild-type and mutant variants) [4]. Here, we have designed a method to estimate the calibration uncertainty that best fits the spread of AFS experimental data. Considering this estimated calibration uncertainty, Monte Carlo simulations are then used to assess the standard deviation of the difference in the mean unfolding force of two proteins under the null hypothesis that there is no difference. With this information, a statistical test is then able to determine the significance of experimentally determined differences in mean unfolding forces. Hence, our method provides a framework to increase confidence in experimental determinations of how protein mechanics is modulated by protein sequence, ligand binding and posttranslational modifications.

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Non-mechanical quantum interactions: spectroscopically probing energy transfer by correlated microscopies (AFM-FLIM)

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Atomic Force Microscopy (AFM) relies on sensing interactions between a probe and the underlying sample. In liquid environment, mechanical interaction is one of the central components of the AFM inner-workings since out of contact interactions are generally small. While AFM has great success in many different areas, it still lacks resolution to image soft samples (in the kPa range) such as living cells. To circumvent this limitation, we developed a sensing mechanism based on the energy transfer between a fluorescent nanodiamond (donor) attached to an AFM tip and the sample (acceptor). To achieve that, we set up an operational scheme that permits to simultaneously acquire AFM and Fluorescence-lifetime Imaging Microscopy (FLIM) images in a cross-talk free manner with no optomechanical effects for different cantilevers [1]. As proof of concept, Fig. 1, we have observed a change in the nanodiamond lifetime (Fig. 1b), out of mechanical contact (Fig. 1c), when approached to a gold substrate in a DBPS buffer. This change is consistent with an energy transfer efficiency of 5-8%. This opens up a novel pathway of quench sensing to image soft samples such as model membranes labeled with organic dyes as acceptors since it does not require tip-sample mechanical contact, in contrast with conventional AFM in liquid. Therefore, very soft samples can potentially be imaged without being deformed if lifetime is used as feedback for imaging.

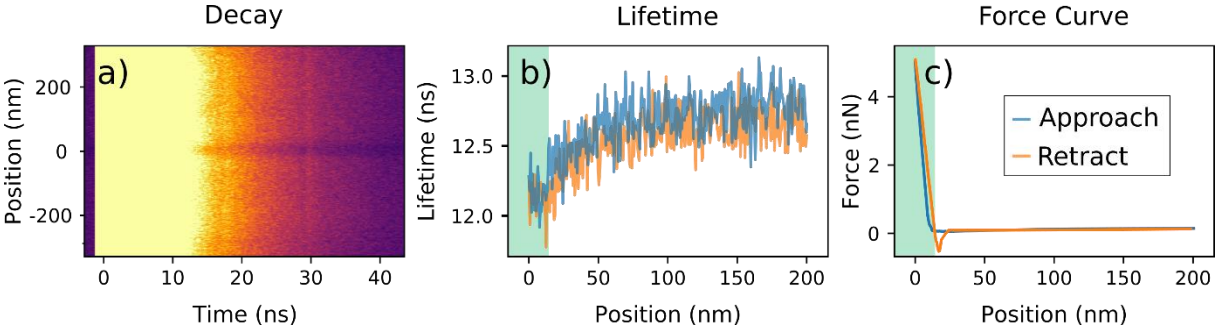

Figure 1. Simultaneous fluorescence decay, lifetime and force curve measurements for a nanodiamond approaching a 10 nm thick gold substrate. (**a**) shows all decay curves for every tip z-position, (**b**) shows the lifetime variations as a function of tip position, and (**c**) shows the force curve. In (**b**) and (**c**), blue and orange lines represent the approach and retract curves, respectively, and the light green region represents the regime of mechanical contact.

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In situ conversion of sphingomyelin to ceramide reveals a local increase of the viscoelasticity of lipid membranes

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Ceramide is produced in cells from sphingomyelin by means of the enzymatic activity of endogenous sphingomyelinase. The presence of ceramide has a high impact in the physical chemical properties of the membrane. Ceramide induces changes in the curvature, phase, segregation and order of the lipid membrane. We are interested in the dynamic nanomechanical changes of the lipid membrane in the conversion of sphingomyelin to ceramide, particularly the impact of chain length and unsaturation of sphingomyelin and ceramide in the overall membrane nanomechanical properties. Atomic Force Microscopy (AFM)-based Force Spectroscopy is an ideal technique to investigate the mechanical properties of lipid bilayers at the nanoscale, their elastic constants [1] but also their plastic deformation and rupture [2]. However, the viscoelastic parameters of lipid membranes have been less explored by these means. In this work, we systematically studied the enzymatic conversion of sphingomyelin-containing supported lipid bilayers to ceramide by adding sphingomyelinase in situ (**Fig.1**). The local production of ceramide induces, in turn, local changes in the membrane mechanics that depend on the chain length and degree of unsaturation of the original sphingomyelin. We assess here the elasticity directly from the AFM force-distance curves and discuss possible approaches to evaluate the viscoelasticity of lipid membranes [3], i.e. using fast mapping with bimodal AFM [4]. The different ceramide localization in the membrane and mechanical properties is relevant in several biological contexts as apoptosis or viral infection.

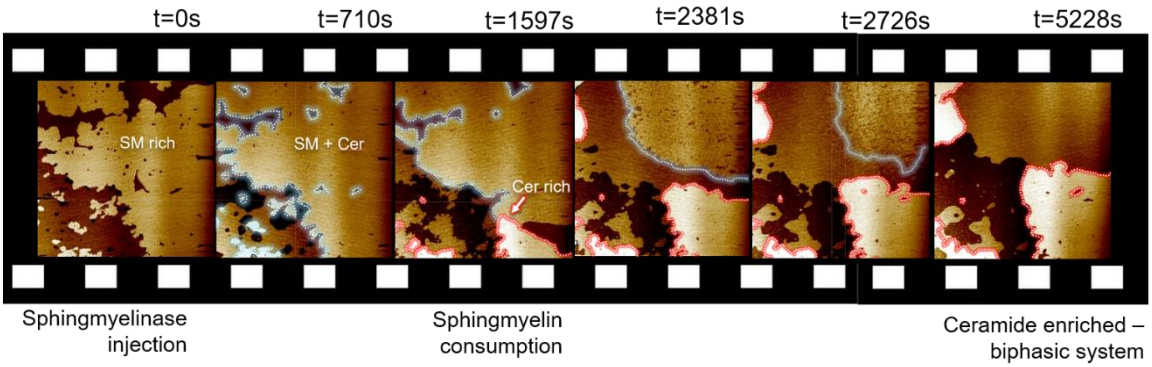

Fig. 1. *In situ* enzymatic conversion of sphingomyelin-enriched to ceramide-enriched domains

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One-step calibration of AFM in liquid

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Nanomechanical measurements of cells and single molecules with atomic force microscopy (AFM) require accurate calibration of two parameters: the spring constant of the cantilever (k) and the optical lever sensitivity (OLS). The most established approach for AFM calibration in liquid environment consists in determining the OLS by acquiring a force–distance curve on a stiff surface and then k is calculated using the thermal fluctuations of the cantilever in liquid (1). Recent works have proposed using cantilevers with already calibrated k, using for example a vibrometer or the Sader method, and then determine the OLS from the thermal spectrum (2). This approach has leads to less variability of nanomechanical measurements than the conventional aforementioned contact based approach (3). Robust and accurate calibration of k using only the thermal spectrum in liquid (not requiring the OLS) would allow one-step calibration of AFM. The Sader or the recent global calibration initiative (GCI) methods do not require knowledge of the OLS to determine k and would allow one-step calibration in liquid(4, 5). Indeed, Sader in liquid is currently implemented in some commercial software packages. However, both Sader and GCI approaches assume high Q-factor cantilevers, not the case for cantilevers commonly used in biological systems in liquid (6). Here we assess the reliability of the Sader method in liquid and propose a method based on the GCI approach to determine k in liquid. We tested the two approaches using only the thermal spectrum in liquid to calibrate k and the OLS on two types of cantilevers with low Q-factor in liquid (AC40 and AC10). While both methods resulted in similar variability, the Sader method led to a systematic bias in k, and subsequently in the OLS. Our results show that the use of GCI approach utilizing only the thermal spectrum in liquid is accurate and allows one-step calibration of AFM(7). To apply this one-step method, accurate knowledge of the factors to correct for both the static to dynamic spring constant and the free-end and loaded-end configurations is essential. These corrections are known for traditional rectangular and V-shaped cantilever geometries. However, the use of cantilevers with non-traditional shapes and large tips (e.g., PFQNM-LC) is becoming increasingly popular, and the traditional correction factors do not apply. Therefore, we have developed a method based on finite element analysis to estimate the correction factors for cantilevers with arbitrary geometry and tip dimensions. We have tested our method on “paddle” shaped cantilevers with pre-calibrated spring constant, confirming the validity of our approach.

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Nanomechanical sub-surface mapping of living biological cells by force microscopy for targeted drug delivery

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Mapping force versus distance curves with an atomic force microscope and the local evaluation of soft samples allows the operator to "see" beneath the sample surface and to capture the local mechanical properties [1]. In this work, we combine atomic force microscopy with fluorescence microscopy to investigate cancerous epithelial breast cells in culture medium. With unprecedented spatial resolution, we provide tomographic images for the local elasticity of confluent layers of cells (Fig. 1a). Strikingly, it is feasible to observe the nanomechanical properties of the fluid-like cytosol beneath a stiff cytoskeletal structure stabilizing the cell membrane that appears to be perforated at unique locations. Contrarily, the highest mechanical strength of the cell was found at locations of the cell cores as cross-checked by fluorescence microscopy, in particular at nucleoli sites as the cumulative elastic modulus of the cell membrane comprising cytoskeletal features and the tight packing ribosomal DNA of the cell [2,3]. The ultimate goal of this work is to utilize the sub-surface mapping of cells for targeted drug delivery to establish a relationship between the penetration sites of nanoparticles with the cell's nanomechanical properties. To this end, ferritin, a protein that is present in the human body for a controlled iron storage and release, is an excellent model system because its empty shell can be modified to carry tailored properties exploitable for targeted and direct drug delivery. We demonstrate, how the pH dependent change in size could be related to the dis- and reassembling of the protein shell of ferritin and apoferritin, respectively. Supplementary imaging by bimodal magnetic force microscopy of ferritin molecules (Fig. 1b-f) accomplished in air revealed a polygonal shape of the core and a three-fold symmetry of the protein shell providing valuable information about the substructure of the nanoparticles [4].

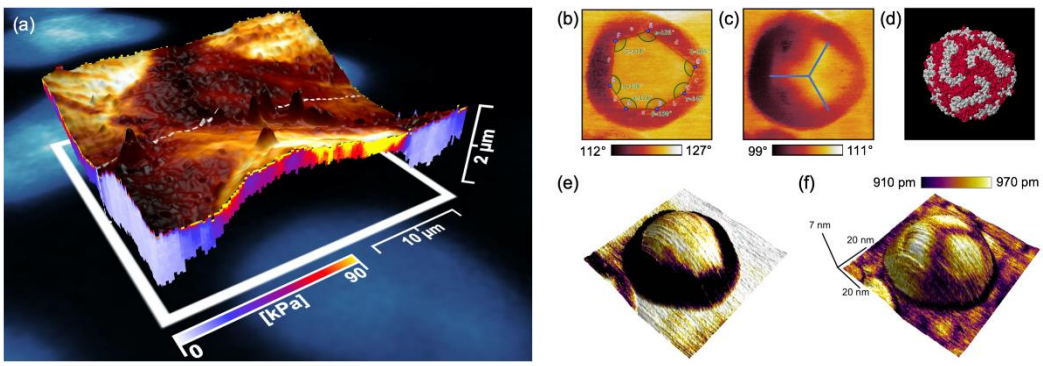

Figure 1. (a) Nanomechanical mapping of epithelial breast cancer cells and bimodal force microscopy of ferritin: (b) first and (c) second eigenmode phase images, (d) crystal structure, (e) 3d topography with color-coded first eigenmode phase and (f) second eigenmode phase.

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Infrared Nanospectroscopy (AFM-IR): Unravelling the Chemical and Structural Properties of Biological Systems at the Nanoscale

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Biological processes rely on a wide class of biomolecular and macromolecular machines whose function emerges from their chemical and structural properties and have characteristic nanoscale physical dimensions. In order to shed light on the rules of life, on dynamic biomolecular processes, on the biophysical properties of individual biomolecules and living organisms, it is crucial to access chemical and structural information at the nanoscale under relevant physiological conditions. However, imaging microscopies are to the most part chemically blind. Mapping a single property at the time, such as morphology or stiffness, is not always sufficient when studying inhomogeneous and complex biological systems, such as biomolecules, cells and living organisms.

Here, we show the application of infrared nanospectroscopy (AFM-IR) as a real breakthrough for the analysis of heterogeneous biological samples at the nanoscale. AFM-IR exploits the combination of the high spatial resolution of AFM (~1-10 nm) with the chemical analysis power of IR spectroscopy. [1] If the wavenumber of the exciting laser radiation pulse matches one of the molecular vibrational energy transition levels of the protein, the IR light is absorbed. This absorption causes a thermal heating and expansion of the protein, which is detected thermomechanically by the AFM cantilever. The IR absorbance at each wavenumber is proportional to the peak-to-peak amplitude of the raw deflection of one of the eigenvalues of cantilever oscillation and to the peak amplitude of its Fourier transform. To increase the resolution and sensitivity of AFM-IR, we used gold probes and a gold substrate to exploit the non-plasmonic rod-like antenna effect and to enhance the field at the apex of the tip.[2] AFM-IR is a versatile technique to study biomolecular processes with nanoscale chemical resolution bridging multiple biological scales, from the recent achievement of single protein sensitivity to protein self-assembly, chromatin organization and cellular systems.[3,4,5,6] Finally, as a major advance in the field, we prove that infrared nanospectroscopy can successfully unravel the chemical properties of biological samples at the nanoscale not only in air, but also in native liquid environment that is of fundamental importance to study biomolecular processes and organisms.[7]

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Hierarchical Self-assembly of Two-dimensional Peptide

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Two-dimensional polypeptides and proteins have a high density of chemical functionality, which are a fundamentally important geometry that can be utilized to direct highly specific intra- and inter molecular interaction. Such peptide based 2D nanomaterials can also display a variety of functionalities. Thus it is of utmost importance to reveal the detailed structure of 2D peptide aggregates. In this talk, I show how a short fragment of the pathogenic proteins can self-assembling into 2D nanostructures, which may lead to the construction of more advanced polypeptide nanostructures for future applications.

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Mechanical compliance of graphene biomaterials for neural repair by force microscopy

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Tissue engineering offers the tools to design biomaterials that, by mimicking the physic-chemical properties of native tissues, serve to promote reparative responses in damaged organs [1]. Specifically, mechanical compliance has been identified as one of the pivotal requirements for such materials. From a traditional point of view, mechanical properties of biomaterials have been measured by using conventional macroscopic tensile and compression tests. In the recent years, force microscopy has opened the possibility of increasing the resolution of those measurements at the nanoscale [2], in line with the dimensions of cellular components and extracellular matrix elements. In this sense, understanding mechanotransduction, the conversion of the mechanical forces that living cells experience into biological signals, will bring important insights in both physiology and pathology [3]. In this work, we explore the regenerative ability of reduced graphene oxide (rGO) scaffolds to support pivotal features of neural repair at 4 months after spinal cord injury by an interdisciplinary approach [4]. 3D randomly porous foams have been prepared in mechanical compliance with neural cells and tissues (Young's modulus of 1.3 ± 1.0 kPa) as demonstrated by atomic force microscopy techniques applied ex vivo. After implantation, the significant increase in Young's modulus caused by massive cell/protein infiltration does not alter the mechanical performance of the contralateral spinal cord but provides mechanical stability to the lesion. Importantly, this mechanical compliance with the spinal cord tissue correlates with positive biological findings as a high degree of vascularization, the growth of neurites, and the reduction of areas of perilesional damage.

