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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 fifteen years ago. The 9th 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.

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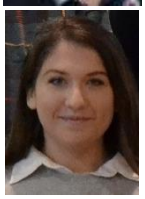

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GENERAL INFORMATION

Poster Session

The poster session will be held during the conference on Wednesday 14th June at the building entrance from 16:30 to 18:00. Pins for mounting the poster on the board will be provided.

There will be a competition in the poster session. The 1st prize is a trophy and a diploma, and the 2nd prize is a diploma.

Internet Access

Wireless internet access is available in the conference venue. Username and password will be provided at the registration desk

Dinner

The conference dinner will be held near the conference venue on Thurdsday 15th at 21:15.

9th MULTIFREQUENCY AFM CONFERENCE SUMMARY

Session	*Invited Speakers*	*Expert Speakers*	*Moderator*	*Room*	
General Session	Takeshi Fukuma	Christine Kranz Pablo Ares	Mingdong Dong	Room 1	**Wednesday 14th June**
2nd Symposium Solid-Liquid Interfaces (I)	Frieder Mugele	Oleg Kolosov Wonho Jhe	Ricardo Garcia	Room 1	
2nd Symposium Solid-Liquid Interfaces (II)	Angelika Kühnle	Yingjie Zhang	Jeffrey Comer	Room 1	
2nd Symposium Solid-Liquid Interfaces (III)	Mingdong Dong Jeffrey Comer		Angelika Kühnle	Room 1	
Magnetic Properties		Hans Hug	Roger Proksch	Room 2	
Cantilevers, modes and resonances			Daniel Ebeling	Room 2	
Advanced AFM Methods I Poster Session	Ruben Perez	Reza Moheimani	Amir F. Payam	Room 2 Entrance	
General Session	Montserrat Calleja Farbod Alijani	Ozgur Sahin	Takeshi Fukuma	Room 1	**Thursday 15th June**
3rd Symposium on Cell and Soft Matter Nanomechanics (I)	Maria C. Serrano		Ricardo Garcia	Room 1	
3rd Symposium on Cell and Soft Matter Nanomechanics (II)	Kislon Voitchovsky	Lorena Redondo-Morata	Monserrat Calleja	Room 1	
Imaging proteins and DNA		Alice Pyne Noriyuki Kodera	Christine Kranz	Room 2	
Advanced AFM Methods II			Farbod Alijani	Room 2	
Plenary Talk	Arvind Raman			Room 1	
General Session	Gabriel Gomila Ken Nakajima	Bart Hoogenboom Matteo Chiesa	Frieder Mugele	Room 1	**Friday 16th June**
3rd Symposium on Cell and Soft Matter Nanomechanics (III)	Andreas Janshoff	Jeanlex de Sousa Johannes Rheinlaender	Jorge Alegre-Cebollada	Room 1	
3rd Symposium on Cell and Soft Matter Nanomechanics (IV)	David Alsteens	Jorge Alegre-Cebollada	Maria C. Serrano	Room 1	
Material characterization	Roger Proksch	Christian Dietz	Matteo Chiesa	Room 2	
Kelvin Probe Microscopy		Liam Collins	Gabriel Gomila	Room 2	

9th Multifrequency AFM Conference, June 14th-16th, 2023

Wednesday 14th June 2023

Time	Duration (min)	Type	Presenter
Room 1	Opening		
09:00 09:15	**15**	Welcome	The Multifrequency AFM Conference Series Conference Chair: Ricardo Garcia
General Session			
Room 1		Moderator	Mingdong Dong
09:15 09:45	**25+5**	Keynote	Takeshi Fukuma
09:45 10.05	**16+4**	Expert	Christine Kranz
10:05 10:25	**16+4**	Expert	Pablo Ares
Coffee Break: 10:30-11:00			

2nd Symposium on Solid-Liquid Interfaces				**Magnetic Properties**			
Room 1		Moderator	Ricardo Garcia	Room 2		Moderator	Roger Proksch
11:00 11:25	**20+5**	Invited	Frieder Mugele	11:00 11:20	**16+4**	Expert	Hans Hug
11:25 11:45	**16+4**	Expert	Oleg Kolosov	11:20 11:40	**16+4**	Extended oral	Thomas Mühl
11.45 12.00	**12+3**	Oral	Elias Nakouzi	11.40 11.55	**12+3**	Oral	Miriaam Jaafar
12:00 12:20	**16+4**	Expert	Wonho Jhe	11:55 12:10	**12+3**	Oral	Victor G. Gisbert
				12.10 12.25	**12+3**	Oral	Jan Soltys
10' Break				**5' Break**			
2nd Symposium Solid-Liquid Interfaces				**Cantilevers, modes and resonances**			
		Moderator	Jeffrey Comer			Moderator	Daniel Ebeling
12.30 12.55	**20+5**	Invited	Angelika Kühnle	12.30 12.45	**12+3**	Oral	Daniel Platz
12:45 13:05	**16+4**	Expert	Yingjie Zhang	12.45 13.00	**12+3**	Oral	Michael G. Ruppert
13.05 13.25	**12+3**	Oral	Sanket Jugade	13.00 13.15	**12+3**	Oral	Teodor Gotszalk
				13.15 13.30	**12+3**	Oral	Gourav Bhattacharya
Lunch Break: 13:30-15:00							

2nd Symposium Solid-Liquid Interfaces				Advanced AFM Methods I			
Room 1		Moderator	Angelika Kühnle	Room 2		Moderator	Amir F. Payam
15:00 15:25	**20+5**	Invited	Mingdong Dong	15:00 15:20	**16+4**	Expert	Reza Moheimani
15:25 15:50	**20+5**	Invited	Jeffrey Comer	15.20 15.45	**20+5**	Invited	Rubén Pérez
15.50 16:05	**12+3**	Oral	Diana M. Arvelo	15.45 16.00	**12+3**	Oral	Daniel Ebeling
16:05 16:20	**12+3**	Oral	Igor Siretanu	**New AFM methods in the market**			
				16:00 16:15	**12+3**	Oral	Nano&More
				16.15 16:30	**12+3**	Oral	Nanosurf
16:30 18:00	**Poster Session (coffee and drinks)**						
End							

9th Multifrequency AFM Conference, June 14th-16th, 2023

Thursday 15th June 2023

Time	Duration	Type	Presenter
Room 1	**General Session**		
		Moderator	Takeshi Fukuma
09.15 09:40	**20+5**	Invited	Montserrat Calleja
09:40 10.05	**20+5**	Invited	Farbod Alijani
10:10 10:25	**16+4**	Expert	Ozgur Sahin
Coffee Break: 10:30-11:00			

3rd Symposium on Cell and Soft Matter Nanomechanics				**Imaging proteins and DNA**			
Room 1		Moderator	Ricardo Garcia	Room 2		Moderator	Christine Kranz
11:00 11:25	**20+5**	Invited	Maria C. Serrano	11:00 11:20	**16+4**	Expert	Alice Pyne
11:25 11:40	**12+3**	Oral	Andra C. Dumitru	11:20 11:40	**16+4**	Expert	Noriyuki Kodera
11.40 11.55	**12+3**	Oral	Guillaume Lamour	11.40 11.55	**12+3**	Oral	Veronika Cencen
11.55 12.10	**12+3**	Oral	Alex Cartagena	11:55 12:10	**12+3**	Oral	Andrea Ridolfi
12.10 12.25	**12+3**	Oral	Livia Angeloni	12.10 12.25	**12+3**	Oral	Ryohei Kojima
5 min Break				**5 min Break**			
3rd Symposium on Cell and Soft Matter Nanomechanics				**Advanced AFM Methods II**			
Room 1		Moderator	Montserrat Calleja	Room 2		Moderator	Farbod Alijani
12.30 12.55	**20+5**	Invited	Kislon Voitchovsky	12.30 12.45	**12+3**	Oral	Vladimir Korolkov
12:55 13:15	**16+4**	Expert	Lorena Redondo-Morata	12.45 13.00	**12+3**	Oral	Gerard.J. Verbiest
13.15 13.30	**12+3**	Oral	Peter Gorelkin	13.00 13.15	**12+3**	Oral	Joan-Carles Escolano
				13.15 13.30	**12+3**	Oral	Hans Gunstheimer
Free time							
20:00 20:15	**Awards ceremony**						
20.20 21.00	**45+5**	Plenary	Arvind Raman				
Conference Dinner: 21.15-22.30							

9th Multifrequency AFM Conference, June 14th-16th, 2023

Friday 16th June 2023

Time	Duration (min)	Type	Presenter
Room 1	General Session		
		Moderator	Frieder Mugele
09:00-09.25	**20+5**	Invited	Gabriel Gomila
09:25-09:50	**20+5**	Invited	Ken Nakajima
09:50-10:10	**16+4**	Expert	Bart Hoogenboom
10:10-10:30	**16+4**	Expert	Matteo Chiesa
Coffee Break: 10:30-11:00			
3rd Symposium on Cell and Soft Matter Nanomechanics			**Material characterization**

Room 1		Moderator	Jorge Alegre-Cebollada	Room 2		Mod.	Matteo Chiesa
11:00 - 11:25	**20+5**	Invited	Andreas Janshoff	11:00-11:25	**20+5**	Invited	Roger Proksch
11:25 - 11:50	**16+4**	Expert	Jeanlex de Sousa	11:25-11:45	**16+4**	Expert	Christian Dietz
11.50-12.05	**16+4**	Expert	Johannes Rheinlaender	11.45-12.00	**12+3**	Oral	Stefano Chiodini
12:05 - 12:20	**12+3**	Oral	Francisco Espinosa	12.00-12.15	**12+3**	Oral	Hung K. Nguyen
10' Break				**5' Break**			
3rd Symposium on Cell and Soft Matter Nanomechanics				**Kelving Probe Microscopy**			

Room 1		Moderator	Maria C. Serrano	Room 2		Mod.	Gabriel Gomila
12.30-12.55	**20+5**	Invited	David Alsteens	12.30-12.50	**16+4**	Expert	Liam Collins
12:45-13:05	**16+4**	Expert	Jorge Alegre-Cebollada	12.50-13.05	**12+3**	Oral	Amir F. Payam
				13.05-13.20	**12+3**	Oral	Yasuhiro Sugawara
13.25-13.30	**5**	Closing words	Ricardo Garcia				
Lunch Break: 13:30-15:00							
Training session simulator: dForce 2.0							

Room 2	
15:00 16:00	Ricardo Garcia & Victor G. Gisbert

9th Multifrequency AFM Conference Poster Session	
1	**Comparative Study of Image Contrast in Contact Mode Scanning Probe Microscopy** Subodh J. Bhosale *Indian Institute of Technology Bombay, Mumbai, India*
2	**Femtolitre Volume Blot-free Electron Cryo-Microscopy Sample Preparation Using Fluid Force Microscopy** Vijayendra Shastri *TU Delft, Netherlands*
3	**Contactless Sensitivity of Micro-Channeled Cantilevers Calibrated by Nanofluidics** Sebastian Sittl *Universität Bayreuth, Germany*
4	**Anomalous Stiffness Dependent Positive Shift in Natural Frequencies in a 2D-MoS2 Coated Microcantilevers** Indranita Lionadi *Nanotechnology and Integrated Bioengineering Centre (NIBEC), United Kingdom*
5	**In-plane and out-of-plane analysis of adsorbate formation, removal, and plasma-induced evolution of defects in multilayer graphene and graphite by multifrequency atomic force microscopy** Marvin Hoffer *Technische Universität Darmstadt, Germany*
6	**Revealing AC Cu-ion transport at the nanoscale in $CuInP_2S_6$ - $In_{4/3}P_2S_6$ flakes during ferrielectric to paraelectric phase transition** Marti Checa *Oak Ridge National Laboratory, USA*
7	**Study of the local conductivity of LCO cathodes for Li-ion batteries by C-AFM** Jesus Díaz-Sánchez *Universidad Autónoma de Madrid (UAM)*
8	**Corrosion Mechanism of Aluminum Alloy Investigated by in-Liquid Nanoscale Potential Measurement Technique** Shinnosuke Yamamoto *Kanazawa University, Japan*
9	**Nanomechanical Mapping of Thermally Evolved Cu-MOF Mediated Porous Copper Oxide Nanoparticles** Gourav Bhattacharya *Ulster University, United Kingdom*
10	**Molecular-Scale 3D-SFM Imaging of Ionic Liquid/Au Electrode Interface Structures and Its Tip/Sample Bias Dependence** Takahiko Ikarashi *Kanazawa University, Japan*
11	**Anomalous underscreening in concentrated aqueous electrolytes: myth or reality?** Frieder Mugele *Univ. Twente, Enschede, The Netherlands*
12	**Inferring Hydration Structure Using Multi-Channel Analysis of FM-AFM Data** Gil Ben Ari *Department of Physics, Technion – Israel Institute*