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Nanoendoscopy AFM: a window into the cell

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The development of new instrumentation methods had always led to significant progress on the cell structure, organization and operation knowledge. One of the key techniques commonly used to understand both cell function and architecture is microscopy, currently applied using several imaging techniques which, however, present the following weak points: 1) electron microscopic techniques are useful tools for imaging the interiors of cells, but do not provide Z maps and must be performed in vacuum, using dehydrated, vitrified or metal shadowing samples, which potentially damage or alter the native supramolecular structure, making it unsuitable to perform studies in living conditions; 2) optical microscopic techniques such as fluorescence imaging are useful for following the dynamics of specific target molecules in living cells, but requires complex sample preparation, using dyes or fluorophores, and presents poor spatial resolution (≈ 200 nm in XY and ≈ 500 nm in Z), which is not sufficient for observing the dynamic structural change of intracellular molecules; and 3) super-resolution microscopy, which cannot resolve structures on the sub-molecular scale (resolution ≈ 10 nm in XY and ≈ 20 nm in Z) and also requires fixing, freezing or fluorescence labeling, leading to drastic changes on structure and functionality. As a result, none of the former techniques can be used to visualize the internal structural dynamics of a single molecule at high resolution in living cells. Therefore, it is necessary to develop a technique that 1) combines sub-nanometer resolution; 2) enables measuring in physiological conditions using native samples; and 3) measures the cell's internal structures non-destructively without disassembling it. Here, we present a novel nanoendoscopy technique based on the atomic force microscope (AFM) to measure cells internal structures, in their own physiological environment without compromising their integrity or disassembling them, obtaining 3D (Fig.1a) and/or 2D (Fig. 1b) images maps that reflect the precise cell's structures and functions.

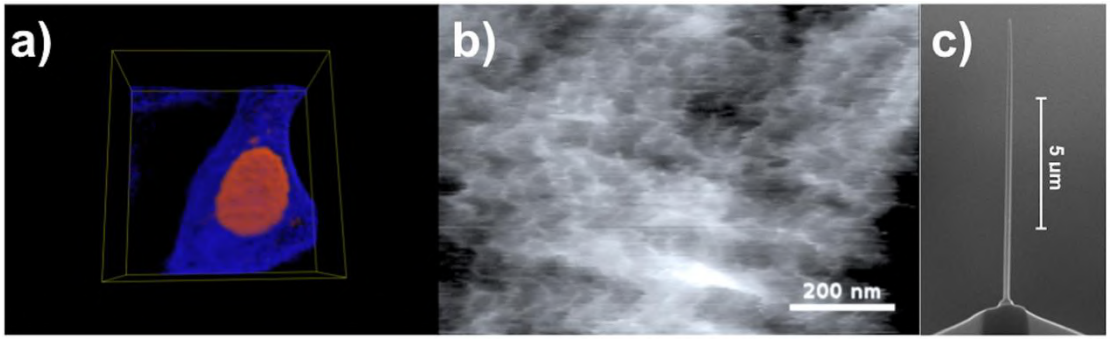

Figure 1. a) HeLa cell 3D-map performed with an AFM. b) Image of the internal side of the apical cytoplasmic membrane of a HeLa cell. c) Example of a microfabricated needle like structure used to penetrate and internally measure cells, fabricated by focused ion beam (FIB) milling.

Recent advances in AFM tip-living cell nanomechanics

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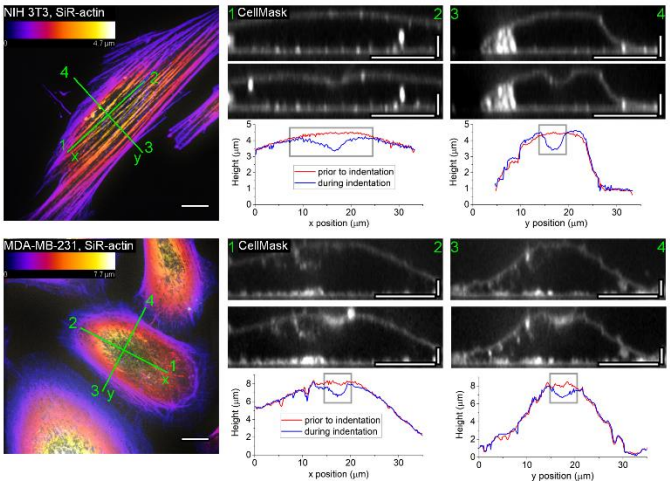
We discuss recent advances in AFM imaging and force spectroscopy on soft materials such as polymers and live cells. These advances rely on significantly improved modeling of tip-sample nanomechanics and microcantilever vibrations, as well as the judicious use of combined instruments.

In the former, new contact mechanics models that explicitly take into account local relaxation and surface forces are needed and their influence on microcantilever dynamics needs to be interpreted. Specifically, we discuss new ways to accurately and quantitatively measure viscous relaxation from standard force curves and discuss approaches using Attard's model to self-consistently include arbitrary surface forces and three –dimensional linear viscoelasticity.

In the latter, data from simultaneous confocal microscopy and AFM needs to be integrated to provide unprecedented insight in the local mechanics of deformation and help correlate mechanical property contrasts to sub-cellular structures. Both computational and experimental results are presented.



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Quantifying mechanical properties of cells and tissue

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In cancer development, cell and matrix stiffness has been demonstrated to be a key indicator of metastatic potential. Atomic force microscopy (AFM) is a commonly used method for extracting mechanical information from soft biological matter including cells and tissue. To assess the mechanical interplay between the cells and extracellular matrix (ECM) during invasion, we combined confocal fluorescence microscopy and AFM indentation to determine the Young's moduli of individual embedded cells and the pericellular matrix [1]. As the samples being studied with AFM become more complex, novel analysis methods must be developed to produce meaningful and quantitative data; thus, new strategies for fitting force-indentation data beyond the standard Hertz model are essential. We developed a method of raw data fitting, which determines the apparent Young's modulus as a function of indentation depth, which provides sensitivity to sample heterogeneities such as subsurface elasticity effects [2]. Further, we studied ECM remodeling in co-culture systems containing cancer cells and cancer associated fibroblasts (CAF) [3]. We found significant remodeling and changes in the mechanical properties of the ECM only in the presence of both cell types. Further we characterized the expression of multiple tumor secreted pro-fibrotic factors and identified the role of platelet derived growth factor (PDGF AA and BB) in breast cancer fibrosis.

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Nanoscale mechanical properties of cells studied by torsional harmonic AFM

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Interaction forces between the sharp tip of atomic force microscope (AFM) probes and surfaces carry detailed information about mechanical and chemical properties of samples. We rely on specially designed T-shaped cantilevers to probe these tip-sample interaction forces while the sample is being scanned by the AFM. Recently, we have applied this approach to image stiffness of live cells at high resolution. We have found that the resulting cell stiffness images exhibited patterns that suggest that the origin of stiffness is intracellular forces, rather than the elastic modulus of the cytoplasm [1]. These patterns facilitated the development a set of mathematical relationships between cell stiffness and intracellular forces, which helped determine physiologically relevant intracellular forces like plasma membrane tension, cortex tension, and tension across actin filament bundles directly from high resolution cell stiffness images. More recently, we applied this imaging approach to investigate mechanical properties of neuronal synapses and found these synapses to be extremely stiff cellular structures. We will present potential biological relevance of these findings.

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Imaging Myosin II-driven Stress Fiber Contraction in De-roofed Cells by High-Speed AFM

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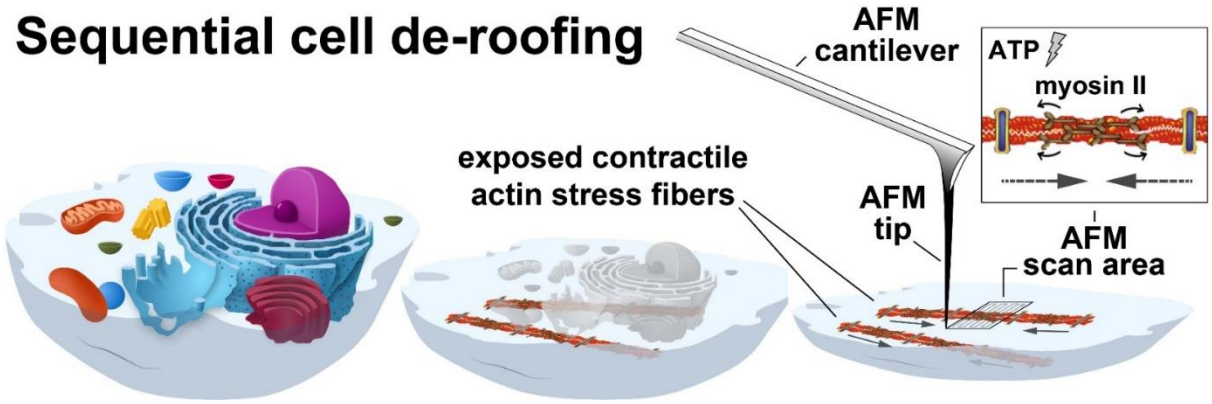
Observing the dynamic action of single protein molecules directly within their native cellular environment is an ongoing experimental challenge requiring innovative high-resolution microscopy solutions. High-speed atomic force microscopy (HS-AFM) is unique because it can directly image the dynamic behavior of individual biomolecules in physiological liquid environments. However, HS-AFM is a surface scanning technique and so far it has not been able to image protein action inside cells, partly because protocols for introducing the AFM tip into the cell have been unavailable. To extend the application of HS-AFM to intracellular structures, different cell de-roofing methods were developed to remove part of the outer cell membrane, thereby exposing intracellular structures, including actin stress fibers (SFs), for subsequent AFM analysis. SFs are contractile fibers of the actomyosin cytoskeleton that help cells to maintain intracellular tension and to migrate. Importantly, exposed SFs in de-roofed cells fully retain their ability for myosin II-dependent contraction after Mg^{2+}·ATP stimulation. Continuous high-speed scanning of contracting SFs revealed unique and real-time insight into the action of individual myosin II motors driving this contraction. Furthermore, high-resolution AFM imaging provides novel insight into sarcomeric SF ultrastructure. Our approach thus establishes an experimental system in which cytoskeletal changes driven by dynamic myosin motor action can be visualized and analyzed on the molecular scale for the first time. Moreover, these experiments generally establish HS-AFM as a powerful tool to visualize dynamic intracellular processes at molecular resolution within native cellular environments.



Viscoelastic Properties of Living Multicellular Epithelial Tissues Determined by Non-Contact Acoustic Frequency-Modulated AFM

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The maintenance of functional epithelial tissues molecular-structural-mechanical integrity requires coordination between cell-cell adherens junctions, tight junctions (TJ), and the perijunctional actomyosin cytoskeleton. First, we addressed the hypothesis that alterations in TJ structure and remodeling of the actomyosin cytoskeleton modify the supracellular epithelial tension, fluidity, and the intra-/inter-cellular adhesive forces. Second, we addressed the hypothesis that lack of horizontal top connectors in the organ of Corti Outer Hair Cells (OHC) stereocilia bundles have impaired suppressive masking while both acoustic and electrical waveform distortions are absent. This unique phenotype suggests that the main source of cochlear waveform distortions may be deflection-dependent hair bundle mechanics resulting from constraints imposed by the presence of horizontal top connectors. Current methods to measure supracellular or localized mechanical properties disrupt intact epithelial tissues, therefore, we developed a novel method to determine the epithelial level or more local mechanics using noncontact acoustic frequency-modulated atomic force microscopy (FM-AFM) [1, 2]. Initially, we tested the method on Madin-Darby Canine Kidney (MDCK) II polarized monolayers. Our results showed that double knockdown (dKD) of zonula occludes ZO-1/ZO-2 elevates the apical epithelial tension and effective viscosity [1]. Interestingly, epithelial tension is more sensitive to inhibition of myosin II ATPase activity than to inhibition of ROCK or MLCK activity, but viscosity is highly sensitive to all [1]. Additionally, we used noncontact FM-AFM to investigate the stereociliary hair bundle stiffness and damping [2]. Hair bundle mechanics were determined from postnatal days P9-P15 stereocilin-deficient mice with detached tectorial membrane ($Strc^{-/-}$/$Tecta^{-/-}$ double knockout) and compared with heterozygous littermate controls ($Strc^{+/-}$/$Tecta^{-/-}$). A significant decrease in bundle stiffness by ~60% was observed when horizontal top connectors were absent from mature cochlea's from P14-P15. Moreover, the bundle viscous damping was significantly reduced by ~74%. Last, we followed the bundle mechanics during OHC development between ages P9-P15 and quantified the observed increase in OHC bundle stiffness and damping in $Strc^{+/-}$/$Tecta^{-/-}$ mice while no significant change was detected in $Strc^{-/-}$/$Tecta^{-/-}$ animals [2]. In conclusion, noncontact acoustic FM-AFM enables the relevant physiological and quantitative investigation of critical mechanical properties in intact biological tissues and could be used to decipher the molecular regulation of epithelial organization and morphogenesis.

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Biophysics, One Molecule at a Time

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POSTERS

Nonlinear Dynamics Perspectives on Intermodulation Atomic Force Microscopy

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This work explores time-varying bifurcations and nonlinear transients in intermodulation AFM (ImAFM) [1]. During ImAFM, the cantilever is excited with two closely spaced frequencies, f_1 and f_2, that fall within the bandwidth of the cantilever's fundamental bending mode. In the time domain, Im AFM excitation is equivalent to slow frequency cycling of the drive excitation at the difference frequency. In the frequency domain, the linear beating in the cantilever's response appears as two closely spaced peaks at f_1 and f_2. The presence of nonlinearity owing to tip-sample interactions generates additional peaks in the frequency domain known as intermodulation products (IMPs), and anharmonicity of the slow flow in the time domain. We seek to develop a theoretical framework for understanding nonlinear dynamics in ImAFM, in particular how the cycling of drive excitation at the difference frequency leads to transitions across bifurcations that separate classical tapping mode attractive and repulsive regimes. Secondly we attempt to understand how the IMP observables patterns and force reconstruction can be effected by the choice of difference frequency. In Fig. 2 (top) we show computational results corresponding to ImAFM at varying levels of the difference frequency. For comparison, the 'unfolded' tapping mode force sweeps (obtained using known semi-analytical relations) are also plotted, which correspond to the ImAFM problem in the limit as. Interestingly, two distinct trends are observed: (1) for low, there is a delay in the bifurcation that increases with increasing, and (2) there is a threshold value of above which the dynamics are attracted to the repulsive branch early and never settle on the attractive branch. Regarding the second trend, theoretical analysis of the domains of attraction show that the onset of interaction with the sample perturbs the cantilever's slow flow in such a way that the dynamics cross the stable manifold early for sufficiently large and remain in the repulsive domain of attraction thereafter. These theoretical predictions were verified over a series of ImAFM experiments corresponding to different cantilevers and samples using a JPK Nanowizard 3. The experimental results corresponding to an AC160 cantilever from Asylum Research and a Mica sample are shown in Fig. 2 (bottom) where strong qualitative agreement with the theoretical predictions is observed.

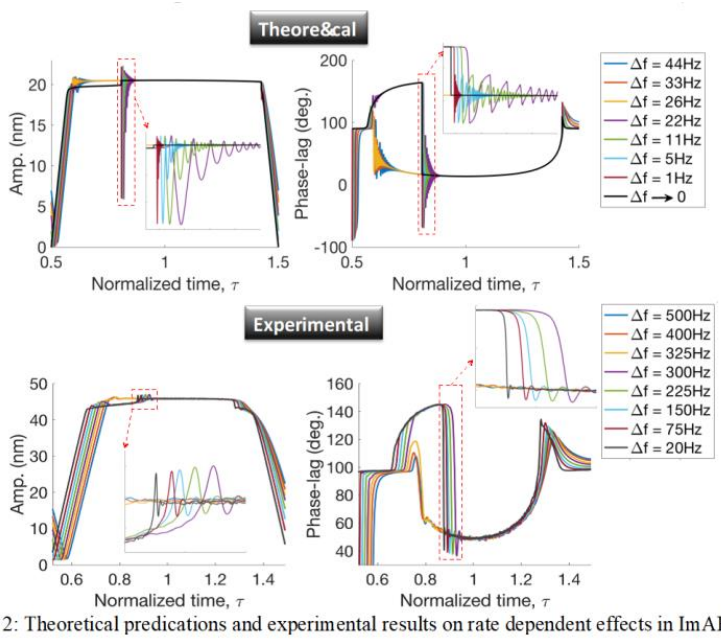

Fig. 2: Theoretical predications and experimental results on rate dependent effects in ImAFM.