13	**Characterization of the mechanical properties of individual bacteria in air by multimode tracking of squared nanomechanical resonators** Alicia Aparicio-Millán *Instituto de Micro y Nanotecnología, Spain*
14	**Probing relative humidity-dependent stiffness of Bacillus subtilis spores** Leonardo I. Ruiz-Ortega *Columbia University, USA*
15	**Monitoring Membrane Insertion of SARS-CoV-2 Fusion Peptide by AFM** Qingrong Zhang Louvain Institute of Biomolecular Science and Technology, Université catholique de Louvain, Louvain-la-Neuve, Belgium
16	**Nanoendoscopy-AFM Measurements of Live Cells: Impact on Proliferation and Stress Response** Hosain Mohammad Mubarak *Kanazawa University, Japan*
17	**Exploring Focal Adhesion Dynamics in Living Cells with Nanoendoscopy-AFM** Mohammad Shahidul Alam *Kanazawa University, Japan*
18	**Effect of functionalization and loading on the mechanical properties of soft polymeric nanoparticles** Aurora Dols-Perez *Institut de Bioenginyeria de Catalunya,Spain*
19	**Light-induced modulation of visco-elastic properties in azobenzene polymers** Stefano Chiodini *Fondazione Istituto Italiano di Tecnologia, Italy*
20	**Towards a real-time imaging of the assembly and disassembly of collagen nanofibers** Clara G. Sacristan *Instituto de Ciencia de Materiales de Madrid, CSIC, Spain*
21	**Studying the anisotropic in-plane nanomechanical properties of cellulose nanocrystals** Catarina Ribeiro *Technische Universität Darmstadt, Germany*
22	**Nanomechanical Mapping of Ultrathin Interfaces with Bimodal Atomic Force Microscopy** Victor G. Gisbert *Instituto de Ciencia de Materiales de Madrid (CSIC), Spain*
23	**Simultaneous quantification of stiffness and dispersion forces of materials with nanoscale precision** Amir Farokh Payam *Durham University, United Kingdom*
24	**Observation of Non-linear Elasticity of a Hygroscopic Biological Material using Atomic Force Microscopy** Christina McBean *Columbia University, USA*
25	**A new modular polyprotein system compatible with single-molecule force spectroscopy by atomic force microscopy and magnetic tweezers.** Diana Velázquez-Carreras *Molecular Mechanics of the Cardiovascular System, CNIC, Madrid, Spain*
26	**Confocal Atomic Force Microscopy** Ruben Guis *Delft University of Technology, Netherlands*
27	**Domain formation in biological membranes investigated by HS-AFM** Ignacio CASUSO *INSERM Marseille, France*
28	**Mechanical properties of hygroscopic polymeric nanofibers through AM-AFM semi-empirical models** Horacio V. Guzman *Jozef Stefan Institute, Slovenia*

MULTIFREQUENCY AFM AND SOLID-LIQUID INTERFACES			
	**Takeshi Fukuma** Kanazawa Univerisity	Visualizing Inside of 3D Self-Organizing Systems by 3D-AFM	**Wednesday 14th June Keynote Speaker**
	**Frieder Mugele** University of Twente	In Situ and Operando Characterization of Photocatalytically Active Faceted Semiconducting Nanoparticles	**Wednesday 14th June**
	**Angelika Kühnle** Universität-bielefeld	Atomic-resolution imaging of ice nucleating mineral-water interfaces	
	**Mingdong Dong** Aarhus Unviersity	Unveiling the Nanotribological Behavior of Two-Dimensional Materials through Atomic Force Microscopy	
	**Jeffrey Comer** Kansas State University	Structure of solvation layers on 2D materials revealed by molecular simulation and comparison to AFM results	
	**Ruben Perez** Universidad Autonoma de Madrid	Molecular identification with AFM images and deep learning	
	**Montserrat Calleja** Instituto de Micro y Nanotecnología	Nanomechanical sensors for biosensing applications	**Thursday 15th June**
	**Farbod Alijani** TUDelft	Eavesdropping single-bacteria nanomotion	

	**Arvind Raman** Purdue University	Recent advances in nonlinear dynamics and machine learning in AFM	**Thursday 15th June Plenary Talk**
	**Gabriel Gomila** University of Barcelona	Multiparametric nanocharacterization of electrolyte gated organic transistors in operando	**Friday 16th June**
	**Ken Nakajima** Tokyo Institute of Technology	Recent Progress of AFM Nanomechanics on Polymeric Materials	
	**Roger Proksch** Oxford Instruments Asylum Research	Quantifying multifrequency electromechanics: Electrostatics, Blind spots and beyond Moore's law ferroelectric materials	

SYMPOSIUM ON CELL AND SOFT MATTER NANOMECHANICS			
	**Concepcion Serrano** ICMM-CSIC	Building mechanically compliant responsive scaffolds for spinal cord injury	**Thursday 15th June**
	**Kislon Voitchovsky** Durham Univeristy	Nanoscale quantification of the molecular mobility in fluid biomembranes with high-frequency AFM	
	**Andreas Janshoff** Georg August University Göttingen	Viscoelastic properties of epithelia in 2D and 3D	**Friday 16th June**
	**David Alsteens** UCLouvain	Deciphering the role of glycans as attachment factors in viral infection using AFM	

ORAL PRESENTATIONS

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

Takeshi Fukuma

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Atomic force microscopy (AFM) has been widely used for investigating various materials at an atomic or molecular scale. However, such high-resolution AFM imaging is possible only when the target atoms or molecules are firmly fixed onto a solid surface. Recently, this situation is about to change dramatically owing to the introduction of 3D-AFM. In 3D-AFM, a tip is scanned in both vertical and lateral directions, and the force applied to the tip during the tip scan is recorded to produce a 3D force map. During the tip scan, the tip interacts with the water and flexible surface structures so that the obtained force contrasts represent their spatial distribution. So far, several groups have demonstrated direct imaging of subnanoscale 3D distributions of hydration structures and flexible molecular chains at the solid-liquid interfaces. In these examples, the molecules are not firmly fixed onto the substrate, yet they are clearly visualized with a subnanoscale resolution. This is owing to the self-organization capability of these target structures. For example, in the case of hydration structure measurements, the tip insertion into the hydration structure will once destruct the ordered molecular density distribution, but it is automatically recovered when the tip is removed due to the self-organizing capability. This mechanism suggests that 3D-AFM may also allow us to visualize the inside of other 3D self-organizing systems (3D-SOSs). 3D-SOS is abundant in the field of in-liquid sciences. Examples include hydration structures, surfactants, swollen polymers in interface sciences, lipid vesicles, mitochondria, chromosomes, nucleus, cells in life sciences, and electric double layers playing critical roles in metal corrosion, catalysis, battery and biosensors in electrochemistry. In most cases, their inside cannot be visualized by the current imaging technology with nanoscale resolution. To overcome this limitation, we have been exploring possibilities of 3D-AFM imaging of various 3D-SOSs. In this presentation, I will overview some examples of such studies from our recent works.

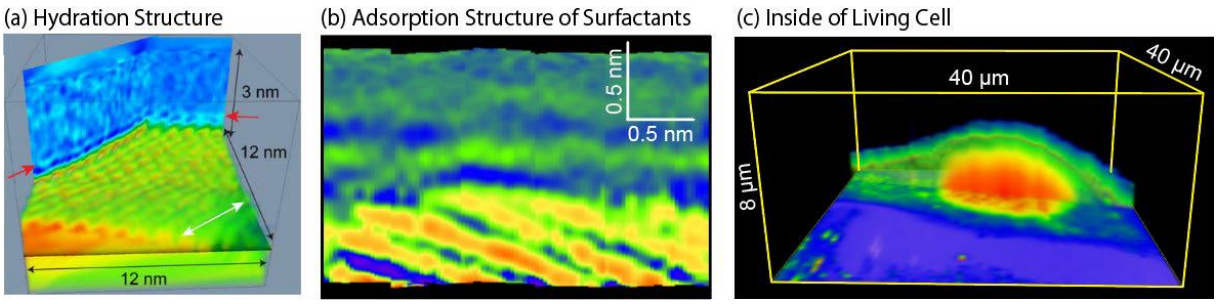

Fig. 1. 3D-AFM images of various 3D-SOSs. (a) Hydration structure formed on a cellulose nano-crystal (CNC) [1]. (b) Adsorption structure of surfactants (C16TABs) in its 10 ppm solution on the sapphire (0001) surface [2]. (c) Inside of live HeLa cell imaged at 37ºC [3].

[1] Yurtsever, A.; Wang, P.-X.; Priante, F.; Morais Jaques, Y.; Miyazawa, K.; MacLachlan, M. J.; Foster, A. S.; Fukuma, T., Sci. Adv. 2022, eabq0160.
[2] Ikarashi, T.; Nakayama, K.; Nakajima, N.; Miyata, K.; Miyazawa, K.; Fukuma, T., ACS Appl. Mater. Interfaces 2022, 14 (39), 44947-44957.
[3] Penedo, M.; Miyazawa, K.; Okano, N.; Furusho, H.; Ichikawa, T.; Alam Mohammad, S.; Miyata, K.; Nakamura, C.; Fukuma, T., Sci. Adv. 2021, 7 (52), eabj4990.

The Role of Surface Charge for the Anti-Biofouling Properties of Polydopamine Films

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Surface charge density and distribution play an important role in almost all interfacial processes including cellular adhesion which governs early steps of biofilm formation. The first attachment of bacteria is one of the most crucial steps in the formation of biofilms (wellorganized aggregates of bacteria protected by extracellular polymeric substances (EPSs)) and is highly influenced next to hydrophobicity and chemical nature of the material, by surface charge. Polydopamine (PDA) is a versatile nature-inspired polymer, which can be formed via a simple dip-coating process from basic dopamine solution [1] or by electro-polymerization [2]. PDA is characterized by a plethora of functional groups like catechol, amine, and imine groups, which partially govern the chemical and physical properties of PDA that can be altered by parameters such as pH or applied potential [3]. For instance, it has been shown that the adhesion properties of PDA and the antimicrobial properties, which are related to the presence of functional groups are strongly influenced by the pH value and the oxidation state of the polymer [4,5].

Here, we present atomic force microscopy (AFM), electrochemical force spectroscopy and force titrations to study the relation of the surface charge density of electrodeposited (e)-PDA under different conditions in respect to the early stages of bacterial attachment of *Escherichia coli*. Next to the characterization of the e-PDA, like point of zero charge, changes in bacterial adhesion, elasticity and morphology of bacterial cells, and biofilm formation are discussed. The latter was also investigated via infrared-attenuated total reflection (IR-ATR) spectroscopy. Finally, single cell force measurements using conductive PDA modified AFMSECM probes will be presented [6].

[1] H. Lee, S.M. Dellatore, W.M. Miller and P.B. Messersmith, Science **318**, 426, (2007)
[2] G. Loget J.B. Wood K. Cho A.R. Halpern and R.M. Corn, Anal. Chem. **85**, 9991 (2013)
[3] J. Kund, S. Daboss T.M. D'Alvise, S. Harvey, C.V. Synatschke, T. Weil and C. Kranz Nanomaterials **11**, 1964 (2021).
[4] V. Ball, Biointerphases **9**, 030801 (2014)
[5] G. Caniglia, A. Teuber, B. Mizaikoff and C. Kranz, Anal. Bioanal. Chem. **415**, 2059 (2022)
[6] S. Daboss, J. Lin, M. Godejohann and C. Kranz, 92, 8404 (2020)

Ferroelectricity in hexagonal boron nitride

Pablo Ares[1,2†], *Colin R. Woods*[1,2], *Harriet Nevison-Andrews*[1,2], *Matthew. J. Holwill1*[1,2], *René Fabregas*[1], *Francisco Guinea*[1,3], *Andre K. Geim*[1,2], *Konstantin S. Novoselov*[1,2,4,5], *Neils R. Walet*[1], *Laura Fumagalli*[1,2]

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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, including a honeycomb lattice very close to that of graphene, exceptional strength, high oxidation resistance at high temperatures and optical functionalities [1]. As a result, it has become a ubiquitous material for the fabrication of van der Waals heterostructures [2]. Like many Group III nitride materials, its covalent bonds are highly polar, presenting the possibility of piezoelectricity [3] and spontaneous polarizations in the correct crystal configurations. In this talk, I will present the occurrence of spontaneous out-of-plane polarization forming ferroelectric-like domains at anomalously stacked hBN interfaces [4]. We have observed these effects using atomic force microscopy (AFM) electrical modes, namely electrostatic (EFM) and Kelvin Probe (KPFM) Force Microscopy, in combination with detailed modelling of in-plane deformation profiles and interface relaxation. Both the in-plane piezoelectricity and the out-of-plane ferroelectricity presented here open up interesting possibilities for precise control of device properties. The experimental approach used here also shows a way to investigate the polarization properties of other materials at the nanoscale.

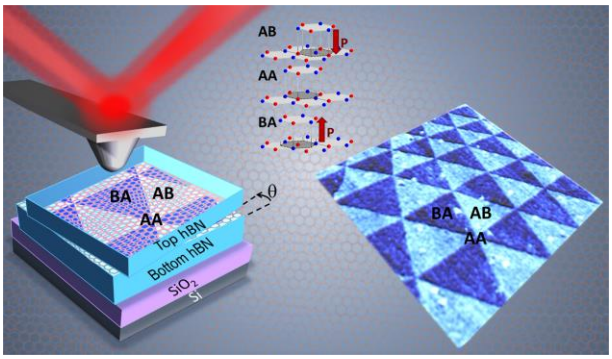

Figure: Ferroelectric-like domains in hBN

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[2] C.R. Dean, A.F. Young, et al., Nat. Nanotechnol. **5**, 722-726 (2010)
[3] P. Ares, T. Cea, M.J. Holwill, et al., Adv. Mater. **32**, 1905504 (2020)
[4] C.R. Woods, P. Ares, H. Nevison-Andrews, et al., Nat. Commun. **12**, 347 (2021)

Multifrequency AFM for Magnetic, Kelvin and Atomic Resolution Contrast

H. J. Hug[1, 2], O. Yildirim[1], A.-O. Mandru[1], D. Rothhardt[3], R. Hoffman-Vogel[3], and A. Kimouche[3]

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Magnetic force microscopy is best performed with low stiffness cantilevers (< 0.5 N/m) with quality factors up to 500'000 obtained under vacuum conditions [1]. The two-passage lift mode techniques typically employed under ambient conditions can then however not be used for tip-sample distance control. Instead, similarly to FM-KPFM, the tip-sample bias is modulated at f_{mod}, generating two pairs of sidebands at $f_0 \pm f_{mod}$ and $f_0 \pm 2f_{mod}$, respectively. While the amplitudes of the first pair of sidebands can be used to map the local Kelvin potential, the amplitudes of the second pair side bands reflect the second derivative tip-sample capacity and can thus serve as a proxy for the tip-sample distance, providing a precise tip-sample distance control. This can be employed to study the evolution of the micromagnetic state with the applied magnetic field, or also to quantitatively compare the MFM contrast obtained with the same tip on different samples [2], or on the same sample with different magnetic states of the tip.

More recently, multimodal operation modes have been used to map the topography of $NiBr_2$ islands on Au(111) with up to atomic resolution, while simultaneously measuring magnetic forces and the local Kelvin potential. This was achieved by driving the first and second flexural modes with oscillation amplitudes of 5 nm and 0.1 nm, respectively. The larger amplitude of the first mode retains high sensitivity for magnetic forces and the measurement of the local Kelvin potential by nulling the sidebands of the first mode. The small amplitude of the second mode (and approximately 40 times higher stiffness) provides high sensitivity for short-range inter-atomic forces. As a result, the sample topography can be imaged with atomic step or atomic resolution, depending on the scan range.

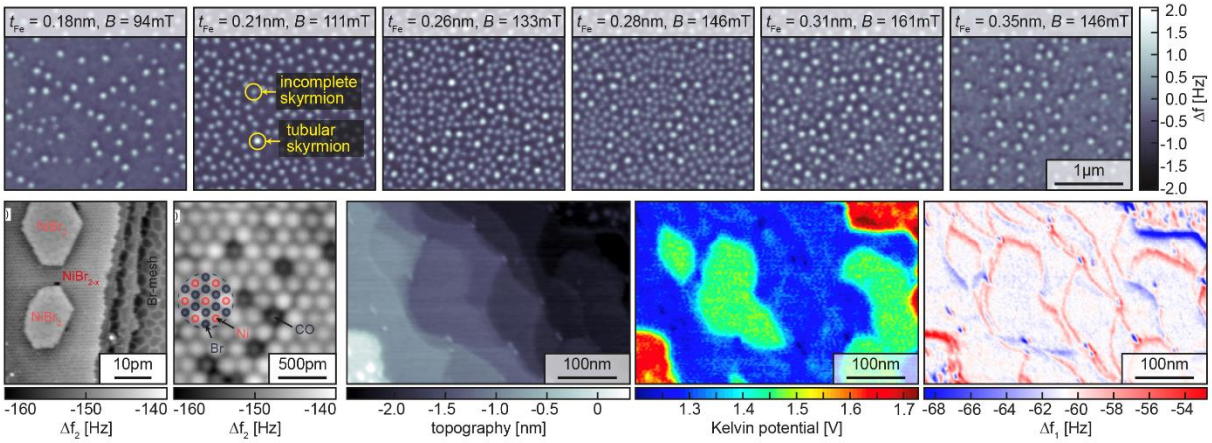

Figure 1: a) to f) MFM data of on F/Fi/F multilayers with different thicknesses t_{Fe} = 0.18 – 0.35 nm acquired with frequency-modulated tip-sample distance control revealing different densitities of tubular/incomplete, i.e. two types of skyrmions. F = [Ir(1)/Fe(t_{Fe})/Co(0.6)/Pt(1)]$_5$ and Fi = [(TbGd)(0.2)/Co(0.4)]×6/TbGd(0.2).
f) $NiBr_2$ islands on Au(111) showing $NiBr_2$, $NiBr_{2-x}$, and Br-mesh phases. g) atomic resolution image of NiBr2. h), i), and j) topography, Kelvin signal, and MFM data acquired simultaneously using multifrequency techniques.

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Simultaneous Magnetic Field and Field Gradient Mapping by Quantitative Multifrequency Magnetic Force Microscopy

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A quantitative, single-pass MFM technique is presented that maps one magnetic stray-field component, B_z, and its spatial derivative, B_z', at the same time [1]. Experimental details, such as the control scheme, the sensor design, which enables simultaneous force and force gradient measurements, as well as the potential and limits of the point monopole description of the magnetic tip moment are discussed.
Our magnetostatically active probe element is an iron-filled carbon nanotube, containing a single domain iron nanowire with an aspect ratio as high as 250. We simultaneously measure the deflection of a tailored low-stiffness cantilever and the frequency shift of one of its flexural vibration modes in fully non-contact operation. Snap-in events are avoided by electrostatic distance control employing a higher order flexural vibration mode. The monopole-type characteristics of the iron nanowire tip allows for a straightforward translation of the cantilever deflection and the frequency shift into B_z and B_z', respectively.
To demonstrate the merit of this technique for studying complex magnetic samples it is applied to the examination of polycrystalline MnNiGa bulk samples.

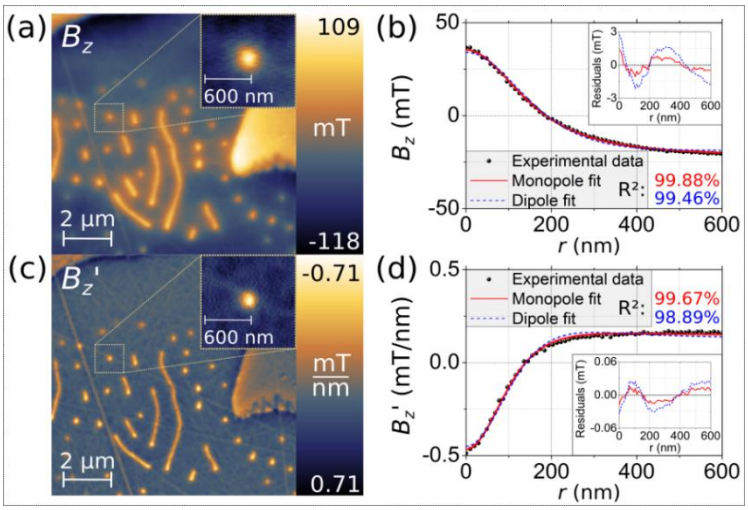

Figure: (a) B_z map of an [001] oriented MnNiGa crystallite. In the inset, a magnified image of an individual bubble domain is shown. (b) An angularly averaged B_z profile for the same bubble is shown together with point monopole and dipole fits. (c) and (d) present the same again but now based on B_z' data.

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Unraveling Dissipation-Related Features in Magnetic Imaging by Bimodal Magnetic Force Microscopy

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Magnetic Force Microscopy (MFM) [1] is the principal characterization technique for the study of low-dimensional magnetic materials. With MFM we detect the magnetic force gradient between the tip and sample with relatively high spatial resolution, simplicity in operation as well as sample preparation. Nonetheless, during years, the samples under study were limited to samples in the field of data storage, such as longitudinal hard disk, thin films, or patterned nanostructures. Nowadays, thanks to the advances and developments in the MFM modes and instrumentation, other fields are emerging such as biological samples. However, in these experiments artifacts in the magnetic images can have strong impact and need to be carefully verified for a correct interpretation of the results. In general, when imaging magnetic materials with MFM the most typical artifacts are the crosstalk between the topography or electrostatic signal and the magnetic signal. However, during MFM imaging the tip stray field (sample stray field) can modify the sample (tip moment) configuration. These reversible or irreversible changes can be significant if the sample (tip) is magnetically soft. For that reason, in this work we will explore new ideas combining the multifrequency modes with the information obtained from the experimental dissipation of energy associated to tip-sample interactions [2].
The multifrequency concept in SFM and its applications has been developed in the last ten years [3] for the characterization at the nanoscale of mechanical or electrical properties in samples with heterogeneous behavior. In addition, bimodal MFM has recently been used for quantitative imaging with high-spatial resolution [4]. The evaluation of the dissipation of energy during the MFM operation by measuring variations in the cantilever oscillation corresponds to the so-called Magnetic Dissipation Force Microscopy (MDFM) mode. The dissipative maps in MDFM have been used to distinguish between Néel and Bloch domain walls, to detect magnetic nanoparticles or to obtain a 3D map of the sample stray field.
Bimodal - MDFM could be a promising technique for increase the possibilities of low dimensional systems characterization.

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Quantitative mapping of magnetic properties at the nanoscale with bimodal AFM

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We demonstrate that bimodal force microscopy enables the mapping of magnetic interactions with high quantitative accuracy and high-spatial resolution (~30 nm). Bimodal AFM operation doubles the number of observables compared to conventional magnetic force microscopy methods, which enables to determine quantitatively in a single processing step several magnetic properties. The theory behind bimodal AFM offers analytical expressions for various magnetic force models, including those characterized by power-law and exponential distance dependencies. Additionally, bimodal AFM provides a self-evaluation protocol that assesses the accuracy of measurements. The experimental results, along with theoretical predictions, demonstrate excellent agreement for two distinct magnetic samples. This supports the application of bimodal AFM as a reliable technique for quantitatively mapping long-range magnetic interactions.

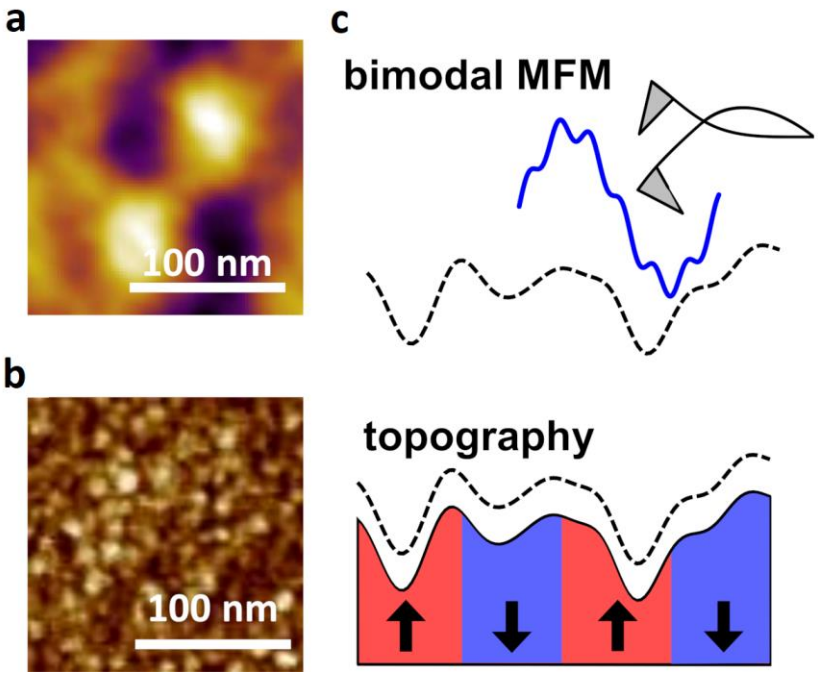

Figure: (a) High resolution image of the bimodal magnetic signal map AFM. interactions (b) Height profile of the surface. (c) Second pass scheme applied in bimodal AFM for imaging magnetic forces.

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Unconventional MFM probe with improved durability

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We have developed magnetic force microscopy tip with a ferromagnetic disk-shaped apex (FMD tip). The fabrication process of the FMD tip is based on the modification of commercial AFM tips by a focused ion beam and subsequent deposition of permalloy (Py). This process leads to a cylindrical tip with the Py disk on top. The unique feature of such a tip is that its mechanical and magnetic properties are disentangled. The ground state magnetic texture of the Py disk is a magnetic vortex where the magnetization circulates in-plane around the disk center. Only a very localized region in the disk center has out-of-plane magnetization called the vortex core [1, 2]. The circular arrangement of the magnetic dipoles creates a closure domain state generating no stray field, leaving the vortex core as the only source of the stray field of the tip. The vortex core acts like a magnetically sharp nanoscale probe while the tip apex is quite large (100 - 300 nm), i.e. robust and wear-resistant.