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Measurement of Surfaces at Cryostatic Temperatures with a Tuning Fork Cantilever Applying a Multifrequency Approach with Intermodulation Products

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A new method to measure three-dimensional force volume data in a single pass using mixing products of different drive tones for high resolution tuning fork based force measurements at low temperature will be presented. Generally force volume data in atomic force microscopy (AFM) are acquired by successive measurements of force-distance curves typically taking more than 10h. This limitation can be addressed by a combination of high resolution imaging at low temperatures using a tuning fork (TF) based AFM and two more advanced measurement methods [2,3]. The new method makes use of a Multifrequency Lock-In Amplifier (MLA, Intermodulation Products AB) detecting the intermodulation products which are generated due to a perturbation by a nonlinear tip-surface interaction. For the measurements the TF-AFM running at a temperature of 4.8K is excited near resonance (~25.5kHz) while a second drive tone is applied to the z-piezo controlling the sample height. This additional modulation of the tip-sample distance has a far lower frequency, in this case at 5Hz (see Fig. 1). These two drive frequencies define at what frequencies mixing products appear in the response signal once the non-linear attractive force regime is entered. Here thirty lock-in channels of the MLA have been equally distributed within the frequency band around resonance, in intervals of the low drive frequency. During a single constant height measurement in roughly 30min the amplitudes and phases of all channels are recorded and are used to reconstruct the force distance curves at every point of the scan resulting in a three-dimensional force field of the sample surface. The dependence of tip height, oscillation and modulation amplitudes and modulation frequency will be presented and discussed as well as the method to reconstruct the force data from the amplitude and phase channels.

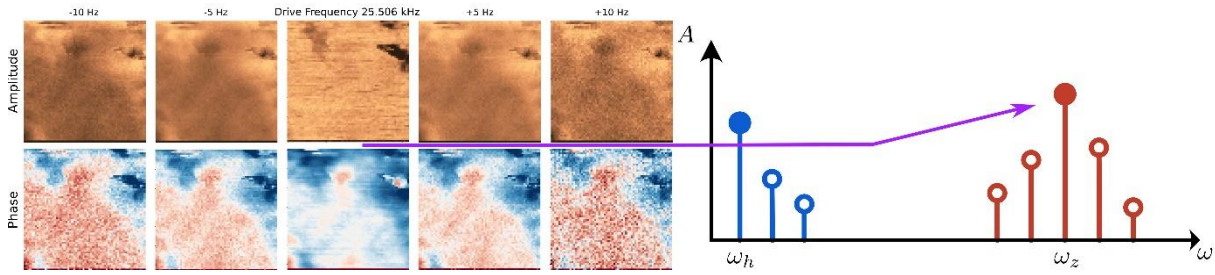

Figure 1: Left: The drive frequency at ~25.5 kHz and the first and second intermodulation products on either side. Right: Blue: z-modulation frequency Red: filled, drive frequency empty, intermodulation products.

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Detector calibration using Intermodulation AFM

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Quantitative AFM relies on accurate calibration of the detector responsivity, i.e. the conversion factor from measured voltage to deflection (nm/V). The detector is typically calibrated using some form of approach curve, where static deflection, amplitude, or resonance frequency is plotted as a function of the height of the cantilever base. Under certain assumptions, for example that the tip does not indent the sample during the approach, such approach curves are used to determine the detector responsivity. These assumptions are approximately valid for a very stiff interaction, where the calibration procedure presents a great risk of damaging a pristine tip. We present a new method of detector calibration based on Intermodulation AFM (ImAFM). ImAFM obtains a force versus deflection curve at *fixed cantilever base height*. It is therefore possible to obtain the detector responsivity by tracking the shift of consecutive ImAFM force curves while slowly approaching the surface. In contrast to the conventional methods, our method allows for change in the tip-surface interaction during the approach, while retaining the ability to accurately determine the distance to the un-perturbed surface (see figure). Calibration is performed directly on very soft materials with the same weak interaction forces used for imaging and quantitative nano-mechanical analysis. Compared to conventional methods, our method gives higher accuracy without risk of damaging the tip.

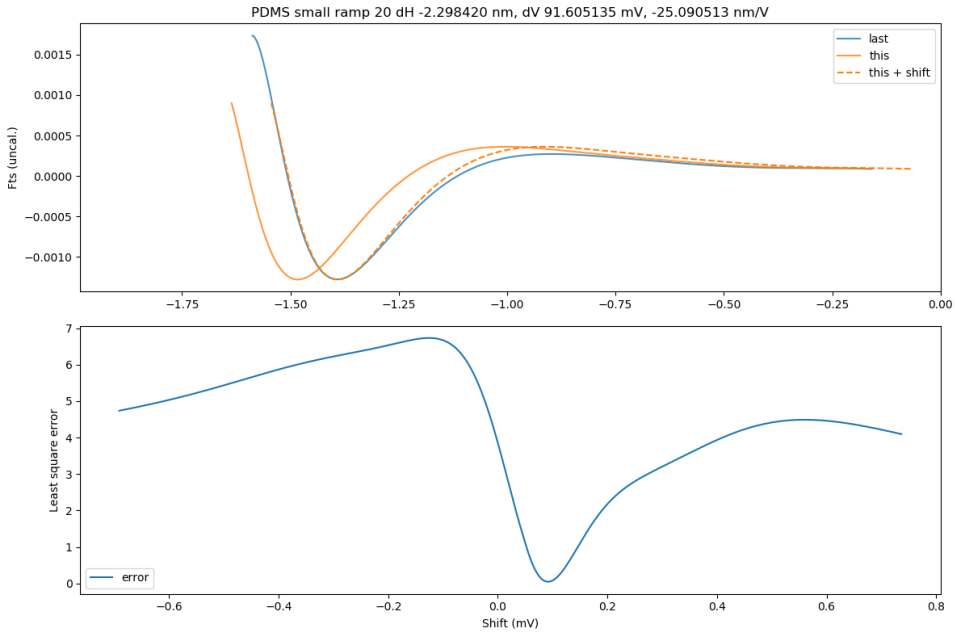

Figure: Top panel, ImAFM force-deflection curves at two different heights (blue and orange). The orange curve has lower peak force and less indentation, but can easily be matched to the blue curve by shifting it along the x-axis (dashed orange). Bottom panel, the shift is found by minimizing or square of the difference between the orange

Detecting non-linear forces on a cavity opto-mechanical sensor via multi-frequency lock-in measurement for AFM

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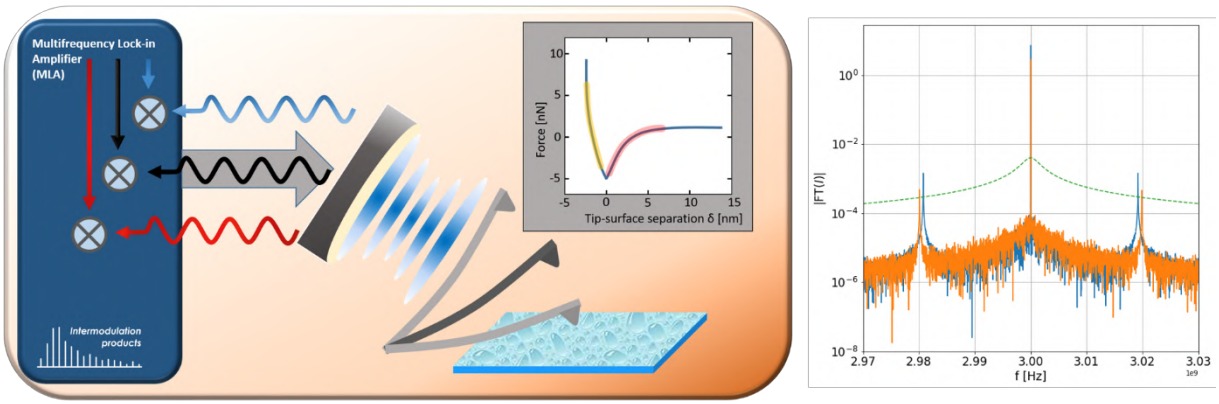
Techniques involving the analysis of the non-linear tip-surface interaction in AFM, such as off-resonance harmonics or intermodulation products near resonance [1], have proven to be the most detailed and accurate methods for rapidly reconstructing the tip-surface force. The accuracy of reconstruction is limited by noise in the force transducer (cantilever) and the speed is limited by noise in the detector. Detection methods beyond optical beam deflection promise enhanced performance and improved resolution. For instance, coupling a mechanical resonator to an optical cavity ideally provides a lossless transduction of the mechanical motion to the phase of the cavity field. This technique found extensive use in gravitational wave detection where the main concern was linear transduction. In our quest toward quantum-limited detection, we must take into account opto-mechanical phenomena [2] such as radiation pressure or back-action. The mechanical motion modulates the cavity field resulting in sidebands in the detected output spectrum, which depend on the strength of the opto-mechanical coupling and the resonance frequency of the mechanical mode. We propose a sensor concept operating in the microwave spectrum where the driving/readout is performed by a tuned multi-frequency synthesizer/lock-in amplifier. With our approach the displacement is tracked via homodyne detection on-resonance, while at the same time detecting the off-resonance sidebands. Alternatively, phase coherent driving at both sidebands introduces the possibility of back-action-evasion.



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Multi-frequency and Multidimensional Low Temperature UHV SFM

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We use a new, home-built UHV low temperature scanning force microscope to simultaneously map vertical and lateral force on the atomic scale. Our instrument uses a fiber-optical interferometer to measure the cantilever deflection. By positioning the fiber outside the cantilever long-symmetry axis, the interferometer can simultaneously measure flexural and torsional oscillations. To drive the cantilever on frequencies of several Megahertz, we found that clean resonance peaks can be routinely obtained by optically driving the cantilever oscillation with a second laser. The system of interest is CO adsorbed on a Au(111) surface which is studied with a CO functionalized metal coated cantilever tip. The cantilever is driven on its second mode flexural resonance frequency of 1.902 MHz at an oscillation amplitude of 40 pm and also on its 1st torsional resonance frequency of 2.218 MHz at an oscillation amplitude of 60 pm. The sample bias is kept at 550 mV to permit the measurement of a tunneling current when the tip is approached to smaller tip-sample distance. We then simultaneously map the 2nd flexural and 1st torsional oscillation mode frequency shift, the corresponding dissipation signals and the tunnel current with a predefined xyz-volume enclosing a single CO molecule. The results show an attractive CO-CO interaction at larger tip-sample distance, that becomes repulsive at a sufficiently small tip-sample distance, when Pauli-repulsive forces between the two oxygen atoms occur. The evolution of the torsional resonance frequency is more challenging to understand: At large tip-sample distance, two negative frequency-shift regions appear on the left and right side of the CO adsorbed on the Au(111) surface, while directly above the CO a positive frequency shift occurs. The latter can be explained by the attractive O-O interaction that "tightens-up" the flexural oscillation mode and increases its resonance frequency like the tension acting on a guitar string. At closer tip-sample distance, where the vertical O-O-interaction force becomes repulsive the torsional mode resonance frequency shift becomes negative on the CO but shows two repulsive peaks on the left and right side of the CO, where the lateral oscillation of the CO on the tip versus the CO on the surface. Our data allows the comparison of the lateral force obtained from E(x,y,z) obtained from $df^{2nd}_{flex}(xy,z)$ data to the actually lateral force measured via $df^{1st}_{tors}(x,y,z)$. This allows the exploration of the limits of the conservative force field, before dissipative force start to play a role.

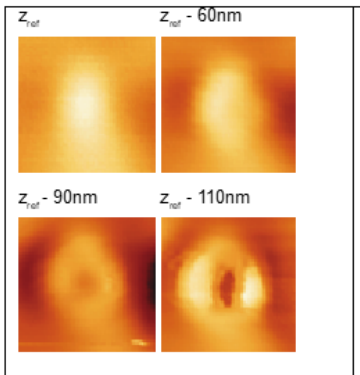

Figure: Here similar data obtained on Cu(111) is displayed. Torsional mode frequency shift images obtained simultaneously with the second flexural mode frequency shift data (not shown here). At larger tip-sample distances, z_{ref}, the torsional mode frequency shift is positive above the CO molecule. This can be attributed to the attractive O-O-interaction force which "tightens" the flexural mode oscillation similar to the effect of increased tension on a guitar string. If the tip is moved z_{ref} -60pm, two distinct regions of negative (attractive) torsional frequency shift appear on the left and right sides of the CO. These result from the attractive lateral force (derivative) acting on the CO on the tip. At z_{ref} -90pm, these attractive force regions move outwards, while close to the CO, the torsional oscillation of the cantilever pushes the tip-CO sideways into the sample CO. The lateral Pauli repulsive force then leads to a positive frequency shift. At z_{ref} -110pm, the boundaries of the repulsive regions become sharp lines indicating flexural instabilities of the tip CO.

Effects of Laser Spot Positioning with Optical Beam Deflection Method on Atomic Force Microscopes

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Due to the advanced probing capabilities of Atomic Force Microscopy (AFM), the technology is not only used for topography but also for material characterization at micro and nano-scales. Regardless of the imaging mode and desired material information, AFM measurements rely on measuring the deflection of a cantilever. This is most commonly done by use of the optical beam deflection method, where a laser is reflected off the backside of the cantilever and into a four-quadrant position-sensitive photodiode (PSD). The voltage readings from the diode change depending on where the laser is reflecting into it, which is directly related to both the slope of the cantilever and the position of the laser spot along the cantilever's backside. Up to this point, the general practice used for laser spot positioning is based on maximizing the signal to noise ratio and placing the laser spot somewhere "near" the end of the cantilever. This work focuses on the importance of laser spot location and suggests a more rigorous positioning methodology, consequently, enhancing reliability of AFM measurements. A numerical study is done that models tip-sample force interactions of a polymer blend sample (polystyrene (PS) and low-density polyethylene (LDPE)) in multifrequency AFM. Based on the established theories from numerical studies, the experimental results from contact mode AFM and bimodal AFM measurements on different sample systems are provided and verified. In both the numerical and experimental studies, three laser spot locations along the cantilever are selected: chosen locations are at 100% of the cantilever's length (directly at the free end), 80% of the overall length and 60%. Based on the findings of the work, an experimental guideline is provided for selecting the *optimum* location of the laser on the cantilever depending on the imaging mode. The results of this study can be helpful in different scanning probe microscopy techniques for characterizing different types of soft matter.

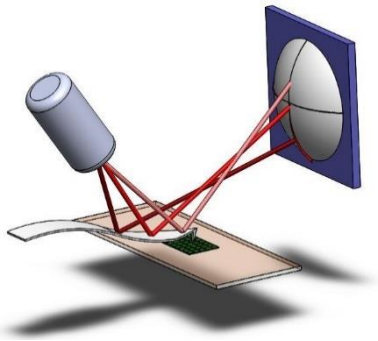

Figure 7 Schematic of Effect of Laser Position on AFM Cantilever

[1] Putnam, J.; Damircheli, M.; Eslami, B.; "Effects of Laser Spot Positioning with Optical Beam Deflection Method on Atomic Force Microscopes." Submitted for Journal Publication.

Mode coupling in non-smooth dynamic atomic force microscopy.

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Dynamic Atomic Force Microscopy (AFM) offers advantages and unique capabilities allowing for the nanoscale characterization of organic and inorganic materials. In dynamic AFM an oscillating cantilever tip interacts intermittently with the specimen while driven close to or at its resonant frequency. The generation of higher order spectral components during cantilever oscillations in dynamic AFM is a direct consequence of the tip-sample interaction and carry information regarding the nanomechanical properties of sample under investigation. To this end, the signal to noise ratio (SNR) of these spectral components are of paramount importance for developing nonlinear identification techniques. Currently, many studies employ intentional mode coupling via physical modification of the AFM probe to enhance specific harmonics of the cantilever. In contrast to these methods, we propose an experimental technique that exploits an unconventional mode coupling between the flexural modes to enhance the sensitivity of higher harmonics. The technique relies on detuning the resonance frequency based on a nonlinear frequency response curve (nFRC) to determine a sweet spot where strong mode coupling is observed. In particular we observe a 7 and 16 fold increase in the sensitivity of the 6th and 17th harmonic, respectively. Additionally, in the sweet spot the phases of the interacting modes are synchronized such that there is reduced sample indentation. Finally, we employ a theoretical model comprising multiple degree of freedom and non-smooth nonlinear interactions between the tip and sample to simulate the experimental data. Our simulations qualitatively conform with the observed physics, and confirms that the mode coupling is the root cause for the observed increase in SNR of higher harmonics. In conclusion, given the simplicity of the technique and the ease of use, we expect the proposed technique to be applied to several dynamic AFM applications.

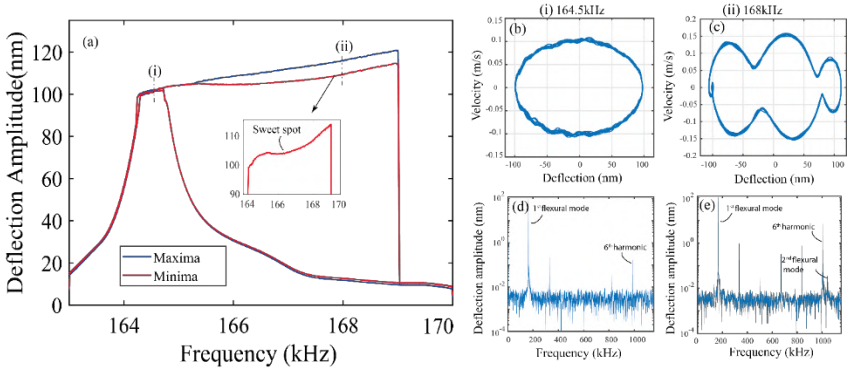

Fig. 1: Experimental nFRC, phase space trajectories and the associated frequency spectra obtained from raw deflection signal. (a) Experimental nFRC, where the blue and green curves represent the maximum and minimum position of the tip, respectively. The inset highlights the gradual curving of the nFRC in a specific range of drive frequency. (b)-(c) Phase space trajectories at 164.5 kHz and 168 kHz of drive frequency showing the influence of one eigen mode and two eigen modes in the cantilever oscillations, respectively. (d)-(e) Frequency spectra of raw deflection signal at 164.5 kHz and 168 kHz showing the presence of higher harmonics and second eigen mode. The variation of phase space from (b) to (c) can be attributed to the appearance of second mode of vibration in (e)

Characterizing the Resolution Limits of Mechanical Resonators

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The mass characterization of nanoparticles is of great interest for many applications in medicine, biology and chemistry. To increase the mass resolution of sensors based on nanomechanical resonators, there is a large research activity on devices that can maximize their quality factors. The efforts to boost the quality factors have been especially important for resonators operating in liquid, targeting the characterization of biological particles. In contrast, a recent publication claims that the ultimate resolution of open-loop resonant sensors improves for lower quality factors [1]. Here we analyse this claim and find that its validity might not hold for practical closed-loop measurement systems, that have a control loop which is necessary for the operation of the resonator as a sensor. To this aim, we present a model that relates the different sources of noise in such a sensing system with the final resolution achieved in a closed-loop sensing scheme. Our model is valid for the noise inherent to the resonator, and other noise sources arising from the peripherals. When a resonator is at its thermomechanical limit and excited at the onset of non-linearity, the theory predicts a mass resolution independent of the quality factor [2]. This finding is verified with experimental data. The dependence of the mass resolution on the quality factor is also calculated for resonators limited by others types of noise, such as frequency fluctuations and instrumentation noise.

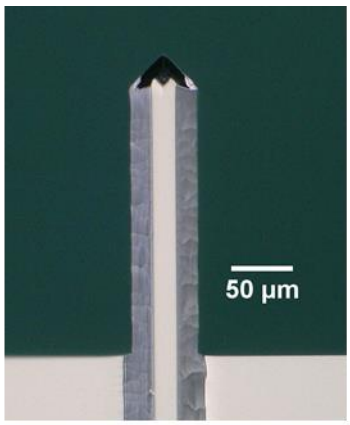

Figure 1: AFM cantilever used to test our model.

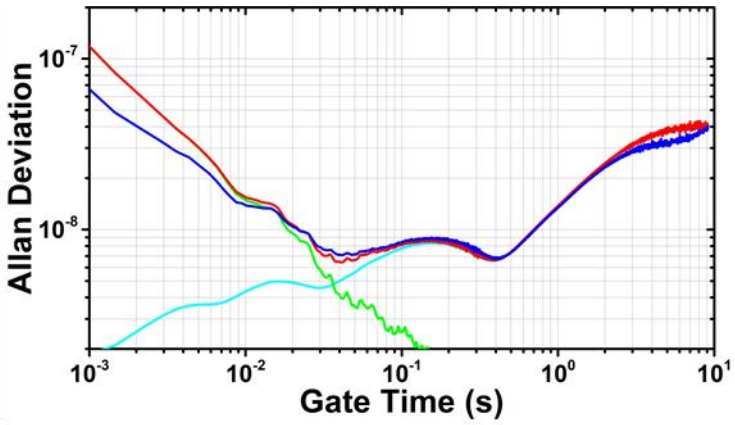

Figure 2: Frequency resolution obtained in closed-loop (blue) and calculated with our model from open-loop measurements (red). Cyan and green curves correspond to models previously used in the literature, only valid over a limited range of gate times.

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Resonant mechanical force sensor based on cavity opto-mechanics

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The field of cavity opto-mechanics has reached the quantum limit for detecting the deflection a micro-mechanical resonator. A key innovation here was the use of a superconducting microwave high-Q resonator, instead of an optical cavity. To achieve quantum-limited sensing, microfabricated devices have been cooled to their ground-state using side-band cooling [1]. Cooling requires that the mechanical and optical modes are strongly coupled and of a sufficiently high Q. We are working to develop a practical force sensor for scanning probe applications based on this development. We seek to combine MEMS-fabrication techniques with circuit QED design ideas to realize a probe suitable for atomic force microscopy (AFM) applications. Our detection principle is based on measuring a change of the resonance frequency of the electrical resonator due to a minute tip displacement. We present an experimental realization of a cantilever probe, where detection is based on mechanically modulating the capacitance of a superconducting series LC-circuit. The inductor is formed from a thin film of NbN in the shape of a meandering nanowire with high kinetic inductance and low loss. The capacitor is formed by a gap in a cut-out membrane, which also forms a mechanical leaver consisting of an inner and outer cantilever flexing in opposition and rotating about a pivot axis close to the tip. This design mechanically amplifies the change in capacitance for a given displacement of the tip.

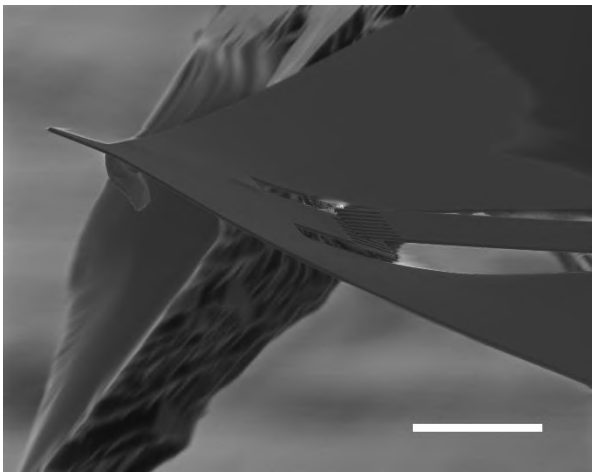

Figure 1 – SEM image of a prototype AFM probe. The inner cantilever forms an interdigitated capacitance in series with the meander inductor. The outer cantilever carries the tip. The scale bar is 20 µm.

[1] Harlow, J. W., Simmonds, R. W., Donner, T., Teufel, J. D., Whittaker, J. D., Li, D., Allman, M. S. (2011). *Nature*, *475*(7356), 359–363.

Abstract Title: Metal AFM probes for measurements of friction

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Main text: Measurement of the friction coefficient with the use of AFM depends on a lot of parameters [1]. This problem is not well addressed for nanoscale in the current state of the art. Most probes on the market are made of silicon with thin coatings. These coatings are easily damaged during friction experiments, therefore they are not suitable for this kind of experiments. Probes made completely of metal could be a solution to this problem. Here presented are both metal probes and novel method of their fabrication.

Processes of manufacturing of metal probes, described in scientific literature, are usually complicated and with many steps [2,3]. The developed method eliminates most of these disadvantages. It uses only cheap and simple tools and safe chemicals. The most important innovation in the process is a metal substrate. It eliminates the need for an additional conductive layer before electrochemical deposition. The second crucial process is an indentation in which mold for tip is created in the substrate. The process can be easily adjusted to manufacture probes from different materials or with different tip and beam shapes. It is also cheap to implement and allows manufacturing in batches. This results in a low cost of the individual probe. The described method was used to manufacture probes from different metals, including gold and nickel. An example is shown in figure 1. Probes were successfully used in commercial AFM in different modes of operation, an example of a result from Lateral Force Microscopy is shown in figure 2. The most important advantage of such probes will be seen during measurements of friction at the nanoscale between different materials. A variety of materials for probes opens the possibility for new research regarding nanofriction and nanowear between materials that could not be previously investigated at the nanoscale. Other possible uses of that probes include measurements of magnetic properties and electric properties.

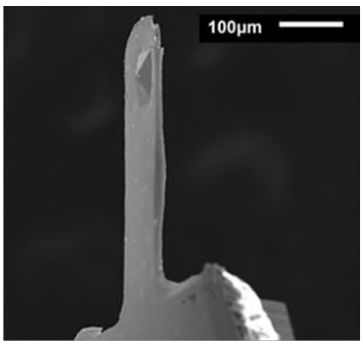

Fig. 1. An image from SEM of a cantilever of a metal probe

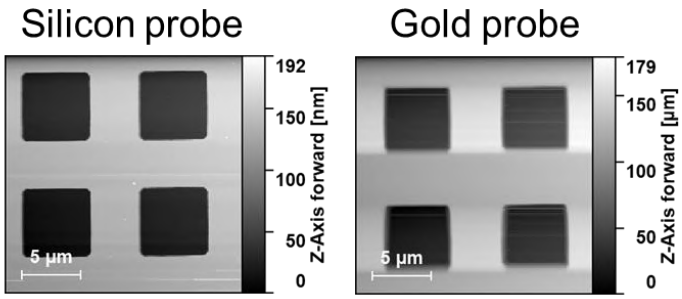

Fig 2. Comparison of LFM images obtained with commercial silicon probe and novel gold probe

[1] B. Bhushan, *Principles and Applications of Tribology*. John Wiley & Sons (2013)
[2] E.J. Luber, B.C. Olsen, C. Ophus, V. Radmilovic, D. Mitlin, Nanotechnology 20, 9 (2009)
[3] J. Zou, X. Wang, *et.al.*, Journal of Micromechanics and Microengineering 14, 204 (2004)

Customized MFM probes

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Magnetic Force Microscopy (MFM) is a variant of the Atomic Force Microscopy (AFM) used to study magnetic structures at the nanoscale [1]. A magnetized tip at the end of a microcantilever scans a sample and detects the tip-sample magnetic interactions. Despite the many improvements achieved by the MFM community throughout the years, the intrinsic drawback of this approach is the increase of the final tip radius and a weak control of the magnetic coating influence over the sample magnetic state. In this work, we present two different routes to prepare high-performance MFM probes with sub-10 nm (sub-25 nm) topographic (magnetic) lateral resolution respectively. On the one hand, we explore the optimization of MFM probes for different applications by means of the fabrication of Focused Electron Beam Induced Deposition (FEBID) magnetic nanorods on top of a non-magnetic AFM tip [2,3]. On the other hand, an accessible route for customizing MFM probes by using sputtering is described [4]. Those methods allow to tailor not only the tip stray field, avoiding tip-induced changes in the sample magnetization, but also to optimize MFM imaging in different environments such as vacuum or liquid media [5] by choosing the cantilevers with the optimum properties for each environment, a technology that is currently not available on the market.

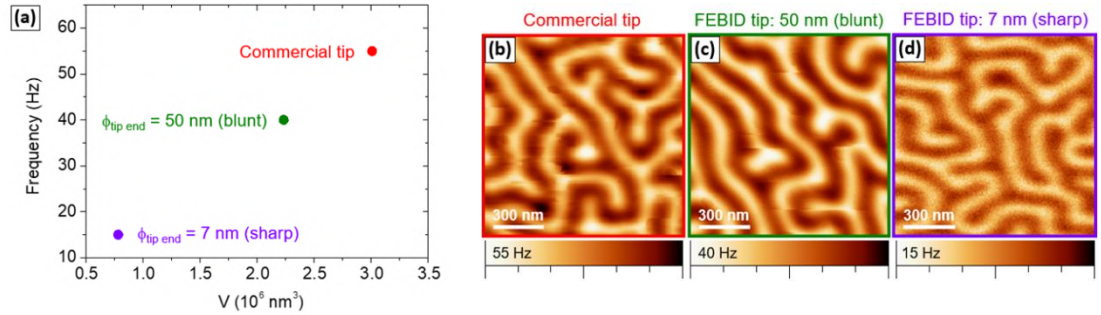

Figure1: Measurement of the frequency shift, related to the tip stray field, for three different probes, represented as a function of the magnetic tip volume. The corresponding MFM images, performed with (b) a standard commercial MFM probe (red), (c) a tip fabricated with an Fe-FEBID nanowire with a blunt tip end of 50 nm (green) and (d) a sharp tip end of 7 nm (purple), are shown.

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[2] M. Jaafar et al. submitted
[3] J. Pablo-Navarro et al. ACS Appl. Nano Mater, **1**, 38-46 (2018)
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[5] P. Ares, M. Jaafar et al. Small **11**, 36, 4731-4736 (2015)

MFM-KPFM characterization of magnetic nanocomposites for bioapplications

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Magnetic nanostructures have been used in different applications in biomedicine and life science such as imaging diagnosis, drug delivery, hyperthermia and molecular detection [1]. Moreover, the effect of different physical stimuli in the cell culture has been studied. In particular, conductive or piezoelectric polymers have been used for tissue engineering applications, due to their ability to create a beneficial electroactive microenvironment to the cells [2, 3]. In this work, a fundamental study of the magnetic and electrical properties of a magnetoelectric nanocomposite is presented, which has shown applications in biomedicine [4]. The composite material is prepared by mixing magnetic nanowires 60 nm in diameter partially embedded into an AAO membrane with interpore distance of 105 nm and a piezoelectric polymer poly(vinylidene fluoride) (PVDF) thin film. Magnetic Force Microscopy-Kelvin Probe Force Microscopy (MFM-KPFM) combined system [5] has been used to characterize the electrical and magnetic properties of this material at the nanoscale. Thanks to this combined system, it is possible to distinguish the magnetic signal coming from the magnetic nanowires and the surface potential of the PVDF layer that varies with its thickness as shown in Figure 1 [6].

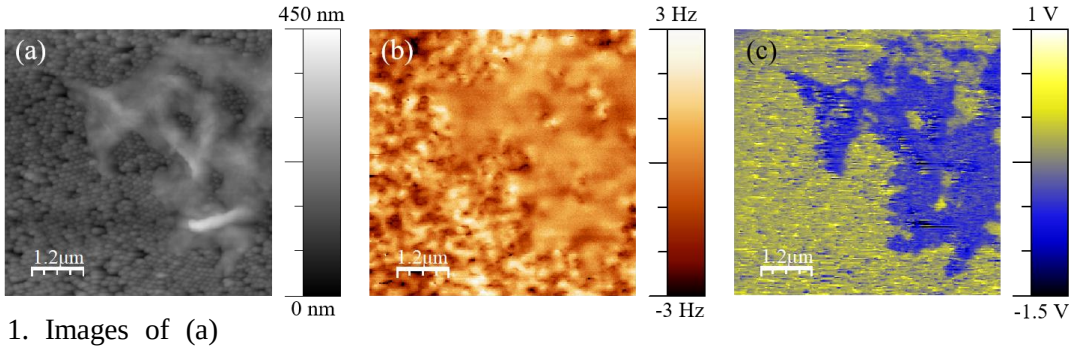

Figure 1. Images of (a) AFM, (b) MFM and (c) KPFM of an area where we distinguish uncovered nickel nanowires and a layer of PVDF-TrFE above them.

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[5] M. Jaafar et al., Beilstein J. Nanotechnol. **2**, 552-560 (2011)
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Nanoscale Analysis of Photocatalytic Reactions by In-liquid Local Potential Distribution Measurement Technique

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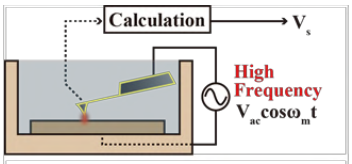

Fig. 1: (a) Experimental setup of OL-EPM

Photocatalysts used for photosynthesis driven by solar energy have been widely investigated. Particularly, bismuth vanadate ($BiVO_4$) has attracted much attention because of its high efficiency and stability for photocatalytic reaction. However, nanoscale mechanisms of such reactions are often elusive due to the lack of a method able to visualize nanoscale distribution of reaction sites at solid-liquid interfaces. To solve this problem, we have recently developed an in-liquid surface potential measurement technique referred to as open-loop electric potential microscopy (OL-EPM) (Fig. 1) [1]. In the previous studies, we demonstrated that OL-EPM can be used for visualizing nanoscale distribution of corrosion cells and catalytic reactions in an electrolyte [2, 3]. In this study, we use this technique to investigate nanoscale distributions of photocatalytic reaction sites at the surface of a photocatalytic electrode consisting of a $BiVO_4$ thin film and an FTO substrate in electrolyte. The $BiVO_4$ thin film was formed by metal organic decomposition method on a FTO/glass substrate. CoPi cocatalysts were deposited by the in-situ photochemical deposition method. The OL-EPM measurements with Au coated cantilever were performed in 1 mM KCl solution containing 10 wt.% ethanol with the electrochemical potential of the electrode controlled to 600 mV or 150 mV vs. Ag/AgCl. The 365 nm light was irradiated onto the sample surface just under the tip. Figs. 2 (a) and (b) show topographic and potential images of the $BiVO_4$ electrodes with and without CoPi cocatalysts, respectively. Before the UV irradiation, the grain boundaries show a relatively low potential. However, after the UV irradiation, some of the grain boundaries show a potential higher than the surrounding area while the rest of them remain relatively low as indicated by the red and black arrows in Fig. 2 (a, b). These results suggest that the grain boundaries can serve as a specific reaction site. In addition, with the CoPi cocatalysts, some of the grain surfaces show a relatively high potential as indicated by the yellow arrows in Fig. 2(b). This result reveal that the photo-reactive spots are created on the grains by loading the CoPi cocatalysts. These results demonstrate the effectiveness of OL-EPM for investigating the nanoscale mechanisms of the photocatalytic reactions in electrolyte.