Here we present our theoretical and experimental study of the FMD tip performance and limitations. We verified its ability by scanning various magnetic structures, including sample with sub-micron-sized domains (bits on HDD) and magnetically soft samples with large domains. Tests on a high-density magnetic recording medium showed that it provides a similar spatial resolution to a commercial MFM probe. It is thanks to the fact that the vortex-core plays the main role in the tip-sample magnetic interaction. However, when scanning large domains, the situation is different. The obtained MFM scans provide a different magnetic image compared to commercial CoCr tips. The image highlights the boundaries between domains with opposite magnetization. The reason for such sensing is due to the induced magnetization of the tip by the sample stray field. This has the consequence that the MFM contrast is mainly generated by the interaction between the sample and the induced magnetic moment rather than with the vortex-core.

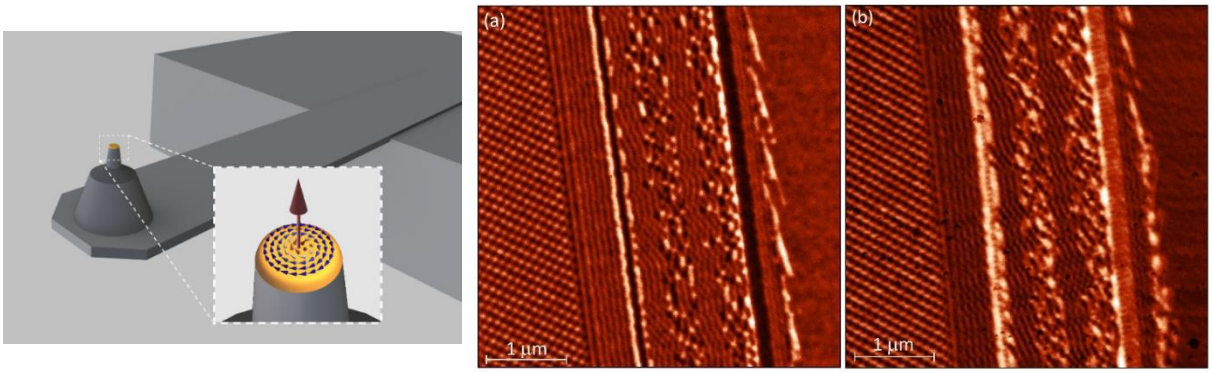

Figure (right) Sketch of the vortex-core tip. The ferromagnetic disk is formed at the flat tip apex. Magnetic vortex core is represented by the out-of-plane brown arrow. (left) Comparison of MFM scans measured by the commercial low-moment tip (a), and by the FMD tip (b).

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[2] S. Krylov , et al., *Journal of Magnetism and Magnetic Materials,* 555, 169357 (2022)

Non-slender MEMS resonators for advanced AFM applications

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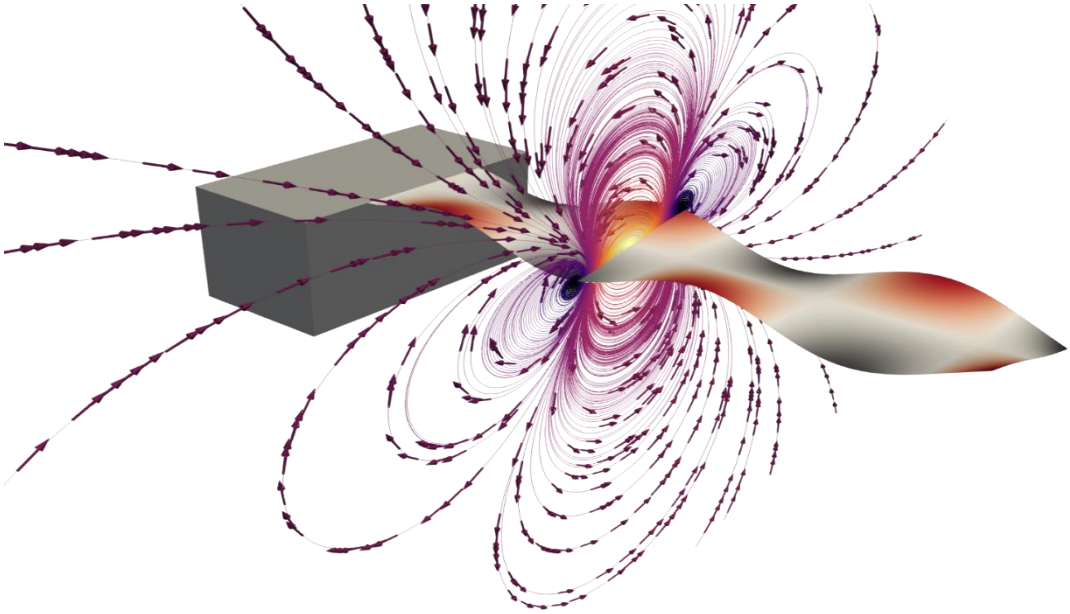
Atomic force microscopy (AFM) took an astonishing development that started from a simple idea and ended at advanced multifrequency methods today. While AFM equipment has generally involved significantly during this development, MEMS resonators with slender beam geometries remain to be a core component in AFM hardware. The focus on this resonator geometry is easy to understand since it facilitates scanning of sample surfaces. Here, we discuss how slight deviations from the standard beam geometry, namely going to plate resonators with finite width, make additional vibrational modes available for dynamic AFM. Going beyond standard beam geometries requires novel methods for modelling the interaction between resonators and fluid environment [1]. Using these methods, we show that the use of plate resonators has the potential of improving AFM in liquids in terms of the resonator's quality factor [2]. However, non-conventional resonator geometries lead not only to modified quality factors. The fluid-structure interaction also couples different vibrational modes with important implications to both single- and multifrequency AFM. In the linear regime, these coupling can also be exploited for fluid sensing. In the nonlinear regime, modal couplings allow for implementing sideband cooling or noise squeezing methods [3].



A cantilevered plate resonator oscillating in a higher order plate mode. Such vibrational modes reach oscillation frequencies in the kHz regime for resonators with widths that are comparable to the resonator length. The arrows surrounding the resonator visualize the flow field in a surrounding liquid (water).

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Experimental Analysis of Tip Vibrations at Higher Eigenmodes of QPlus Sensors

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QPlus sensors are non-contact atomic force microscope probes constructed from a quartz tuning fork and a tungsten wire with an electrochemically etched tip. QPlus sensors are routinely used to visualize the chemical structure of adsorbed organic molecules via the so-called bond imaging technique [1]. Recent work using higher-order resonance modes has also resolved the chemical structure of single organic molecules [2], however, the image contrast can differ significantly from the conventional bond imaging contrast, which was suspected to be caused by unknown vibrations of the tip. This work investigates the source of these artefacts by using a combination of mechanical simulations and laser Doppler vibrometry measurements of the qPlus sensor prong and tip to characterize a range of sensors with different tip wire geometries [3]. The results show that increased tip mass and length cause increased torsional rotation of the tuning fork beam due to the off-center mounting of the tip wire and increased flexural vibration of the tip. These undesirable motions cause lateral deflection of the probe tip as it approaches the sample, which is rationalized to be the cause of the different image contrast. The results provide valuable insights into imaging artefacts observed with higher eigenmode and multifrequency bond imaging and can serve as a guide for future probe development.

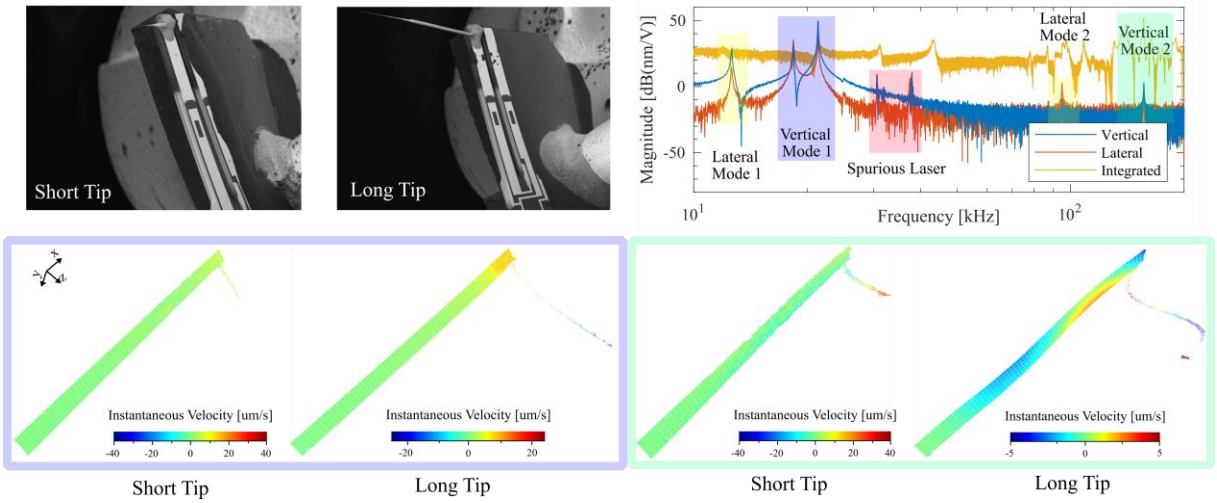

Figure: Top row: SEM images of two of the experimentally investigated qPlus sensors and frequency response of the long tip qPlus sensor measured with a laser Doppler vibrometer (MSA-100 3D) from the top (vertical) and side (lateral) and using an integrated current amplifier (integrated). Bottom row: Lateral deflection mode shapes of the short and long tip qPlus sensor prong and tip at the first and second vertical mode (color coded according to the frequency response). Significant lateral tip vibrations can be observed at the first and second vertical mode.

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Application of active piezoresistive cantilevers in high-eigenmode surface imaging

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In this presentation we will show the application of the so called active piezoresistive cantilevers in the high-eigenmode surface imaging. The active piezoresistive cantilever integrates a piezoresistive deflection sensor and a deflection actuator [1]. The actuator can operate thermomechanically, which means that by the dissipation of the heat within the structure mechanical stress induces the microbeam displacement [2]. Alternative technology is to immerse the active piezoresistive cantilever in the magnetic field [2]. By controlling the current flowing thorough the actuator it is possible to exert the electrodynamic force and actuate the tip movement. The piezoresistive deflection detector responses to the stress occurring when the beam is deflected. In this way the active piezoresistive cantilever forms a micro-electromechanical system (MEMS), which is used in our experiments in the surface metrology. The higher eigen mode cantilever operation makes it possible to increase the scanning throughput as the probe vibrates over the investigated surface with much higher frequency (basic resonance frequency of ca. 53 kHz and second eigenmode vibration of ca. 349 kHz)

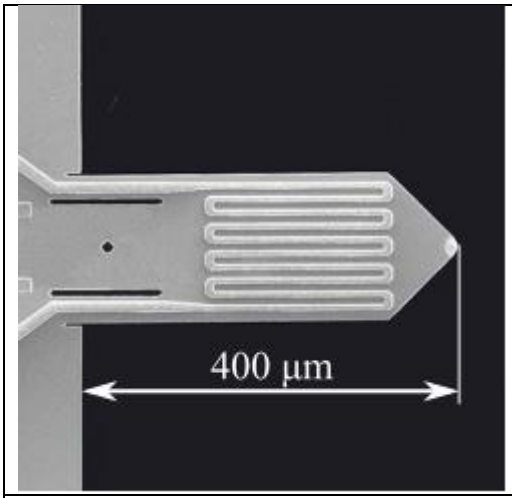

Fig. 1. Active piezoresistive cantilever (https://www.nanoanalytik.net)

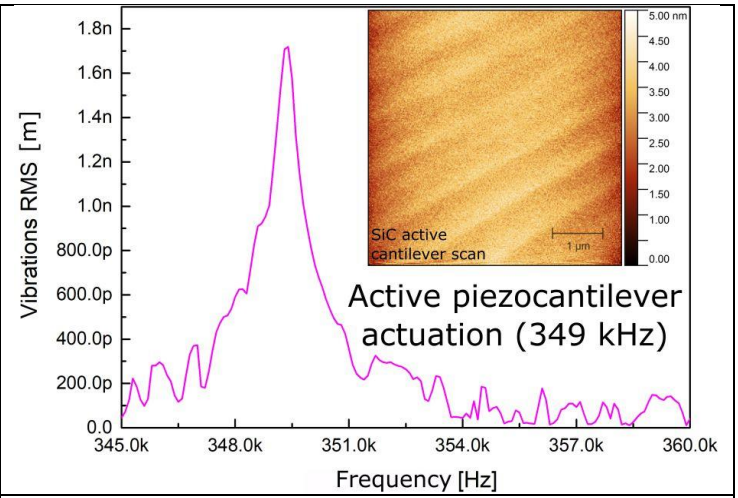

Fig. 2. Second eigenmode of the active piezoresistive cantilever and the topography of the silicon carbide surface recorded in the tapping mode

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Tailored Microcantilever Optimization for Multifrequency Force Microscopy

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Microcantilevers are at the heart of atomic force microscopy (AFM) operation and play a significant role in AFM- based techniques. With the recent advancement in multifrequency AFM, which needs simultaneous excitation and detection of multiple eigenfrequencies of microcantilevers, there is a need to study the structural designing and optimization of microcantilevers [1]-[2]. In this work, the cantilever is modified with gold nanoparticles using a dip-coating method and changes the natural frequency of the cantilever. The added mass loading decreases the resonance frequency of the cantilever. Higher eigenmodes are tuned, and the fourth eigenmode becomes an integer harmonic of the modified resonant frequency. The theoretical and simulative model reported that integer harmonics enhance the coupling in multifrequency AFM measurements and improve image quality and resolution [3]. To confirm the predictive model results, we have used tapping-mode AFM and the bi-modal Amplitude Modulation (AM-AM) AFM technique to explore and quantify the effect of higher-order eigenmode tuning on the imaging quality of polystyrene-polymethylmethacrylate (PS-PMMA) block co-polymer assembly deposited on a glass slide. We collect the AM-AM images for the pristine and gold-modified cantilevers for the 1st-4th eigenfrequencies. Figure 1 represents the topography images at 4th eigenfrequencies for the cantilevers and the second phase mapping. A much-improved resolution can be observed for the modified cantilevers when the higher eigenmode matches the integer harmonics. The findings are well aligned with the theoretical prediction. The different statistical methods and image analysis techniques are further employed to quantify the changes in the resolution and image quality.