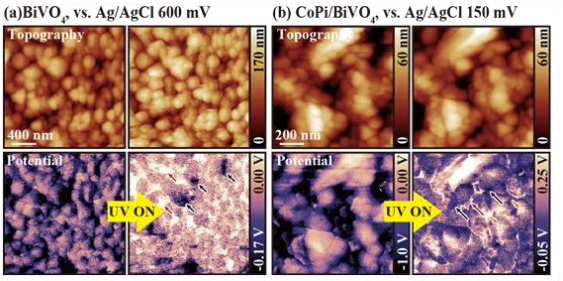

Fig. 2: Photo-induced changes in the topographic and potential images of the $BiVO_4$ electrodes (a) with and (b) without CoPi cocatalysts obtained in 1 mM KCl aq. containing 10 wt.% ethanol.

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[3] M. Morimoto et al., ***Electrochimica Acta*** 290 (2018) 255-261

Study of the structural and conductivity properties of chemical delitiated $LiCoO_2$ films by synchrotron XRD, SEM and C-AFM

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Lithium-ion batteries (LIBs) are widely used in current consumer electronics, and their demand in electric and hybrid vehicles and renewable energy-related energy storage applications is expected to grow in the near future. LIBs consist of positive and negative electrodes, an electrolyte, a separator, and battery casing. The most common positive electrode material in LIBs for consumer electronics is lithium cobalt oxide ($LiCoO_2$, LCO). However, much still needs to be done to understand the physical mechanisms behind the operation of LCO and the potential room for improvement.

In this work, we investigate the changes in crystal structure, texture and conductivity properties in polycrystalline Li_xCoO_2 films with chemical delithiation. Thin films of Li_xCoO_2 grown by magnetron sputtering are exposed *ex-situ* to oxalic acid ($C_2H_2O_4$) to gradually remove lithium in broad ranges. The effects of the delithiation is studied complementarily using synchrotron x-ray diffraction (XRD), photoelectron spectroscopy (UPS), scanning electron microscopy (SEM) and conductive atomic force microscopy (C-AFM).

Simultaneous viscosity and density measurement of small volumes of liquids using a vibrating microcantilever

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Accurate and rapid determination of the density and viscosity of liquids is central to countless industrial, technological and scientific processes [1]-[2]. Such measurements can be time consuming and often require sampling substantial amounts of the liquid. These problems can partly be overcome with the use of microcantilevers but most existing methods depend on the specific geometry and properties of the cantilever, which renders simple, accurate measurement difficult [3]-[4]. Here we present a new approach able to simultaneously quantify both the density and the viscosity of microliters of liquids. The method, based solely on the measurement of two characteristic frequencies of an immersed microcantilever, is completely independent of the choice of cantilever. We derive analytical expressions for the liquid's density and viscosity and validate our approach with several simple liquids and different cantilevers.

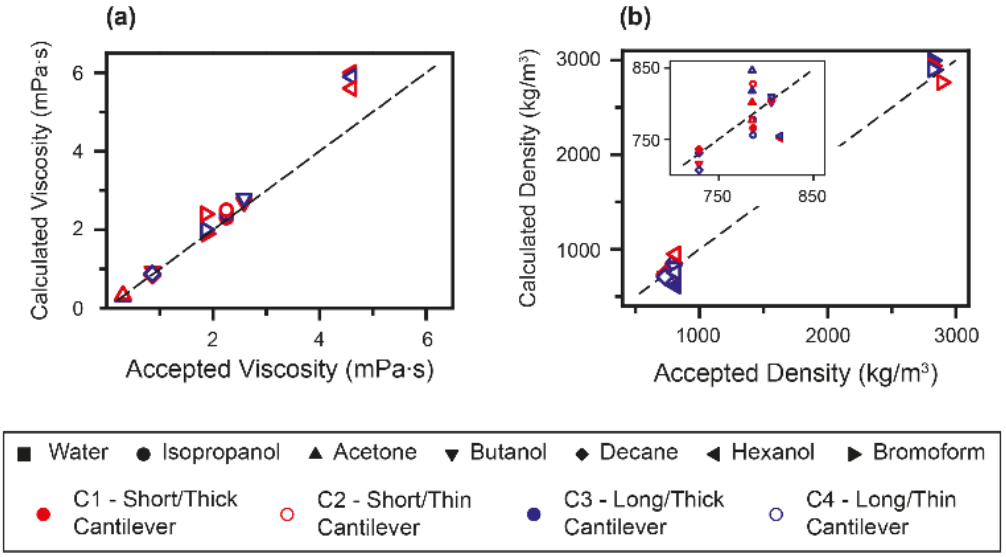

Fig 1: Comparison between the accepted and calculated viscosities (a) and densities (b) of the probed fluids. The modelled η and ρ compare well with the accepted values, as evidenced by their collapsing onto the line of unity gradient. The inset in (b) highlights the data points at lower densities [5].

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Development of Fatigue Testing System for in-situ Observation of Micro/Nano scale Fatigue Mechanism by High Speed-AFM

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There are three principal steps which may be observed in the fatigue process of engineering structures: initiation, including nucleation and micro growth, crack growth and ultimate failure [1]. The first sign of fatigue damage which can lead to crack initiation is intragranual slip band formation evident on the free polished surface of a stressed specimen [1]. While several theoretical and computational methods have been proposed to study fatigue crack initiation and growth such as [2]–[3], physical evidence and high resolution validation data for fatigue nucleation, initiation and growth relies upon the development of experimental techniques and observations. Most historic observations of fatigue crack initiation have been performed by optical microscopy, limited TEM and/or SEM [4]. Although notable insight has been obtained, the fidelity of information on local plasticity was compromised by limited resolution of these microscopes and/or their inability to measure true sample topography. Since 1994 [5] AFM has been used to study fatigue and to obtain quantitative information on slip spacings and slip height displacements of alloys. However, all such measurements suffer from significant time constraints associated with slow AFM scan which limits the quantification of slip bands during cyclic fatigue tests to very small areas [4]-[5]. To overcome the mentioned limitations, we propose a miniature stage, which provide the ability of in-situ observation of cycle by cycle evolution of microcracks and slip bands while the specimen is under the load. Moreover, to overcome the limitation of quantitative analysis of fatigue initiation and growth by considering large images contain several grains, we demonstrate the application of high-speed AFM with angstrom resolution on local step height.

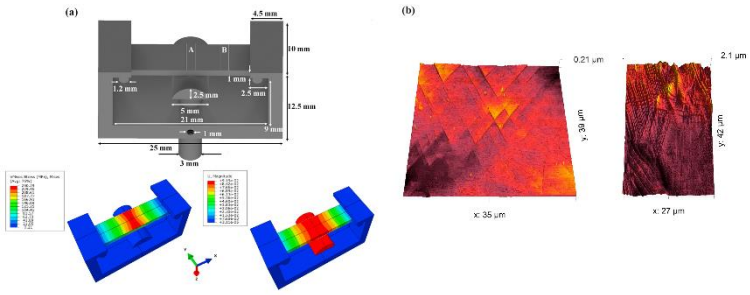

Fig.1. a) Schematic of stage with FE simulatin b) HS-AFM map of slip bands after 1000 and 10000 cycles, respectively [4].

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Bimodal AM-FM nanomechanical mapping of Pentacene thin films

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Successful demonstrations of organic electronic devices have led to an increasing interest in organic semi-conductive materials, with pentacene being one of the most relevant material for p-type OFETs; indeed, pentacene has demonstrated having one of the highest hole and electron mobility among organic small molecules [1]. The material exhibits a strong tendency to form highly ordered films which depend on the growth conditions and the substrate, especially when grown by high-vacuum sublimation [2]. Mechanical properties of organic semiconductors have become relevant since their nowadays wide application in stretchable and flexible electronics [3]. We have applied bimodal AFM [4] to generate nanomechanical maps of pentacene thin film surfaces. Specifically, we have implemented bimodal AM-FM which consists of an amplitude modulation feedback acting on the first mode and a frequency modulation feedback on the second mode of the cantilever. This bimodal AFM configuration enables fast, accurate and subnanometer-scale Young's modulus mapping on a wide range of materials in air and liquid. The elastic modulus map shows a value of about 15 GPa similar to what expected from literature values. We then perform experiments on few layer thick pentacene films. A recent theory taking into account the effect of the substrate is used to interpret the data [5].

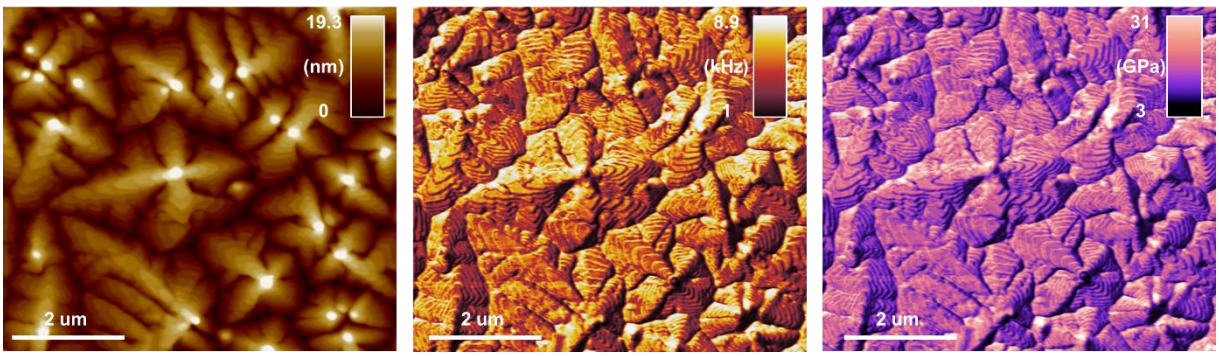

Figure 1. (a) Topography image of a 15 nm thick pentacene film. **(b)** Second mode frequency shift and **(c)** elastic modulus map.

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Effects of active screen plasma nitriding to the surface morphology

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Plasma nitriding is a surface treatment widely used in the industry to improve the hardness, the wear and in some cases the corrosion resistance of steels. One type of this technology is the active screen plasma nitriding (ASPN), which is becoming increasingly widespread among the surface hardening techniques.

Active screen plasma nitriding experiments were performed at 490–510 °C, for 4 h in a 75 % N_2 + 25 % H_2 gas mixture using tempered 42CrMo4 type low alloy steel. For surface characterization a Veeco diInnova type atomic force microscope (AFM) was used in contact-mode with an ART D160 diamond probe (spring constant: 5 N/m). The images were obtained in 2 μm×2 μm and 10 μm×10 μm scan sizes, with a sampling resolution of 512×512 and 1 Hz scan rate.

Surface roughness, morphology and average grain size were examined. It can be clearly seen in Fig. 1 that the surface roughness of the nitrided samples can even be 30-40 times higher than the polished reference sample. With this measurement, the dimension of the active screen can be optimized according to the properties of the formed nitride layer.

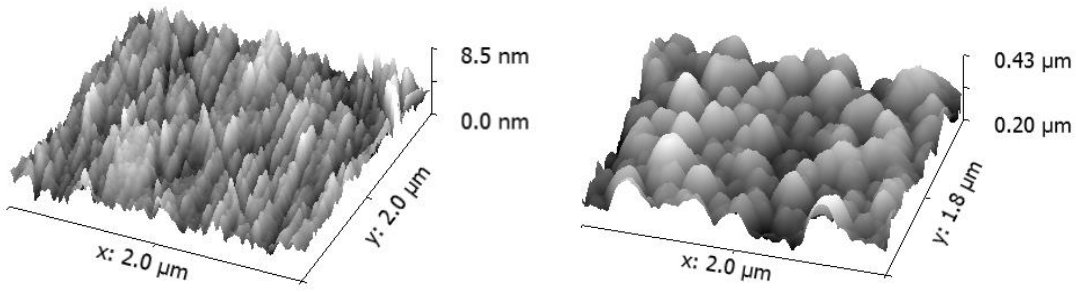

Figure 1. Surface morphology of the *left:* polished, *right:* plasma nitrided samples

Know your full potential: Quantitative Kelvin probe force microscopy on nanoscale electrical devices

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KPFM is widely used to map the nanoscale potential distribution in operating devices, e.g. in thin film transistors, battery materials and solar cells. Quantitative surface potential measurements are crucial for understanding the operation principles of functional nanostructures in these electronic devices. Nevertheless, KPFM is prone to certain imaging artifacts, such as crosstalk from topography or stray electric fields. We compare different amplitude modulation (AM) and frequency modulation (FM) KPFM detection methods on a reference structure consisting of a glass-platinum interdigitated electrode array. This structure allows to modify the surface potential externally and minimizes corrosion, while mimicking the sample geometry in device measurements. In particular, we investigate how quantitative different KPFM methods can measure a predefined externally applied voltage difference between the electrodes. We found that when operated with a feedback, FM KPFM methods provide more quantitative results that are less affected by the presence of stray electric fields compared to AM KPFM methods [1].

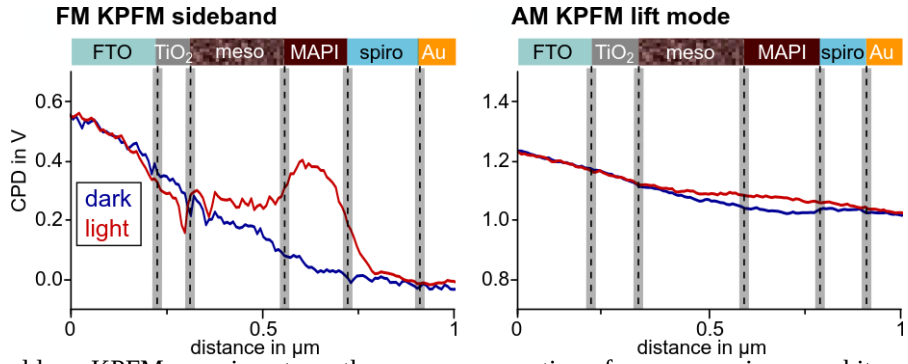

Figure 1: CPD line profiles of two closed loop KPFM experiments on the same cross section of a mesoscopic perovskite solar cell under short circuit conditions with and without illumination, visualized by the red and blue line, respectively. The cell consisted of a fluorine-doped tin oxide (FTO) electrode, a compact TiO_2 electron extraction layer and a mesoscopic TiO_2 layer (meso) filled with the perovskite light-absorber methylammonium lead iodide (MAPI). The mesoscopic layer was followed by a compact MAPI capping layer, the hole transport material spiro-OMETAD and a gold electrode. Prior to the measurement, the cross section of the solar cell was polished with a focused ion beam (FIB) to minimize topographic crosstalk. The CPD line profiles on the left were extracted from double side band frequency modulation KPFM (FM sideband) scans in single pass with VAC of 3V [2]. The CPD line profiles on the right were extracted from amplitude modulation KPFM (AM lift mode) scans in lift mode with a tip-sample distance of 10 nm, an oscillation amplitude of ~80 nm and a tip voltage U_{AC} of 1 V. Each line profile is an average of three adjacent scan lines. [1]

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Mechanically soft domain walls in ferroelectrics

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There is currently a vigorous research effort on the functional properties of ferroelectric domain walls. An important part of their appeal is that domain walls possess functional properties distinct from the host material. Their distinct functionality foments new concepts in electronic nanodevices where domain walls act as mobile two-dimensional elements.

Among the many properties of domain walls, mechanical response appears to have been largely neglected. In this presentation, we will show our experimental measurements on stiffness of domain walls. In particular, we have used Atomic Force Microscopy to investigate the difference in stiffness between domains and purely ferroelectric (non-ferroelastic) domain walls separating antiparallels 180^{o} domains. Surprisingly, they have a distinct mechanical response, markedly softer than that of the domains they separate.

Initially, Piezoresponse Force Microscopy (PFM) was used to image the polarized domains of the materials and consequently to identify different types of domain walls appearing in the materials. Thereafter, Contact Resonance Frequency (CRF) mode was used to identify the stiffness of the domain walls. In CRF, the stiffness of the material is determined from the measured resonance frequencies of the cantilever when the tip is in contact with the sample, as these contact resonance frequencies depend on the tip sample mechanical coupling and undergo distinct shifts when the tip is scanned over areas with different stiffness. Theoretical calculations, based also in flexoelectric effects, enhance our experimental results.

Multi-scale characterisation of a semi-crystalline polymer reveals hidden ferroelectricity above the Curie transiton

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Ferroelectrics exhibit a phase transition to a paraelectric state driven by temperature - called the Curie transition. It is known that a ferroelectric material shows piezoelectric and/or electrostrictive behaviour. However, semi-crystalline ferroelectric polymers such as the copolymer of vinylidene fluoride and trifluorethylene (P(VDF-TrFE)) show some unexpected characteristics, with ferroelectric behaviour above the Curie transition, but without piezoelectric nor electrostrictive response[1,2]. Here, we demonstrate that electromechanical response of ferroelectric crystalline domains does exist above the Curie transition and explain why it is not observed in macroscopic measurements. To resolve these seemingly contradictory observations, we have performed high-resolution nanomechanical measurements on P(VDF-TrFE) films and *in situ* X-ray diffraction measurements on P(VDF-TrFE) capacitors (see Fig. 1), in both, the ferro- and paraelectric states. We demonstrate that the particular semi-crystalline microstructure in the paraelectric state, formed of ferroelectric crystalline domains embedded into a softer amorphous phase, lead to the local electromechanical response to be counterbalanced by the amorphous phase, effectively masking its macroscopic effect[3].

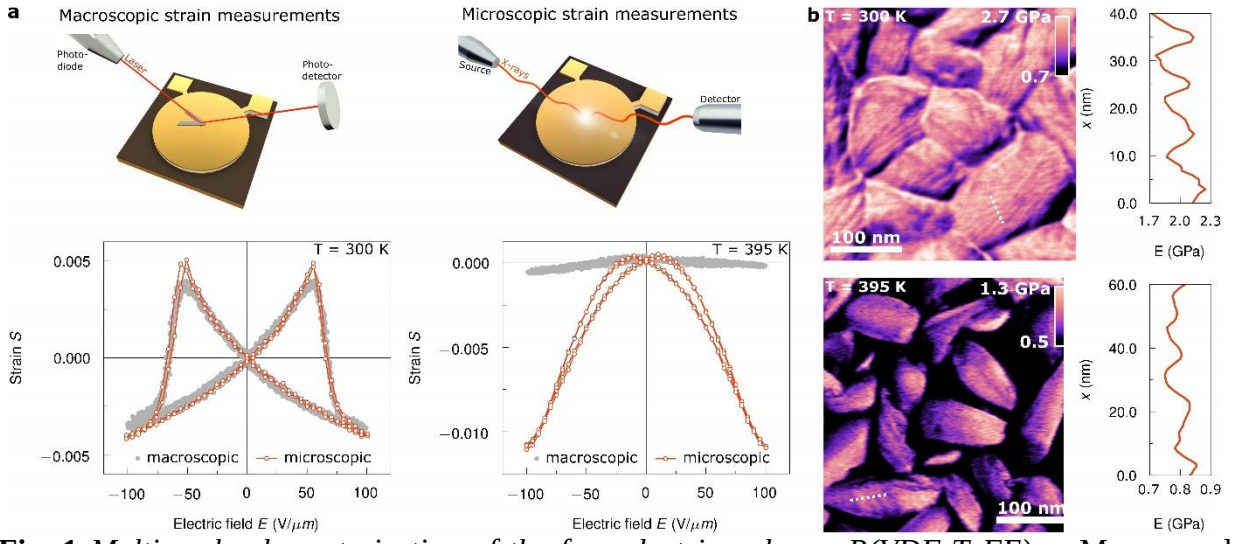

Fig. 1 *Multi-scale characterisation of the ferroelectric polymer P(VDF-TrFE).* **a** Macro- and microscopic electromechanical response of P(VDF-TrFE) in the ferro- and paraelectric states. **b** Nanomechanical maps of P(VDF-TrFE) in the ferro- and paraelectric states. The nanomechanical maps have been generated by bimodal AFM.

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In-plane and out-of-plane nanomechanical characterization of HOPG at the atomic scale

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Multifrequency atomic force microscopy [1] enables high resolution imaging of flat surfaces such as HOPG down to the atomic scale. The technique is based on the simultaneous excitation and detection of two or more cantilever eigenmodes. Depending on the type of the oscillation modes (flexural, torsional or lateral), out-of-plane elastic and dissipative sample properties or the in-plane shear behavior can be analyzed [2]. Here, a bimodal approach was developed where the flexural component of the first torsional eigenmode amplitude was used for the topographical feedback. Therefor the coupling of the second flexural and the first torsional eigenmode, which results from the cantilever geometry, was exploited. Additionally, the first lateral eigenmode was excited at a constant amplitude while the frequency shift was recorded. Using the described setup atomic resolution was achieved in both imaging channels at ambient conditions, yet in the flexural topography images only every second carbon atom could be resolved, resulting in a triangular appearance. This effect is a result of the Bernal stacking of graphite monolayers, leading to two distinguishable carbon atom sites. Mapping the lateral frequency shift, however, provided a more comprehensive image, resolving the complete hexagonal arrangement of the carbon atoms. We aim to study the change in nanomechanical properties originating from single defects artificially generated within the structure by oxygen plasma treatment.

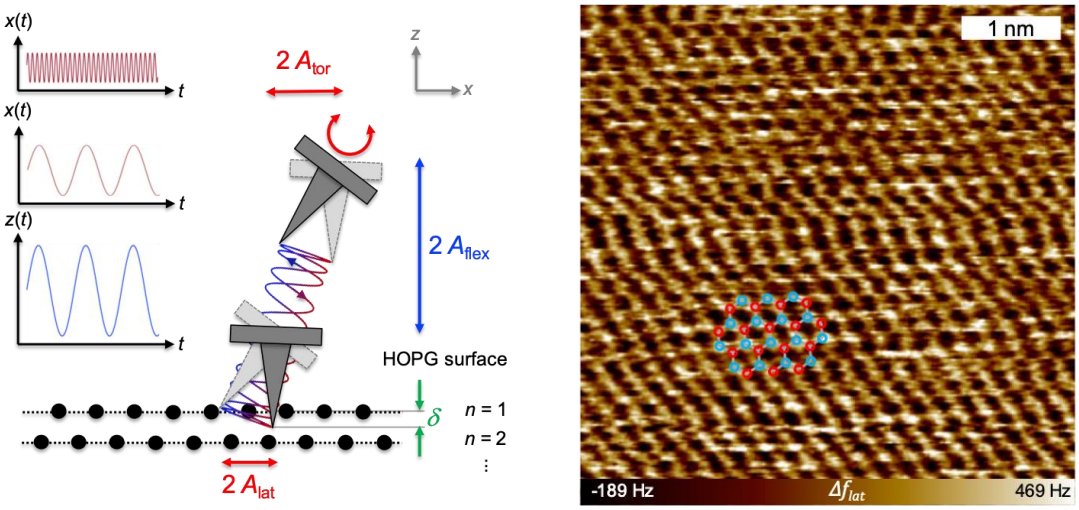

Figure: Schematic illustration of the setup for the nanomechanical characterization of HOPG surfaces via multifrequency atomic force microscopy (left) and lateral frequency shift image, showing atomic resolution of a HOPG surface (right).

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Nanomechanical Probing of Graphene-Liquid Interface using Dynamic Atomic Force Microscopy

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Several interesting phenomena can occur at a 2D material-liquid interface like wherein a single atomic layer is in contact with a liquid surface. Atomic Force Microscopy (AFM) can help us in probing the nanomechanics of such unique interfaces. We have developed a simple and effective platform using dynamic AFM to probe suspended monolayer graphene interacting with liquid underneath it. A chemical vapor deposited (CVD) graphene is suspended over 10 μm deep micro-channels fabricated on a PDMS substrate using the modified direct transfer method. IPA-water mixture is made to flow into the micro-channels using capillary effect. Phase-imaging in dynamic AFM provides a striking contrast to clearly distinguish wrinkles, cracks, edges, holes/tears and pristine regions of graphene. The best possible phase contrast was achieved for an amplitude set-point between 0.5 and 0.6. This observed phase-contrast was mainly due to the much larger force gradients on liquid than on suspended graphene with liquid underneath it.

To investigate how the dynamics of suspended graphene is altered with the presence of underneath liquid, we calculated energy dissipation in tip-suspended graphene in air (GoA) interaction and tip-suspended graphene with underneath liquid (GoL) interaction. The peak value of energy dissipation for GoA varies from 0.5-1.8 keV while that for GoL is around 0.5 keV. The suspended graphene membrane in air oscillates due to the tip-membrane adhesive forces incurred in every oscillation cycle of the cantilever. We found that these oscillations become more prominent resulting in artifacts when imaging in second eigenmode of the cantilever at 2 MHz which is close to the resonance frequency of the suspended membrane. The presence of liquid below the suspended membrane not only increases its stiffness but also significantly damps the oscillations of membrane induced by tip-membrane adhesion. As a result, energy dissipation on GoA is much larger than on GoL. Additionally, we performed amplitude-distance spectroscopy to study the dynamics of the drying process as the liquid underneath the suspended membrane dries off. We observed some abrupt changes in the amplitude of cantilever thus providing insights into the dynamics of suspended membrane as the liquid is drying. These measurements could also be particularly useful to probe the nature of defects in graphene.

Bottom-effect in atomic force microscopy nanomechanics

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† These authors have equally contributed to this work

Nanomechanical properties are an essential tool for soft matter characterizations. Atomic Force Microscopy (AFM) nanomechanical experiments typically rely on the acquisition of force-distance curves to be analysed with an appropriate contact mechanics model to extract, for instance, the sample Young modulus. Several models have been proposed and, among them, Hertz and Sneddon approaches are the most commonly used. These theories are based on the approximation that the substrate is too far from the indentation region to affect the elastic modulus. However, Dimitriadis [1] has theoretically demonstrated that the substrate contribution is in general not negligible. This effect is called *bottom-effect artefact*. Recently, Garcia [2] has extended the bottom-effect theory giving a more general description and providing also numerical simulations. However, a direct experimental proof of the existence of the bottom-effect artefact is still missing, together with an experimental demonstration of Garcia's approach validity. In this contribution, an AFM force-spectroscopy experiment is performed onto a supported lipid bilayer deposited on mica by the Langmuir-Schaefer technique. The sample exhibits, in a liquid environment, two different morphologies: 2-layers and 4-layers regions. Remarkably, when the force-indentation curves are fitted with Sneddon's formula two clearly different Young modulus values are obtained corresponding to these two film regions, while when Garcia's approach is used the same elastic modulus is restored, as expected for an intrinsic material property. Finally, finite element method (FEM) simulations are provided, obtaining a good agreement with the experimental data, therefore confirming once again the robustness of Garcia's bottom-effect theory.

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S.C., S.M. and P.C. are grateful for financial assistance from the European MagicCellGene Project (M-ERA.NET COFUND call 2016, Ministerio de Economía y Competitividad from Spain in the framework of project PCIN-2017-127). P.C. and S.M. also acknowledge support from DGA and Fondos FEDER for funding Platon research group (E31_17R). K.K. and D.B.W. are grateful to the German Science Foundation (SFB 803, project A05) for financial support.

High-speed, Non-contact AFM Imaging of Nanoscale Silicon Structures

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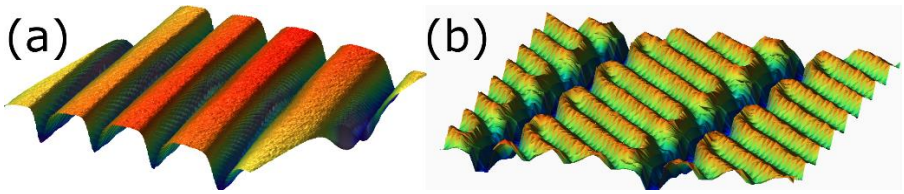
High-speed atomic force microscopy has proven it can image a unique set of nanoscale samples and processes. For example, it has captured protein-protein interactions and the growth of protein crystals [1]. Typically, these images and movies are generated with tapping mode, and sensitive samples such as soft mammalian cells, or semiconductor components may be perturbed or damaged by the imaging process. This work aims to alleviate these issues by developing a fast AFM system that is also non-contact. Such a system should also be able to capture dynamic nanoscale processes, but without any physical disruption.

At the heart of the non-contact AFM system is the constant-height feedback loop, working to maintain a constant distance between cantilever tip and sample while scanning. In this research, this feedback loop is redesigned for speed. This includes a new control methodology as well as a redesign of the hardware. On the control side, the method for resonating the cantilever was chosen to be self-excitation [2], instead of the traditional phase-locked loop. This control scheme allows the user to trade bandwidth for a reduction in image noise with a single parameter, the loop gain. The complete linearized feedback loop was derived with the root-locus (RL) [3], a graphical control technique that provides intuition on the closed-loop dynamics. With the RL, it can be shown that the bandwidth of this new system can be made extremely high, limited only by the slew rate of the piezo stage amplifier. On the hardware side, all data acquisition, communication, control and signal generation were implemented on a single Field Programmable Gate Array (FPGA), instead of individual analog components. Parallel, high-speed FPGA-PC data lines were also added. The result is a greatly simplified non-contact AFM system, that is fast, robust and intuitive to tune. Such a system is suitable for high-speed, true non-contact imaging. The methodology was validated experimentally by taking high-speed images in air of nanoscale silicon samples provided by the semiconductor industry. It is hoped that in future work, this system will prove itself with new recordings of protein-protein interactions, without physical disruption.



Non-contact scans in air of silicon chips provided by Applied Materials. (a) Image dimensions: $3.6\mu m \times 3\mu m$, Feature Height: $16nm$, Scan Rate: $0.90\mu m^2 / second$. (b) Image dimensions: $1.5\mu m \times 1.5\mu m$, Feature Height: $50nm$, Scan Rate: $0.25\mu m^2 / second$.

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ESCRT-III spirals are loaded springs that govern spontaneous membrane deformation

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The endosomal sorting complex required for transport-III (ESCRT-III) system is crucial in many cellular processes that imply membrane deformation and fission, where the ESCRT-III proteins are in the cytoplasm and the membrane bud faces away from the cytoplasm [1]. The ESCRT-III subunits polymerize and form filaments that assemble in high-order structures like spirals on flat membranes [2] or helices in cylindrical membrane tubules [3]. High-speed atomic force microscopy (HS-AFM) has provided information about the polymerization growth, the dynamics and subunit turnover of ESCRT-III spirals on supported lipid bilayers on mica [2,4]. Here, we use Polydimethylsiloxane (PDMS), a polymer whose elasticity ranges from 100kPa to 20MPa, as the substrate for supported lipid bilayers to allow for topological changes of ESCRT-III assemblies that are hampered in solid supports typically used in AFM and optical microscopy experiments such as mica or glass. After formation of a homogeneous lipid bilayer on PDMS, we supply ESCRT-III (Snf7) and observe and analyze the structural dynamics of ESCRT-III on such soft supports. Our novel data show that on the soft substrate the spirals reduce their inter-filament distance and concentrate into smaller disks. Eventually the inner ring undergoes a transition downwards deforming the bilayer and the substrate beneath the spiral, in analogy to the native function of ESCRT-III, providing direct proof of the 'loaded spiral model' [2].