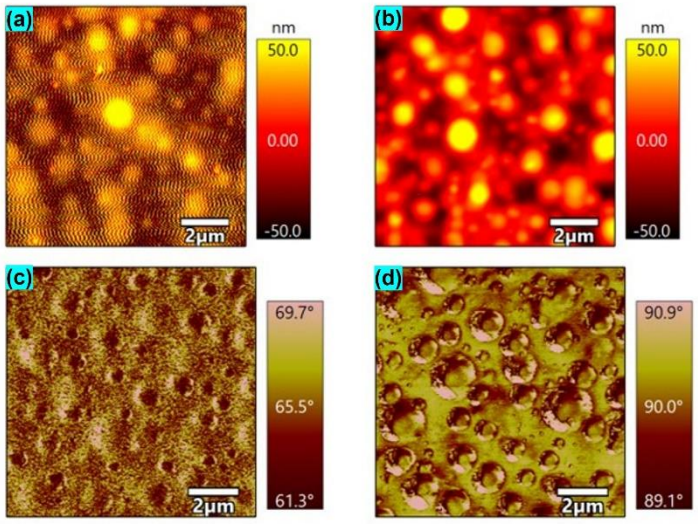

Figure 1. Topography images of (a) Nunano (b) Gold-modified Nunano from the fourth eigenmode and second-phase mapping of (c) Nunano (d) Gold-modified Nunano cantilevers from 1st and 4th eigenmodes of bimodal AFM.

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Control of the Scanning Tunneling Microscope for Atomic-Precision Hydrogen Depassivation Lithography

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Improvement in manufacturing precision has been the driving force behind technological advancements throughout history. In recent years, the scanning tunneling microscope's atomic-precision placement accuracy has enabled several research groups to engineer atomic-scale silicon quantum devices like qubits for emerging quantum computers. Hydrogen depassivation lithography (HDL) is the first step in fabrication of these silicon quantum devices. Future commercial success of this technology hinges on reliable, repeatable, and high-throughput operation of the scanning tunneling microscope. However, the STM is a characterization tool and its use for nanofabrication leads to challenges. In this talk, we demonstrate that many of these challenges can be traced back to the poor performance of the STM's feedback control system and explain how to these issues may be resolved [1,2,3].

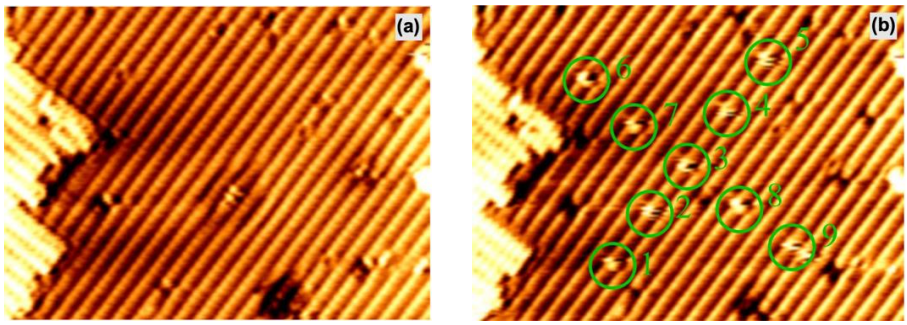

Figure 1. Atomic precision lithography on hydrogen passivated silicon

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[2] H. Alemansour, S. O. R. Moheimani, J. H. G. Owen, E. Fuchs, and J. N. Randall. High signal-to-noise ratio differential conductance spectroscopy. *Journal of Vacuum Science & Technology B*, 39(1):010601 (5pp), January 2021.

[3] H. Alemansour, S. O. R. Moheimani, J. H. G. Owen, J. N. Randall, and E. Fuchs. Ultra-fast method for scanning tunneling spectroscopy. *Journal of Vacuum Science & Technology B*, 39(4):042802 (7pp), 2021.

Molecular identification with AFM images and deep learning

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High resolution non-contact atomic force microscopy (HR-AFM) with CO-functionalized metal tips reveals the internal structure of adsorbed organic molecules with unprecedented resolution. resolving intermolecular features, determining bond orders, and characterizing intermediates and final products generated in on-surface reactions [1]. Recent advances in the interpretation of the AFM contrast observed in porphycenes [2] and on self-assembled molecular layers driven by either halogen [3] or hydrogen bonds [4], shows that there are clear connections between fundamental chemical properties of the molecules and key features imprinted in force images with submolecular resolution.

Inspired by these results, we address the problem of the complete identification (structure and composition) of molecular systems solely based on AFM images, without any prior information, exploiting deep learning (DL) techniques. In a first step, we restrict ourselves to a small set of 60 flat molecules and demonstrate the automatic classification of AFM experimental images by a DL model trained essentially with a theoretically generated data set [5]. We analyze the limitations of two standard models for pattern recognition when applied to AFM image classification and develop a model with the optimal depth to provide accurate results and to retain the ability to generalize. We show that a variational autoencoder (VAE) provides a very efficient way to incorporate into the training set, from very few experimental images, characteristic features that assure a high accuracy in the classification of both theoretical and experimental images.

Learning from the successes and the limitations of this proof-of-concept, we have developed QUAM-AFM, the largest data set of simulated AFM images generated from a selection of 685,513 molecules that span the most relevant bonding structures and chemical species in organic chemistry [6]. QUAM-AFM contains, for each molecule, 24 3D image stacks, each consisting of constant-height images simulated for 10 tip–sample distances with a different combination of AFM operational parameters, resulting in a total of 165 million images. The data provided for each molecule includes, besides a set of AFM images, ball-and-stick depictions, IUPAC names, chemical formulas, atomic coordinates, and map of atom heights. In order to simplify the use of the collection as a source of information, we have developed a graphical user interface that allows the search for structures by CID number, IUPAC name, or chemical formula. Using QUAM-AFM, we have designed and trained different deep learning models to go beyond the classification of limited groups of molecules and achieve the complete identification of an arbitrarily complex, unknown molecule, including multimodal recurrent networks (M-RNNs) [7] and Conditional Generative Adversarial Networks (CGANs) [8].

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Chemical bond imaging using torsional and flexural higher eigenmodes of qPlus sensors

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Non-contact atomic force microscopy (AFM) with CO-functionalized tips allows visualizing the chemical structure of individual adsorbed molecules and enables in-depth studies of on-surface reactions and self-assembly processes. The bond imaging performance of qPlus sensors when using torsional and flexural higher eigenmodes and bimodal AFM was systematically analyzed. [1] The torsional eigenmode is perfectly suited for lateral force microscopy (LFM) with single bond resolution (see corresponding AFM image below). This enables easy switching between vertical and lateral bond imaging without replacing the sensor simply by actuating the same sensor at a different frequency. In case the 2nd flexural eigenmode is applied for bond imaging, particular contrast features appear in the AFM images (see yellow and red arrows). These features are caused by a diagonal (i.e. in-phase vertical and lateral) oscillation of the AFM tip as indicated by an analysis of the mode shapes via laser Doppler vibrometry. Our results give valuable information for future designs of qPlus sensors, that can help to reduce imaging artifacts and increase the signal-to-noise performance.

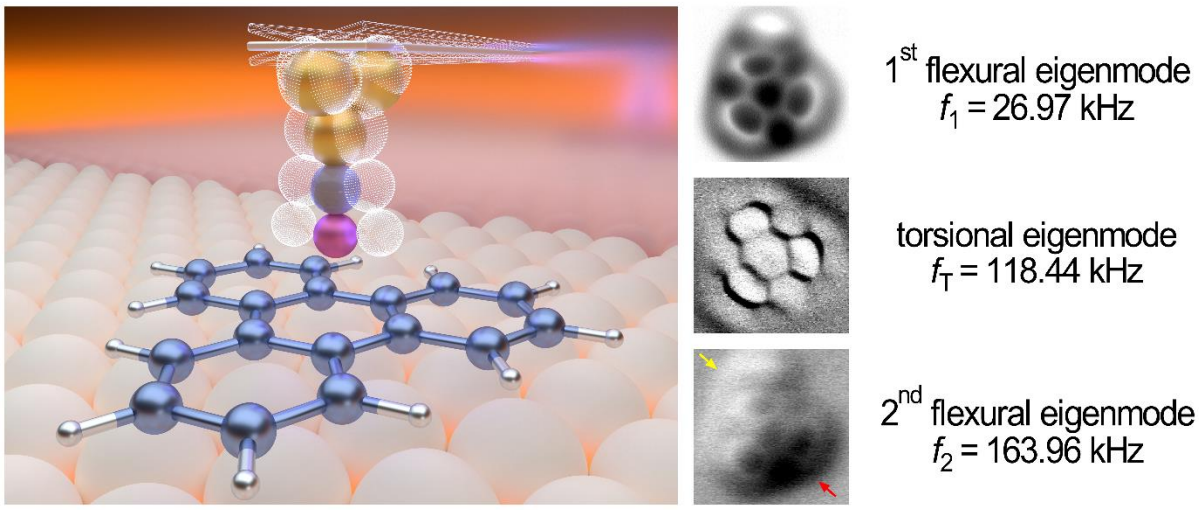

Figure: Using the torsional eigenmode and the 2nd flexural eigenmode of a qPlus sensor for bond imaging AFM with CO functionalized tips at low temperatures in ultrahigh vacuum. When the torsional eigenmode is excited the tip oscillates nearly parallel to the surface (see left scheme). This is useful for performing lateral force microscopy with single chemical bond resolution (see AFM image in the right middle).

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AFM Probe Information and Resources on NanoAndMore's Website

Peter Besmens

NanoAndMore GmbH; Steinbuehlstr. 7; 35578 Wetzlar; Germany

NanoAndMore, founded in 2002, has established itself as the No. 1 one-stop-shop for various AFM probe brands. We serve different needs from budget friendly AFM probes for e.g. educational use to high quality AFM probes for leading edge research.