[1] Tang, S., Henne, W.M., Borbat, P.P., Buchkovich, N.J., Freed, J.H., Mao, Y. et al. (2015) Structural basis for activation, assembly and membrane binding of ESCRT-III Snf7 filaments. eLife 4, 1–22
[2] Chiaruttini, N., Redondo-Morata, L., Colom, A., Humbert, F., Lenz, M., Scheuring, S. et al. (2015) Relaxation of loaded ESCRT-III spiral springs drives membrane deformation. Cell 163, 866–879
[3] Cashikar, A.G., Shim, S., Roth, R., Maldazys, M.R., Heuser, J.E. and Hanson, P.I. (2014) Structure of cellular ESCRT-III spirals and their relationship to HIV budding. eLife.
[4] Mierzwa, B. E., Chiaruttini, N., Redondo-Morata, L., Moser von Filseck, J., König, J., Larios, J. et al. (2017) Dynamic subunit turnover in ESCRT-III assemblies is regulated by Vps4 to mediate membrane remodeling during cytokinesis. Nat. Cell Biol. 19, 787–798

Development of 3D-AFM for visualizing 3D structures of inside of chromosomes with nanometer-scale resolution

Keisuke Miyazawa[1, 2], *Ryohei Kojima*[1], *Makiko Meguro*[1], *Shinichi Horike*[1], *Takashi Sumikama*[2], *Naoko Okano*[2], *Konan Imadate*[3], *Kaori Hirahara*[3], *Takeshi Fukuma*[1,2]
Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
WPI-NanoLSI, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
Osaka University, Yamadaoka2-1, Suita 565-0871, Japan
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In three-dimensional atomic force microscopy (3D-AFM), an AFM tip is three-dimensionally scanned at a solid-liquid interface, and we record a 3D distribution of the interaction force applied to an AFM tip. In previous studies using 3D-AFM, hydration and flexible molecular structures of crystal surfaces [1] and lipid bilayers [2] were visualized with subnanometer-scale resolution. In addition, we recently applied 3D-AFM technique into more practical samples in various research fields. For example, we have visualized fibrillary structures of lubricant molecules for a magnetic hard disk. Based on these results, 3D-AFM has great possibility to visualize more complicated and inhomogeneous fibrillary 3D structures in liquid. In this study, we have developed 3D-AFM technique for visualizing human chromosome. Chromosome has 3D folding structures containing fibrillary DNA, histones and proteins with gene function of an organism, and it shows X shape with the thickness of ~700 nm as shown in 2D-AFM image in Fig. 1a. In order to visualize 3D folding structures of chromosomes by 3D-AFM, it is necessary to insert AFM tip into inside of chromosome. Thus, we fabricated thin (< 30 nm) and long (>500 nm) cylinder tip by using carbon nano-tube (CNT) as shown in Fig. 1c(i). Fig. 1b shows the 3D force image obtained by using CNT tip, and it shows local contrasts correlating to the part of inside structures of a human chromosome. In addition, we compared force curves (Fig. 1c(ii)-1d(ii)) measured by the conventional Si tip (Fig. 1c(i)) and the CNT tip (Fig. 1c(ii)) on the chromosome, respectively. The force curve obtained by CNT tip (Fig. 1d(ii)) clearly shows oscillatory profiles correlating to penetrations of the CNT tip into the surface and the inside folding structures of the human chromosome. This results opens up a wide range of new application fields of 3D-AFM in various research fields.

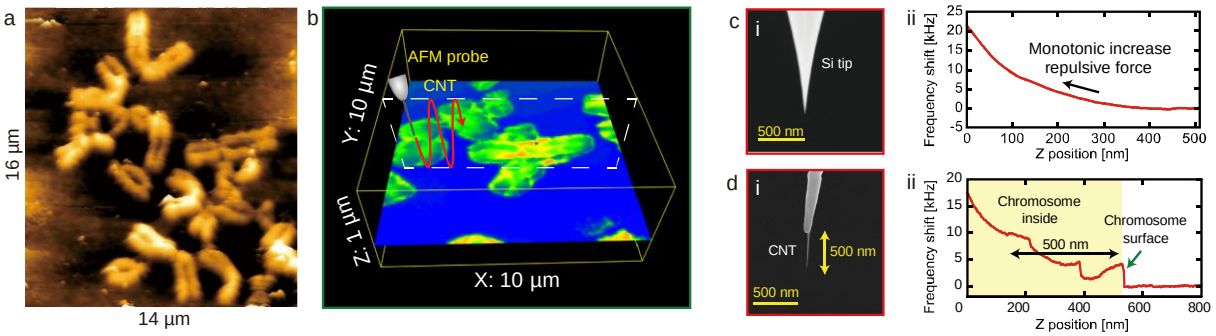

Figure 1: (a) 2D- and (b) 3D-AFM images of human chromosome. (c-d) (i) SEM images of tips and (ii) force curves obtained on human chromosome measured by (c) a conventional tip and (d) a home-made CNT tip.

[1] T. Fukuma et al., Phys. Rev. Lett. **104**, 016101 (2010)
[2] K. Miyazawa et al., Nanoscale **8**, 7334 (2016)
[3] H. Asakawa et al., ACS NANO **6**, 9013 (2012)

The mechanics of single cross-links which mediate cell attachment at a hydrogel surface

Arzu Çolak, Bin Li, Johanna Blass, Aranzazu del Campo, and Roland Bennewitz

INM – Leibniz Institute for New Materials, Saarbrücken, Germany
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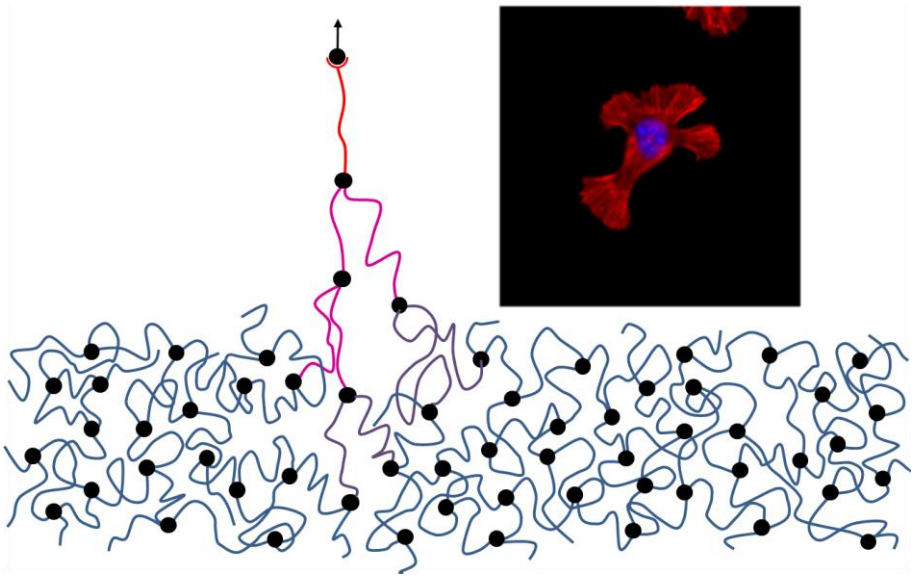
The response of cultured cells to the mechanical properties of hydrogel substrates depends ultimately on the response of single crosslinks to external forces exerted at cell attachment points. We prepared hydrogels and confirmed fibroblast spreading on the hydrogel after a hydrogel linker was functionalized with the RGD cell adhesive motif. We performed specific AFM force spectroscopy experiments on the same linkers in order to probe the mechanics of single cross-links which mediate the cell attachment and spreading [1].

We compared hydrogels of varying elastic modulus between 4 and 41 kPa which exhibited significant differences in cell spreading. An effective spring constant for the displacement of single cross-links at the hydrogel surface was derived from the distributions of rupture force and molecular stiffness. A factor of ten in the elastic modulus E of the hydrogel corresponded to a factor of five in the effective spring constant k of single crosslinks, indicating a transition in scaling with the mesh size ξ from the macroscopic $E \propto \xi^{-3}$ to the molecular $k \propto \xi^{-2}$.

The quantification of stiffness and deformation at the molecular length scale contributes to the discussion of mechanisms in force-regulated phenomena in cell biology.



[1] A. Çolak et al., Nanoscale, 2019, 11, 11596

Carcinomas with occult metastasis potential: Diagnosis/prognosis accuracy improvement by means of force spectroscopy

Anahid Amiri,[1] Florian Hastert,[2] and Christian Dietz[1]

[1]Physics of Surfaces, Institute of Materials Science, Technische Universität Darmstadt, Alarich-Weiss-Str. 2, 64287 Darmstadt, Germany

[2]Cell Biology and Epigenetics, Department of Biology, Technische Universität Darmstadt, Schnittspahnstraße 10, 64287 Darmstadt, Germany

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Accurate diagnosis of cancer stage is inevitable for the following prognosis in patients struggling with these lesions to promote patient's health and survival rate. Previous studies on survival rate statistics show in some cases failure in cancer stage surveys in which metastasis or recurrence of the disease was not accurately prognosed [1,2]. Morphology study of cancer cells advances our understanding about cancer behavior and its progression, in which, in our study [3] on invasive cancer cells we observed a fewer formation of cytoskeleton components compared to their counterparts. Here we show that carcinomas with an occult propensity of metastasis depict a number of poorly differentiated cells with decreased amount of cytoskeleton components in a near-well differentiated population. Force spectroscopy in conjunction with fluorescence microscopy of lung cancer, liver hepatoma and melanoma provided a general view of cells architecture leading to the conclusion that the scarce abnormal-shaped cells with low formation of structural filaments conveys the high risk of metastatic potential of the tumor. The results demonstrate that force spectroscopy complements conventional diagnostic approaches by an accurate cytoskeleton assessment and can improve the following prognosis in epithelial cancers with occult metastasis risk.

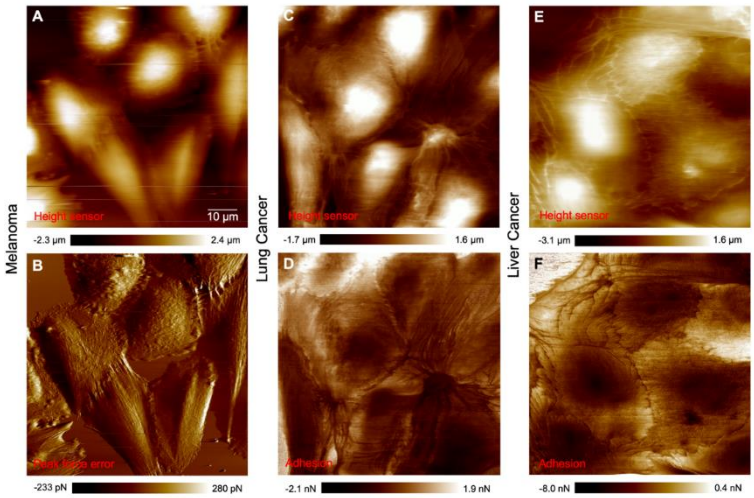

Figure. Force spectroscopy images of (A-B) melanoma, (C-D) lung cancer, and (E-F) liver cancer cells.

[1] B. R. Gastman, P. Gerami, S. J. Kurley, R. W. Cook, S. Leachman, and J. T. Vetto, J. Am. Acad. Dermatol. 80, 149 (2019)

[2] D. C. Whiteman, P. D. Baade, and C. M. Olsen, J. Invest. Dermatol. 135, 1190 (2015)

[3] A. Amiri, F. Hastert, L. Stühn, and C. Dietz, Nanoscale Advances (2019)

Biomechanical properties of the human lens capsule assessed with AFM and nanoindenter

A.A. Frolova[1] ,Yu.M.Efremov[1], B.S.Shavkuta[1,2], S.L.Kotova[1,3], K.S.Avetisov[4], N.A.Bakhchieva[4], I.A.Novikov[4], A.A.Akovantseva[2] , S.E.Avetisov[4,5], P.S.Timashev[1,2,3]

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The lens capsule (LC), a thin specialized basement membrane that encloses the crystalline lens, is essential for both the structural and biomechanical integrity of the lens. Knowing the LC mechanical properties is important for understanding its physiological functioning and for providing better surgical treatment of a cataract. Using AFM and nanoindentation, we performed the morphology imaging and biomechanical testing of the human LC in order to reveal the age-related changes and influence of a disease (pseudoexfoliation syndrome, PES) as well as the influence of the application of the trypan blue staining during the surgery.

The LC samples were harvested during a routine cataract surgery and were studied without further treatment in a PBS buffer at room temperature. The age of patients ranged from 56 to 86 years. The Young's moduli were obtained in the Fast Force Volume mode on the epithelial (Ep) and anterior (An) sides of the lens capsule.

Similar to the previous studies, we found the that Young's modulus of the Ep side was higher than that of the An side by several times. Although the absolute Young's modulus values did not directly depend on the patients' age, the Ep/An ratio decreased with age, thus the difference between the sides was less pronounced in older patients. In general, the Young's moduli of the PES samples were somewhat higher than those of the normal capsules corresponding to the same age. No significant changes were found in the mechanical properties of ALCs of patients with PES versus the control group, as well as in the ALC with and without trypan blue staining. The mechanical properties of the majority of samples were rather heterogeneous, with the edges being softer than the capsule's center.

The Young's moduli obtained by nanoindentation were similar to those obtained by AFM, with small variations due to the differences in the indentation regimes. However, the dependencies found by AFM were almost entirely reproduced in the nanoindentation studies.

We have found no significant differences in the samples' morphology, as shown by AFM imaging.

This work was supported by the Russian Scientific Foundation 15-15-00132.

Harmonic analysis of the local piezoresponse in ferroelectric ceramics of PZT
Bacterial cell wall mechanical damage studied by simultaneous nanoindentation and fluorescence microscopy

Adrián Del Valle[a], *Joaquim Torra*[a], *Patricia Bondia*[a], *Caterina M. Tone*[a], *Virginia Vadillo*[b] *and Cristina Flors*[a]

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[b]Department of Applied Physics, University of Extremadura, Avda de Elvas s/n, 06006 Badajoz, Spain.
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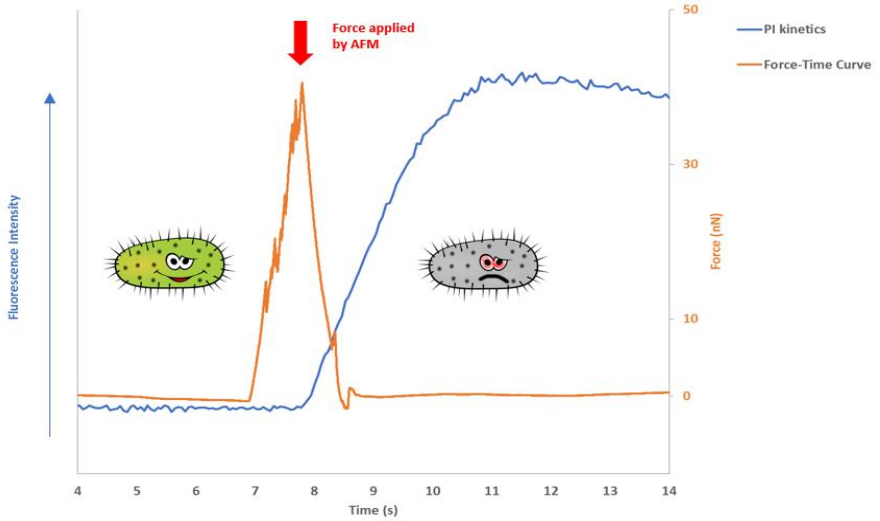
We have developed two experimental protocols to perform simultaneous AFM nanoindentation and fluorescence imaging on immobilized bacterial cells, with the goal of quantifying the forces necessary to produce critical damage to the bacterial cell wall. The first method is assessed by quantifying the fluorescence enhancement kinetics arising from propidium iodide (PI), a marker for membrane integrity, upon indendation. Our main observation is that a correlation exists between the magnitude of the force applied to rupture the cell wall and the delay of the PI fluorescence response. The second method monitors bacterial metabolism upon AFM indentation by following oscillations of the Min system, which has been proposed as an indicator of the metabolic state of bacteria [1]. The latter experiments confirm that metabolic activity ceases after critical damage. While previous studies have shown that bacteria are rather resilient to AFM nanoindentation [2], our experimental strategy using simultaneous fluorescence in a systematic and quantitative way may help to provide a deeper insight into the range of forces that are relevant to "mechano-bactericidal" mechanisms of action [3].



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[2] Z. Suo, R. Avci, M. Deliorman, X. Yang, and D. W. Pascual, "Bacteria survive multiple puncturings of their cell walls," Langmuir, vol. 25, no. 8, pp. 4588–4594, 2009.
[3] N. Lin, P. Berton, C. Moraes, R. D. Rogers and N. Tufenkji, "Nanodarts, nanoblades, and nanospikes: Mechano-bactericidal nanostructures and where to find them," Adv. Colloid Interface Sci., vol. 252, pp. 55–68, 2017.