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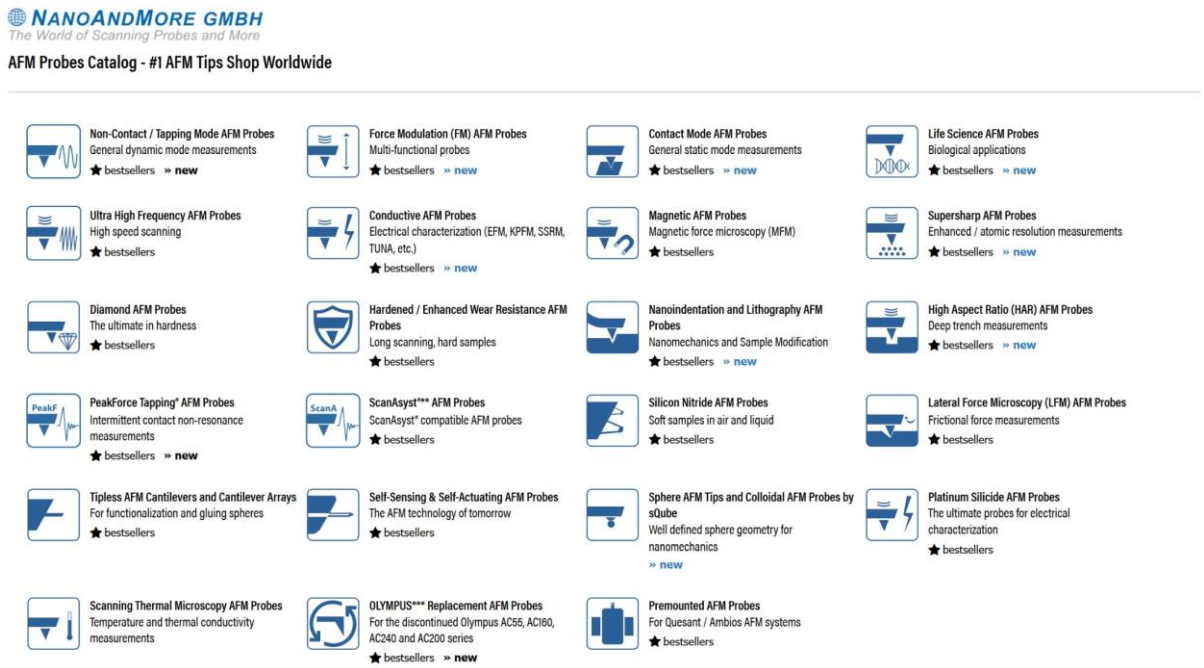

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Developments in photothermal excitation for AFM sample imaging, characterization, and manipulation

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Atomic force microscopy (AFM) is a powerful and multifunctional instrument capable of imaging, characterization, and manipulation through interaction of the tip and the sample. In most AFM systems, a light source is used to detect the cantilever motion using the beam deflection method. Including a second light source in the AFM for actuating the cantilever through the photothermal effect has proven in recent years to be a valuable addition to the AFM instrument. Beyond exciting the cantilever at its resonance frequency, photothermal excitation offers numerous additional ways of actuating the cantilever. These include low-frequency manipulation of cantilever deflection and temperature, actuation at sub-resonance frequencies, and excitation of higher eigenmodes, opening new approaches for using the AFM.

In this presentation, we will provide an overview of new developments and methods in using photothermal excitation in AFM for imaging, characterization, and manipulation of samples at the nanoscale. These include off-resonance imaging techniques that make use of photothermal excitation for overcoming traditional speed limits in off-resonance imaging [1], mass and mechanical property measurements of cells and particles [2, 3], and manipulation of samples through photothermal control of the tip temperature.

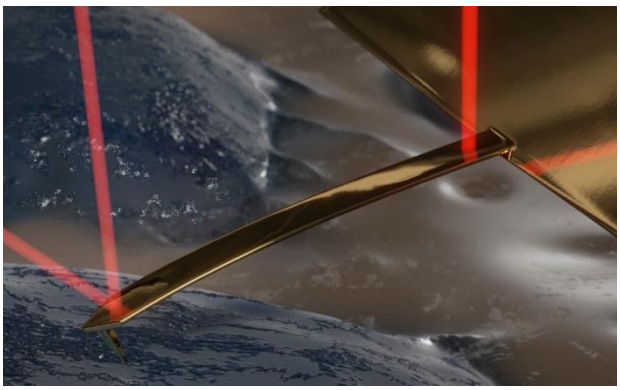

Figure 1: schematic representation of using photothermal actuation to directly actuate cantilever bending, through a second, intensity-modulated light source that is directed towards the base of the cantilever.

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Nanomechanical sensors for biosensing applications

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Resonant nanomechanical structures used as sensors have provided extreme mass sensitivity, in the yg range, achievable due to the low mass of the devices.[1] Progress in downscaling the devices has been accompanied by the development of non-invasive displacement detection techniques with sub-picometer sensitivity, with optical readout of the resonators by a laser beam probe being a very successful approach in recent decades.[2,3] The flexibility to fabricate devices of different sizes and geometries has allowed the characterisation of biological analytes with a wide mass range, from small proteins to bacteria and even human cells;[1,4,5] however, the application of nanomechanical resonators in the field of biology has faced an additional challenge: the small sensing area of the sensors hinders the interaction of the analytes, which are usually found in liquid media, with the micro- and nanoscale sensors, which have to be placed in vacuum to maximise the mass responsivity, leading to very low throughput.[6] In this talk we will explore different approaches to overcome these challenges. We will discuss a multi-frequency nanomechanical mass spectrometer prototype designed to focus, guide and soft-land nanoparticles, bacteria and virus particles on a nanomechanical resonator surface placed in vacuum.[7] We will also discuss the integration of microchannels inside transparent resonators for cancer cell characterisation, allowing simultaneous measurement of single cell buoyant mass and reflectivity with a throughput of 300 cells/min[8] and present preliminary results on the integration of open nanofluidics with nanowire resonators for highly sensitive mass measurements.[9]

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Eavesdropping single-bacteria nanomotion

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Over the last decade, the advancements in microsystems technologies have made it possible to use mechanical probes for characterizing biological substances. In this context, Atomic Force microscopy has been used to detect nanoscale vibrations of colonies of bacteria [1]. Although holding a great promise, the number of bacterial cells required to detect this nanomechanical motion has remained relatively large. By reducing the thickness of the probe, the sensitivity of the nanomotion detection technique can be enhanced significantly.
In this talk, I will show how by utilizing ultrathin (< 1 nm) graphene drums the nanomotion of a single bacterium can be probed in its growth environment [2]. By experimenting with a series of genetically modified bacterial cells and blocking one-by-one different routes of nanomotion, I discuss the nature of these random vibrations and elaborate on the role of motility on the observed signal. Finally, I will demonstrate how vibrations of graphene drums can detect antibiotic resistance at the single cell level and discuss the prospects of graphene membranes as a next generation antibiotic susceptibility testing platform.

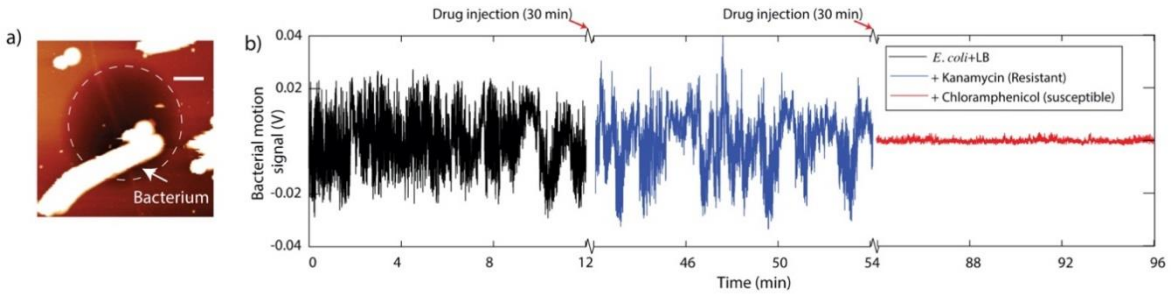

Fig. 1. (a) Atomic force microscope image of a graphene drum (shown by dashed circle) in the presence of single bacterium in liquid; (b) Motion of the drum with *E. coli* before and after adding Kanamycin (Resistant) and Chloramphenicol (Susceptible). White scale bar in Fig 1a is 4μm. In Fig 1b "LB" stands for Luria Bertani.

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Discovery of a fundamental class of solid matter with the atomic force microscope

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Atomic force microscopes (AFMs) are powerful tools to characterize the structure and properties of matter. Using a combination of different AFM techniques including topographic imaging, nanomechanical cantilever sensors, force-distance curves, and frequency-dependent dynamic nanomechanical measurements, we investigated mechanical properties of the hygroscopic spores of a common soil bacterium. We encountered several results that are difficult to rationalize with well-accepted mechanical models of solid matter. To explain our findings, we developed a simple theory based on the hydration force[1]. The theory not only provides explanation to several perplexing results, but also predicted highly unusual mechanical phenomena. Those predictions are also verified by AFM measurements. The findings indicate the existence of a fundamentally distinct class of matter that derives its mechanical properties from water[2].

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Uncovering the invisible complexities of the genome

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Technological developments in high resolution Atomic Force Microscopy (AFM) now enable us to routinely visualise single biomolecules, with sub-molecular resolution. This allows us to observe and quantify changes in the structure of individual DNA molecules in liquid, quantifying their twist, writhe, and topology as they 'explore' their complex conformational space [1]. These measurements are "invisible" to microscopy techniques that rely on labelling or averaging; however, AFM is still not routinely used to help solve problems inaccessible to the traditional tools of structural biology.

One of the rate-limiting steps for the widespread adoption of AFM is a lack of open software pipelines to analyse the increasing volumes of data produced. We have developed TopoStats, a high-throughput, open-source Python package designed to process and analyse raw AFM images, reducing the burden of user oversight including processing of images one-at-a-time for downstream analysis. TopoStats can read and process raw AFM image files, automating image filtering, segmentation, and feature extraction to produce clean, flattened images and powerful statistical information. This enables the user to identify and quantify the structure of individual biomolecules within a broader population [2].

Here we investigate a protein with a poorly understood role within the nucleus, NDP52. We combine our high-resolution AFM and image analysis methods to quantify how NDP52 binding affects the structure and conformation of individual DNA molecules. We are able to resolve the substructure of NDP52 as formed of two globular domains, with a single coiled-coil between them [Figure 1]. We observe that NDP52 is able to dimerise, noting this occurs via the globular regions in addition to the coiled-coil as previously postulated. We detect NDP52 binding with high affinity to DNA and inducing strong bends into the DNA structure. We also observe NDP52 bridging between multiple DNA strands, affecting their higher-order conformation. We quantify these effects to suggest, together with extensive biochemical studies, a possible function for NDP52 in chromatin regulation [3].

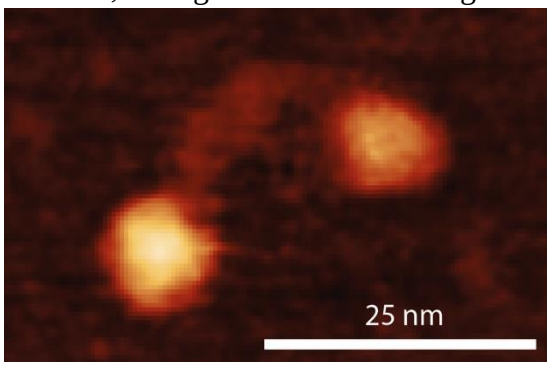

Figure 1: AFM reveals the structure of the protein NDP52

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Recent progress in high-speed atomic force microscopy

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High-speed atomic force microscopy (HS-AFM) allowed us to directly visualize the dynamic behaviors of biological molecules in action at nanometer spatial and sub-second temporal resolution. The power of HS-AFM has been demonstrated by an increasing number of imaging studies on biological molecules [1-3]. For example, from our group, pooling of the translational factors around the ribosomal stalk complex [4], structural dynamics of intrinsically disordered proteins [5], DNA reeling and cleavage reactions by CRISPR-Cas3 [6] were directly captured. However, the vast majority of biological processes have not yet been visualized by HS-AFM. In order to apply HS-AFM to a wider range of biological phenomena, further improvements of HS-AFM are thus necessary.

In recent years, we have been carrying out research and development to improve the temporal resolution of HS-AFM system. Using a tiny piezo and a new holding method of piezo, the resonant frequency of the Z-scanner was improved from ~0.2 MHz to ~1.1 MHz [7]. We also developed an electrical circuit called "resonance controller (Reso-con)" which can control the resonant frequency and quality factor of a Z-scanner without changing the mechanical part. For the amplitude detection, we invented a differential-based ultrafast amplitude detection method with zero intrinsic latency based on the basic trigonometric theorem [8]. By reviewing the optical parts in the optical beam deflection system of HS-AFM, the leaser spot on a cantilever could be reduced to less than 30% of that of conventional system, allowing us to use a smaller cantilever with a higher resonant frequency. We then fabricated such smaller cantilevers by processing small cantilevers with a focused ion beam lithography, by which the resonance frequency of the cantilever became ~10 MHz in liquid. These developments combined with the only-trace imaging mode [9] will enable us to perform HS-AFM imaging ~10 times faster than before.

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Optimization of parameters in HS-PORT-AFM imaging

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High speed atomic force microscopy (HS-AFM) is already a widely used technique for visualising biological processes in real time and at single molecule resolution[1]. We use a variation of off-resonance tapping mode, the photothermal off-resonance tapping mode (PORT), where only the cantilever is actuated and moved up and down[2], to monitor biomolecular assembly patterns. This limits the damage of the sample while still achieving appropriate imaging speeds. We can use these advantages to track delicate interactions and monitor their patterns. So far, we have been able to apply the technique for a variety of dynamic in-vitro studies, such as DNA tripod supramolecular assembly and clathrin mediated endocytosis[2]–[4]. Using blunt-end DNA tripods as an organic model system, we test the effects of various imaging parameters and the range of possible properties for measurement.