Characterizing nanomechanical properties of comedones after treatment with sodium salicylate

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[2]Research and Development, GlaxoSmithKline, Weybridge, Surrey, UK.
Zeinab Al-Rekabi

Excessively oily skin can often cause unwanted skin traits in patients, such as excessive shine, enlarged pores, frequent outbreaks or acne. Comedones are small skin-colored papules frequently found on the T-zone (forehead, nose and chin). Sodium salicylate (NaSal) is an ingredient commonly used in anti-inflammatory drugs, anti-bacterial agents, anti-blemish and anti-aging cosmetic products. Although the exact mechanism of NaSal on comedones is not fully understood at present, it appears to exhibit a significant exfoliation effect on the skin after repeated use. Recent advances in metrology have led to novel methods being implemented using atomic force microscopy (AFM) to probe the structure of biological samples and materials. Indeed, characterization of the nanomechanical properties of comedones at sub-cellular levels remains key in understanding the dynamic processes of comodone outbreak. Herein, we investigated the physical properties of comedones pre- and post-treatment using 2% NaSal under ambient temperature and humidity. When treating comedones with 2% NaSal, samples appeared significantly softer when compared to their pre-treated measurements. Furthermore, the force-volume maps generated, showed that after NaSal treatment, areas in the comedone appeared softer suggesting the beneficial impact of the 2% NaSal solution on loosening the inner content of comedones. Our results provide evidence that NaSal is indeed beneficial as an active ingredient in topical creams aimed at targeting eruptive skin conditions.

Correlation at the nanoscale of chemical, structural and conductivity properties of non-stoichiometry Li-ion battery cathodes

J. Díaz[1], S. Quílez[1], E. J. Fuller[2], A. A.Talín[2], E. Vasco[3], H. P. van der Meulen[4,5,6] and C. Polop[1,5,6]

[1.] Dpto Física Materia Condensada, Universidad Autónoma de Madrid, Spain
[2.] Sandia National Laboratories, Livermore (CA), USA.
[3.] Instituto de Ciencia de Materiales de Madrid, CSIC, Spain
[4.] Dpto. Física de Materiales, Universidad Autónoma de Madrid, Spain
[5.] Instituto Nicolás Cabrera, Universidad Autónoma de Madrid, Spain
[6.] Condensed Matter Physics Center (IFIMAC), Universidad Autónoma de Madrid, Spain

Lithium-ion batteries (LIBs) are widely used in current consumer electronics, and their demand in electric and hybrid vehicles and renewable energy-related energy storage applications is expected to grow in the near future. Lithium cobalt oxide (LiCoO2, LCO) is the most common cathode material used in LIBs. In this work, we investigate the correlations between chemical composition, morphology, and conductivity in no-stoichiometry LCO films, which are ad-hoc prepared with in-plane composition gradients. We observe a strong correlation between these gradients and the local structure and electrical properties of the films. A kinetic model based on differential scattering and diffusivities of the involved metal species is proposed to address the experimental results.

EXHIBITORS

Asylum Research will be hosting a workshop on high-speed AFM on Thursday, October 29th from 17:45-18:30

https://zoom.us/j/94592819328

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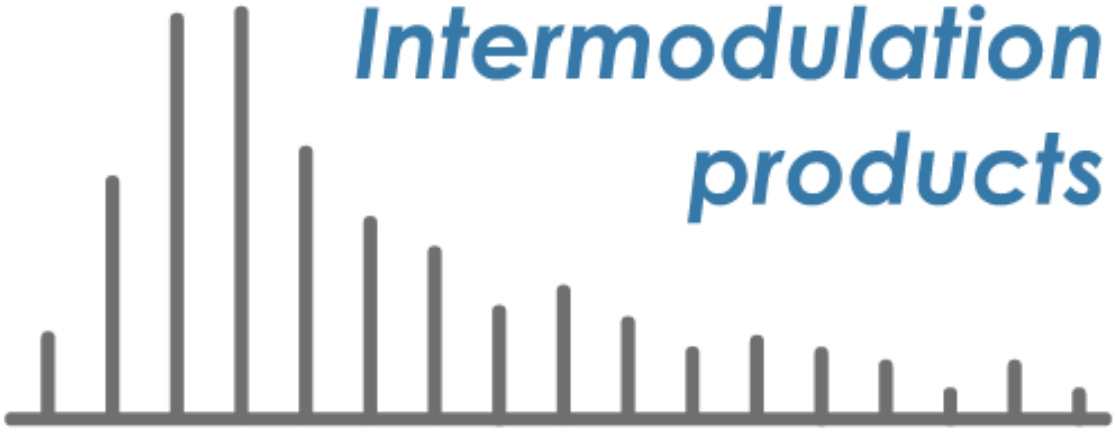
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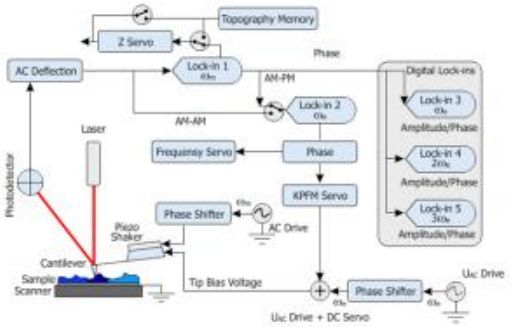

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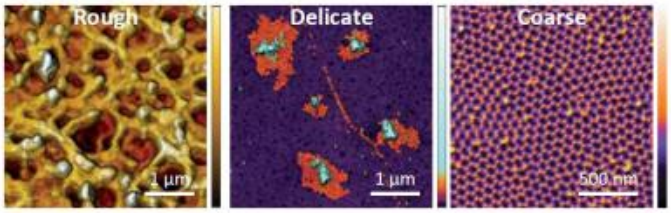
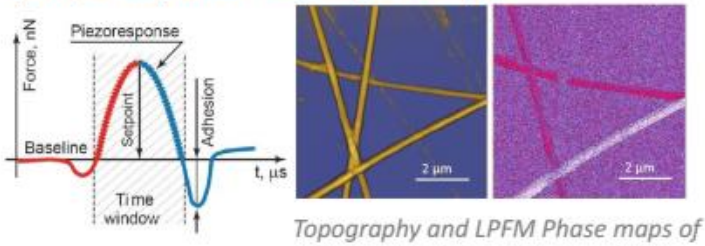


Topography images of NC membrane with a rough surface, F14H20 particles loosely bound to the surface and Al_2O_3 aka "Tip Grater".

HybriD PFM – Complex electromechanical studies (PFM, QNM) of soft and fragile samples



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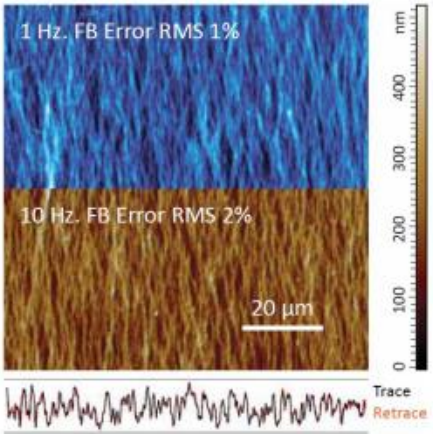

90×90×0,5 µm image of collagen fibers captured at 1 & 10Hz scanning rates in closed-loop mode

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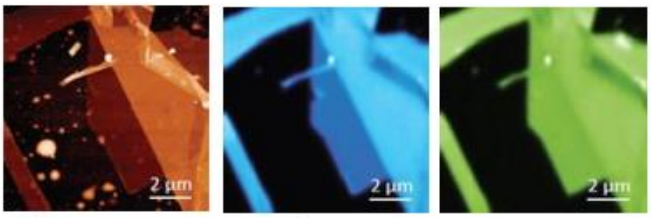
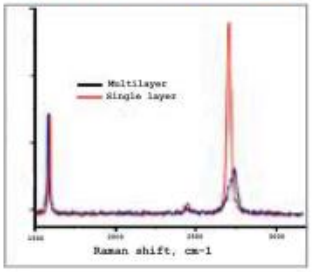
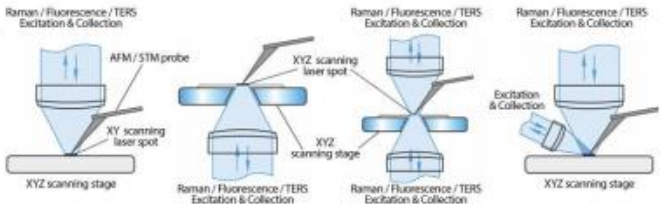


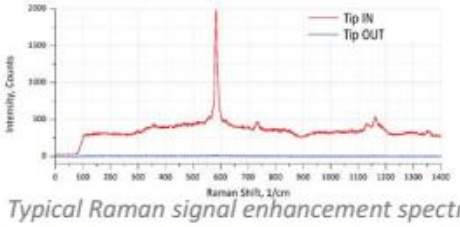


Topography, G band intensity and 2D band intensity maps of graphene flakes on Si/SiO2 with corresponding Raman spectra

Easy optical access for all configurations



TERS-ready experimental setup



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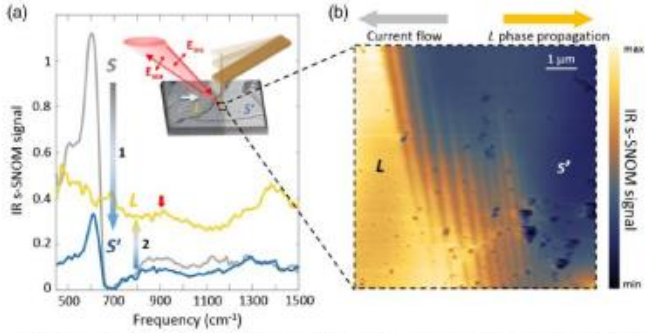
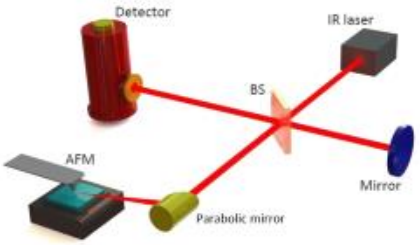


Nano IR spectroscopy and microscopy of Mott insulator Ca_2RuO_4

(a) IR nano spectra of S, S', and L states. (b) IR s-SNOM imaging (second harmonic) of the L-S' boundary stripes at the phase boundary at a frequency of 900 cm^{-1} marked by a red arrow in (a).

Credits: J. Zhang et al, *Physical Review X*, vol. 9, no. 1, 2019.



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Park Atomic Force Microscopy

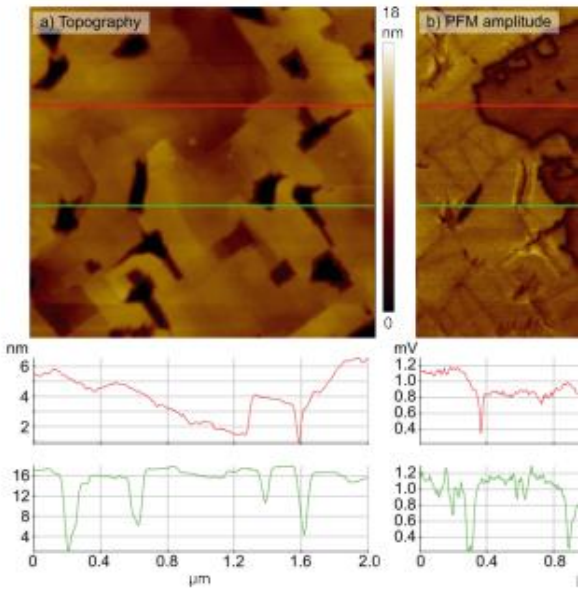
Dual Frequency Resonance Tracking for

Stabilizing the Piezoresponse of Rough

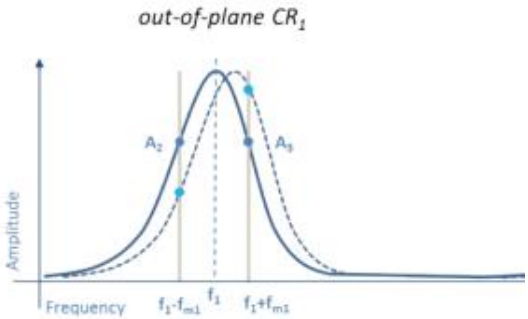
DFRT PFM for reliable characterization of domain pa

Investigating the electromechanical properties of functional ferroelectric materials for electronic or optoelectronic applications requires a high spatial resolution of vertical and lateral domains.

Dual frequency resonance tracking (DFRT) can enhance and stabilize the local piezoelectric information available via **Piezoresponse Force Microscopy (PFM)**, which significantly improves the accuracy and quality of acquired data.



Out-of-plane and in-plane DFRT piezoresponse simultaneously captured by driving the electrical excitation of the cantilever at the contact resonance of the vertical deflection, as well as the torsional resonance on ferroelectric thin films, including bismuth ferrite and lead zirconium titanate.



Piezoresponse Force Microscopy

DFRT PFM

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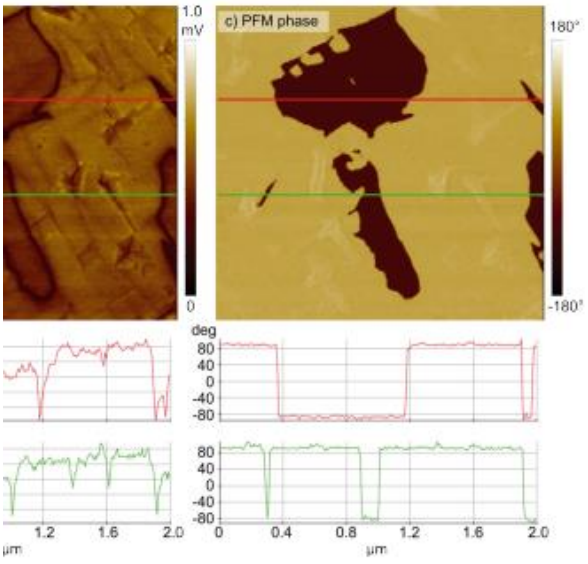
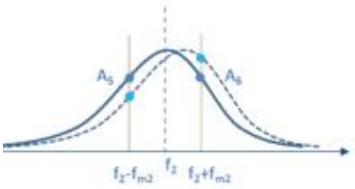
ı Ferroelectric Materials

tterns on rough, polycrystalline samples



in-plane CR_2



Due to the surface roughness of the BFO, single frequency resonance-enhanced PFM suffers severe instabilities, most prominent in the PFM amplitude. Imaging the sample in **DFRT-PFM** reduces the overall topographic cross-talk from step edges or dents in the sample surface significantly.

Image: DFRT PFM measurement on BFO, showing the sample topography (a), PFM amplitude (b) and PFM phase (c) with line profiles through different regions of the image. The AC excitation = 1 V.

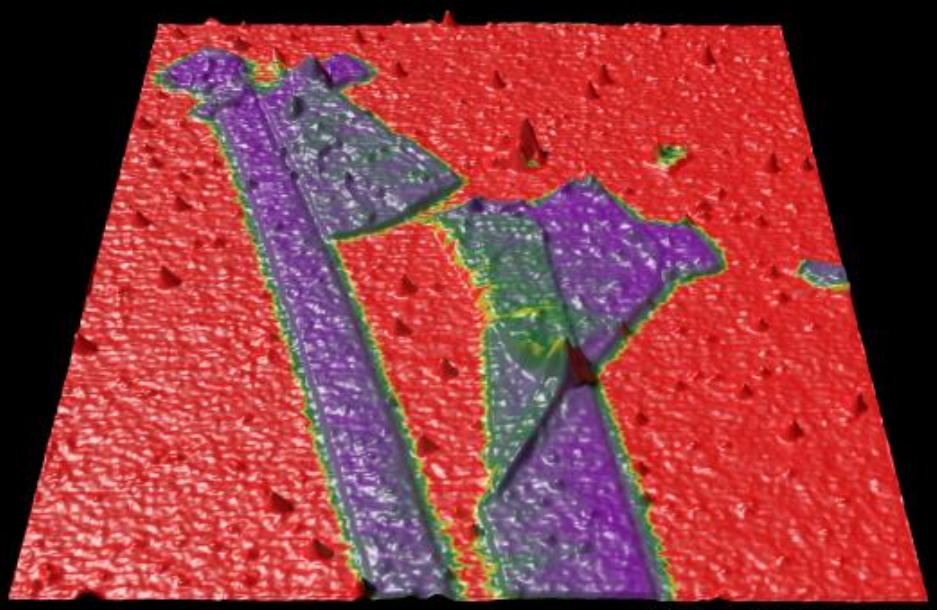
"Simultaneous vertical and lateral resonance tracking PFM on ferroelectric"

8th Multifrequency AFM Conf.:
Thursday, 26 March,
12:40 p.m.

Presented by Ilka Hermes,
Principal Scientist, Park Systems Europe

Talk to us about the future

New developments, innovative approaches, cutting edge instruments: find out what's coming!



KPFM measurement on graphene

Visit Nanosurf in the exhibition hall

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