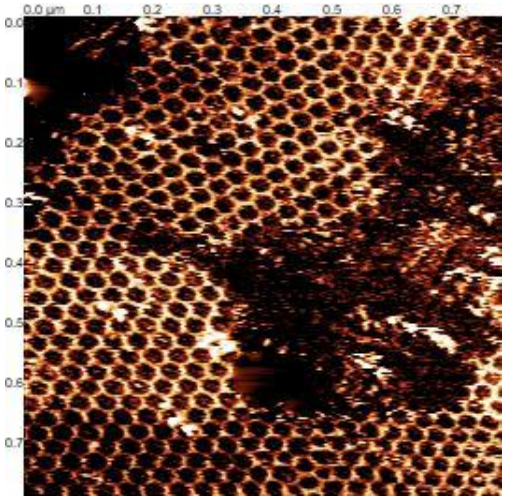

Figure 2: Assembly of blunt-end DNA tripods at steady state, acquired with HS-PORT AFM

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Structural and mechanical heterogeneity of metaphase chromosomes probed by Atomic Force Microscopy

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Faithful genome separation is deeply related to the structural and mechanical properties of metaphase chromosomes[1]. Despite numerous models describing the higher order structure of metaphase chromosomes, the highly condensed chromatin organization is still hindering the experimental characterization of sub-chromosomal structures to most single-molecule techniques. We herein employ Atomic Force Microscopy (AFM) and AFM-based Force Spectroscopy (AFM-FS) to study the structure and mechanics of human metaphase chromosomes within a liquid environment that closely resembles the physiological conditions of mitosis, without the use of fixatives that could induce alterations to the chromosome properties. Our AFM images shed light on the finer details of the unperturbed chromosome structure, providing new insights into the heterogeneous organization of chromatin in different regions of the chromatids. Using AFM-FS, we then probe chromosome mechanics in specific parts of the chromosome body, namely the arms, telomeres and centromere. More precisely, for each region we map the elastic and plastic mechanical response across the two sister chromatids. According to our analysis, the chromatin architecture of metaphase chromosomes possesses a viscoelastic nature, dependent on the strain rate and is heterogeneous across the chromatids. Furthermore, different parts of the chromosome body possess different mechanical characteristics, possibly due to distinct structural features. Our AFM characterization supports and adds new information to recent findings about the structural and mechanical heterogeneity that underpins the architecture of metaphase chromosomes[2]. The presented results provide new insights into sub-chromosomal structures and show how AFM can be effectively employed for studying metaphase chromosomes under physiological conditions.

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Visualization of 3D internal structures of human chromosomes by 3D-AFM using a carbon nanotube tip

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Three-dimensional atomic force microscopy (3D-AFM) is capable of obtaining a 3D force image with subnanometer-scale resolution. Previously, 3D-AFM visualized solid-liquid interfacial structures such as hydration[1] and flexible molecular-structures[2]. In addition, nanoendoscopy-AFM was recently developed based on 3D-AFM, and visualized intracellular structures in a living cell by a needle tip[3]. However, it is still difficult to visualize organelles such as chromosome, nucleus, golgi and mitochondria with nanometer-scale resolution due to the technical difficulty in reducing the diameter of the needle tip less than 40 nm. Here, we performed a 3D-AFM measurement of a chromosome extracted from a human cancer cell (HeLa) (Fig. 1a). Chromosome is a 3D-folded structure of a chromatin fiber (DNA and proteins) with a diameter of 30 nm, but the folded structures is not understood well. To visualize 3D-folded structures of human chromosome by 3D-AFM, we fabricated a carbon nanotube (CNT) tip to reduce the diameter of the needle tip less than 25 nm (Fig. 1b). We obtained the 3D frequency shift (Δf) image (Fig. 1c) by scanning the CNT tip in the chromosomes in 100 mM PBS solution. Figure 1d, a XZ slice from the 3D-Δf image on a chromosome along line AB as indicated in Fig. 1c, shows the local distributions until 500 nm in depth from the surface of the chromosome. To enhance the short-range interaction, the long-range interaction in Z direction from the XZ slice in Fig. 1d was subtracted in Fig 1e, in which local contrasts in the XZ slice were enhanced and the complicated overlapping fiber-like features inside the chromosome were visible. These results indicate that the obtained 3D-Δf image shows internal structure of chromosomes. It suggests that 3D-AFM has a great possibility to visualize the 3D structures of organelles taken from a cell with nanometer-scale resolution and expand applications of 3D-AFM into biological and medical science fields.

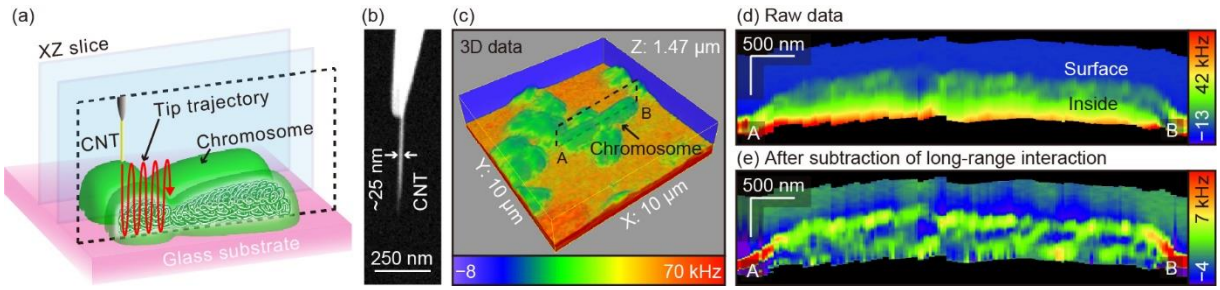

Fig. 1: (a) Schematic illustration of 3D-AFM measurement of a human chromosome using a carbon nanotube (CNT) tip. (b) SEM image of the CNT tip. (c) 3D-Δf image of human chromosomes obtained by 3D-AFM using the CNT tip in (b). (d-e) XZ slices taken from 3D-Δf image in (c) before and after subtraction of long-range interaction, respectively.

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How far we can push the limits of AFM resolution in soft matter?

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Understanding the intricate details of exquisite molecular architecture is pivotal in most areas of soft mater. This ranges from molecular packing in polymers, supramolecular systems on surfaces to capsomere assemblies in viruses.

In this work, we will present a number of case studies where ambient Atomic Force Microscopy has been successfully applied to understand molecular ordering in real-world polymers, unveil the structure of hydrogen bonded molecular networks and study the evolution of mosaic viruses in the ambient and liquid environment.

The key to such high resolution is a newly developed higher eigenmode imaging modality. We will demonstrate how the use of higher eigenmodes imaging provides a routine approach to achieving molecular, and in some instances submolecular resolution, on a wide range of soft matter samples. This approach, unlike others, does not require any special cantilevers or custom modified AFM components. Here, we have implemented this technique on a commercial Park Systems AFM to achieve molecular resolution on a real-world samples of Teflon, polyethylene and i-polypropylene We will discuss challenges and advantages of applying higher eigenmode imaging technique in structural studies of polymers and supramolecular assemblies.
Figures: maximum two figures.

Acoustic subsurface-atomic force microscopy: Three-dimensional imaging at the nanoscale

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The development of acoustic subsurface atomic force microscopy, which promises three-dimensional imaging with single-digit nanometer resolution by the introduction of ultrasound actuations to a conventional atomic force microscope, has come a long way since its inception in the early 1990s [1,2,3]. Recent advances provide a quantitative understanding of the different experimentally observed contrast mechanisms, which paves the way for future applications. In this work [4], I first review the different subsurface atomic force microscope modalities (see Figure 1a-f): ultrasonic force microscopy, atomic force acoustic microscopy, heterodyne force microscopy, mode-synthesizing atomic force microscopy, and near-field picosecond ultrasonic microscopy. Then, I highlight and resolve a debate existing in the literature on the importance of the chosen ultrasound excitation frequencies with respect to the resonance frequencies of the cantilever and the observed contrast mechanisms. Finally, I discuss remaining open problems in the field and motivate the importance of new actuators, near-field picosecond ultrasonics (see Figure 1g), and integration with other techniques to achieve multi-functional non-destructive three-dimensional imaging at the nanoscale.

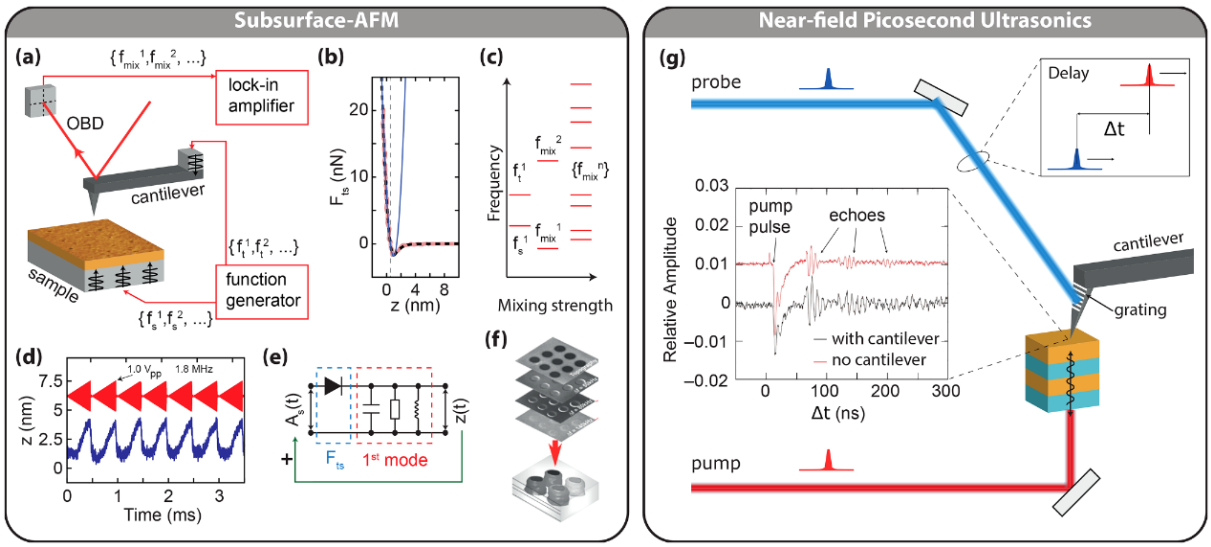

Figure 1. (a) Acoustic subsurface AFM uses one or multiple ultrasound excitations of the sample and/or cantilever and records the cantilever motion at their mixing frequencies using the optical beam deflection (OBD) method. These mixing frequencies are generated by the nonlinear tip–sample force F_{ts}, as depicted in (b) and (c). (d) UFM uses an amplitude modulated excitation signal on the sample (red). The cantilever only follows the modulation frequency, resulting in a rectifying effect (blue). (e) The cantilever response z(t) also modulates F_{ts}; hence, it couples back into the F_{ts} (green arrow) complicating the modeling of the acoustic subsurface AFM. (f) By combining signals from different mixing frequencies, it is under certain conditions possible to reconstruct a 3D image of the measured device. (g) Near-field picosecond ultrasonics uses an ultrashort laser pump–probe technique in combination with a cantilever probe in order to perform pulse-echo measurements in the nanoscale (inset). By varying the delay Δt between the pump and probe pulse, the measurement depth can be controlled.

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Large area automated structural and mechanical analysis of developing cells and tissues

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Active forces in biological systems define the interactions between single molecules, growing cells and developing tissues. Atomic force microscopy (AFM) can be successfully applied for comprehensive nano-mechanical characterization of such samples under near physiological conditions. Currently, the trend is to extend this by studying the mechanobiology of living cells while evaluating their structure and the interaction with their cell culture substrates. It is interesting to understand how cell behavior is driven by the cytoskeletal dynamics and cell mechanics in typical cell culture scaffold scenarios.

We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be studied with high-speed AFM and associated with spatially resolved cytoskeletal reorganization events. We will further extend this with high-speed mechanical mapping of confluent cell layers, which in combination with optical tiling can be applied to automated analysis of large sample areas.

As a tool for analyzing the complex cellular mechanobiology, we went beyond purely elastic models, and performed sine oscillations (up to 500 Hz, amplitude 5-60 nm) in Z while in contact with the surface to probe the frequency-dependent response of living fibroblasts. We will further discuss how to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus (E', E'') distribution in such samples.

In the past, investigating large and rough samples such as tissues and hydrogels using AFM was challenging due to the limited z-axis of the AFM. Using osteoarthritic cartilage as an example, we will demonstrate multi-region AFM probing over a large, rough sample area while providing additional correlative optical data sets.

Nanomechanical analysis of surfaces utilizing AFM with off-resonance photothermal excitation

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Atomic force microscopy (AFM) is a powerful tool for studying the mechanical behavior of materials and biological systems at the nanoscale. Among the various AFM imaging modes, off-resonant imaging modes have become popular, because of their robustness and ease of use during imaging, along with the ability to extract mechanical characteristics of a sample in a straightforward manner [1]. Recently, an off-resonance imaging mode using photothermal excitation was presented [2], overcoming the traditional speed limitation to off-resonance imaging modes that use the AFM z-scanner to perform the required tip-sample distance modulation. This mode may also enable the extraction of mechanical sample characteristics, as during operation the cantilever deflection undergoes a cycle similar to conventional force-distance spectroscopy.

Here, we present the extension of photothermal-based off-resonance imaging modes towards the extraction of mechanical sample characteristics. We will address the technical challenges involved in developing this measurement technique, such as the handling and analyzing of large amounts of data generated during imaging. Furthermore, we will discuss experimental measurements and finite-element simulations of the cantilever deflection during imaging in this mode and how this relates towards the mechanical characteristics that can be extracted, and highlight a number of imaging applications.

Our findings enhance the understanding of different techniques for nanomechanical analysis and show that photothermal off-resonance tapping is a promising mode for high-speed imaging and extraction of mechanical sample characteristics.

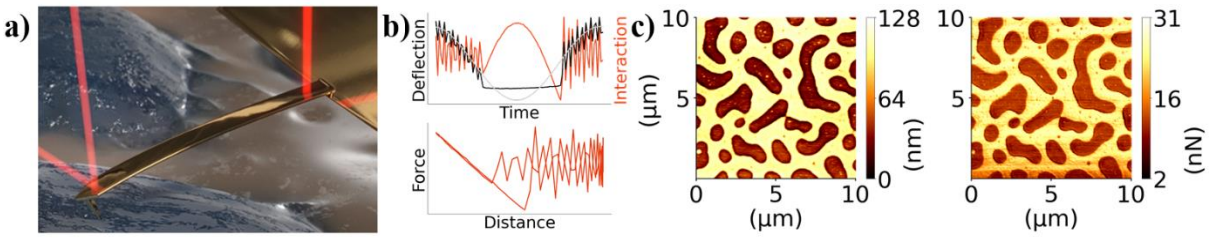

Figure 3: a) Artistic view of the laser configuration for photothermal off-resonance imaging. **b)** Photothermal off-resonance imaging signals acquired on a mica surface, captured as time-series data (above) and post-processed into force-distance representation (below). **c)** Topography (left) and adhesion map (right) of a PS-SBS block copolymer spin-coated on a mica surface acquired at 20 kHz excitation frequency with a comparable soft cantilever WM0.6 (k = 0.6 N/m, f_0 = 350 kHz, Nanosurf).

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Recent advances in nonlinear dynamics and machine learning in AFM

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In this talk, I will discuss two recent advances in AFM methods. The first is related to nonlinear dynamics in Intermodulation Atomic Force Microscopy. Through theory and experiments we show that this important multi-frequency AFM method also features the possibility of bi-stability, bifurcations, and co-existence of solutions. By controlling the difference frequency one can in fact control access to two different regimes of operation, one dominated by attractive forces and another by repulsive forces, each with a different spectrum of intermodulation products. In the second part, we will focus on new computational advances in Peak Force Tapping (PFT) and machine-learned approaches to extracting local adhesive and viscoelastic parameters from experimental PFT data on elastomers.

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Multiparametric nanocharacterization of electrolyte gated organic transistors in operando

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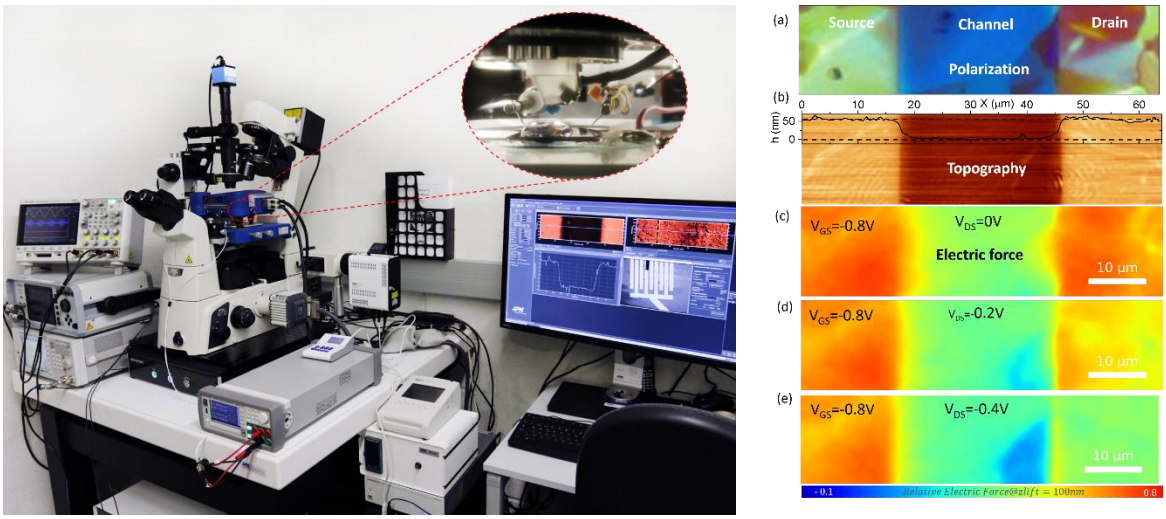
Electrolyte-Gated Transistors (EGTs) have emerged as an integral part of numerous applications in biosensing and bioelectronics, owing to their remarkable ability to efficiently transduce biological events into amplified electronic signals while stably operating in aqueous electrolytes. A typical EGT is a three-terminal device consisting of a semiconducting channel between source and drain electrodes capacitively coupled with the gate electrode through ions in the electrolytes. Understanding these devices at the nanoscale is paramount in order to leverage their respective or combined functionality for various applications. An optimized level of crystallinity or a balance between ionic and electronic conduction within the semiconductor might be desired, which directly relates to the physical and chemical nature of the semiconducting material and its response to applied electric fields. However, probing the nanoscale properties under operating conditions has been challenging due to the complications arising from the electrolyte environment.

In this presentation, I will review the progress made in our research group towards developing an advanced scanning probe microscopy technique to probe different functional properties of the semiconductor materials (morphology, electrical, mechanical) at the nanoscale in operating electrolyte-gated transistors (EGTs). The technique is based on in-Liquid Scanning Dielectric Microscopy (in-Liquid SDM) to which we added automated functionalities and multiparametric characterization capabilities for comprehensive and simultaneous probing of the nanoscale electrical, mechanical and morphological properties in operating EGTs. Examples of applications to Electrolyte Gated Organic Field Effect Transistors (EGOFETs) [1], [2] and Organic Electrochemical Transistors (OECTs) will be presented.



Left: Automated Multiparametric in-liquid SDM setup. Right: Polarization, topographic and electric force images at different applied voltages of the same region of an EGOFET.

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Recent Progress of AFM Nanomechanics on Polymeric Materials

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Atomic force microscope (AFM)-based nanomechanics [1] is a powerful tool to investigate a wide variety of topics in polymer science and technology, which gives maps of Young's modulus, adhesion etc. at nano-scale resolution. The author has been devoted himself to developing the method and applying it to various problems, which will be reviewed in this presentation.

1. Heterogeneous stress formation of filled rubber materials upon a mechanical stimuli [2] and the comparison with FEM calculatinos [3]
2. Heterogeneous distribution of cross-linking density of glassy materials [4], and the validation of modified Halpin-Tsai equation for filled epoxy sample [5]
3. Visualization of dynamic stress network formed in block-copolymer based thermoplastic elastomers and some mathematical analyses on it [6]
4. Sub-surface information detected by AFM nanomechanics and its validation with transmission electron microscope tomography [7] and machine learning

The author will also explain the current state-of-the-art techniques such as nano-scale viscoelastic measurement based on nanorheology AFM [8] and its several examples at the site.

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Molecular-resolution and biochemically specific AFM on the outer membrane of living

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The outer membrane is a formidable barrier that protects Gram-negative bacteria against harsh environments and against many antibiotics. While its biochemical composition is well established, its organisation and assembly appear non-trivial. Here I will describe our AFM experiments on the outer membrane of living E. coli. Combining label-free, molecular-resolution imaging with mutagenesis and nanobody-labelling approaches, we show that the outer membrane is phase-separated into, on one hand, a cell-spanning network of outer membrane proteins and, on the other hand, protein-depleted, glycolipid-enriched domains. These experiments redefine the textbook view of the E. coli outer membrane and exemplify the power of AFM for high-resolution imaging of living cells.

Power law investigation using multifrequency AFM

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The development of multifrequency theory has enabled the quantification of material properties and the acquisition of property maps during imaging[1]. A force model needs to be assumed, and a set of equations must be simultaneously solved, in order to extract material properties[2-4]. In the long range, the tip sample force is frequently modelled using an inverse power law. The inverse power law is of relevance to describe many other physical phenomena including electrostatic[5–8], ferroelectric[9–10], and magnetic[4–11–13] phenomena. Here we focus on long range forces modelled in terms of power laws and employ the multifrequency theory to explore these forces and the relevant parameter space to minimize errors. We solve the governing integral equations analytically and via numerical integration of the equation of motion for powers 2 to 5. We demonstrate that conditions exist where the corresponding errors can be as low as 10% across all powers examined, settings that are compatible with experimental imaging. Roughly this happens when the free amplitude of the first mode ~ 1 nm and the free amplitude of the second mode is about 10% the value of the first amplitude for sufficiently high but standard set points, i.e. $A_1/A_{01} > 0.8$. 10% the value of the first amplitude for sufficiently high but standard set points, i.e. $A_1/A_{01} > 0.8$. We provide the means to 1) explore, image, and quantify relevant nanoscale forces modeled in terms of power law in multifrequency AFM, 2) solve the relevant set of equations, 3) provide and interpretation and analysis of a relevant set of conditions where imaging should be carried out to minimize errors. We know that the results are of interest to the broader multifrequency community and look forward to discussing our results during the upcoming 9th Multifrequency Conference.

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Quantifying multifrequency electromechanics: Electrostatics, Blind spots and beyond Moore's law ferroelectric materials

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Last year marked the 30th anniversary since piezoresponse force microscopy (PFM) was demonstrated on ferroelectric polymers by Guthner and Dransfield. Since then, it has emerged as the preeminent nanoscale approach to characterizing electromechanical response and is commonly used as a verification for ferroelectricity on the nanoscale. The recent advent of weaker, beyond Moore's law materials has accelerated the reporting of "strange ferroelectrics" – synonymous with materials that are mistakenly interpreted as ferroelectric. Motivated in many cases by the need for ever smaller dimensions in micro- and nano-electronics, there has been a rapid expansion of research on thin film ferroelectric materials. Ideally, materials that go into these beyond Moore's law devices should be compatible with currently used CMOS technologies, leading, for example to Hafnia based materials and layered van der Waals ferroelectrics. These materials commonly have inverse piezo coefficients that are small, often less than 1pm/volt. They also often have small switching voltages, required for low power device performance but that also limits the range of the excitation potential. In this talk, I will discuss some recent results that demonstrate quantitative PFM measurements at noise floors as low as 100fm/Volt. I will also report on a large series of systematic studies exploring so called blind spots where the measured amplitude and phase are independent of electrostatic crosstalk. These exhaustive measurements were simultaneously made with both a standard optical beam (beam bounce) detector and a quantitative interferometric detector. The results are in good agreement with Euler-Bernoulli beam theory and allow a quantitative comparison of various strategies for quantifying electromechanical response and for eliminating crosstalk. These results have broad implications for AFM-based nanomechanical measurements in general.

In-plane and out-of-plane force deconvolution and interaction analysis of adsorbates of graphitic surfaces by multifrequency atomic force microscopy

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Multifrequency atomic force microscopy (AFM) is shown to be an excellent tool for imaging crystal structures at atomic resolution in different spatial directions. However, determining the forces between single atoms remains challenging, particularly in air under ambient conditions. We developed a trimodal AFM approach that simultaneously acquires torsional, lateral and flexural frequency-shift images as well as spectroscopic data to transfer these observables into in-plane and out-of-plane forces between single bonds of highly oriented pyrolytic graphite (HOPG) at atomic resolution in air under ambient conditions based on the Fourier method (Figs. 1 a, b). Significant differences were observed in the in-plane forces depending on the orientation of the carbon bonds relative to the direction of torsional oscillation [1,2].

A non-negligible implication when imaging graphitic samples under ambient conditions is the accumulation of airborne adsorbates at the surface. The developed multifrequency technique revealed friction anisotropy at distinct areas of a graphene-/graphite-flake after 14 days of storage when exposed to air, albeit not throughout the whole flake, indicating different types of adsorbates. High-resolution imaging revealed that friction anisotropy occurred not only at positions where stripe like structures were visible, but also on areas which were completely covered with adsorbates (Figs. 1 c, d). Additionally, we demonstrated that after removal of the adsorbate material by oxygen-plasma treatment, no friction anisotropy was observable on the graphene-/graphite-flake, corroborating adsorbate driven nature of friction anisotropy on graphitic surfaces [3].

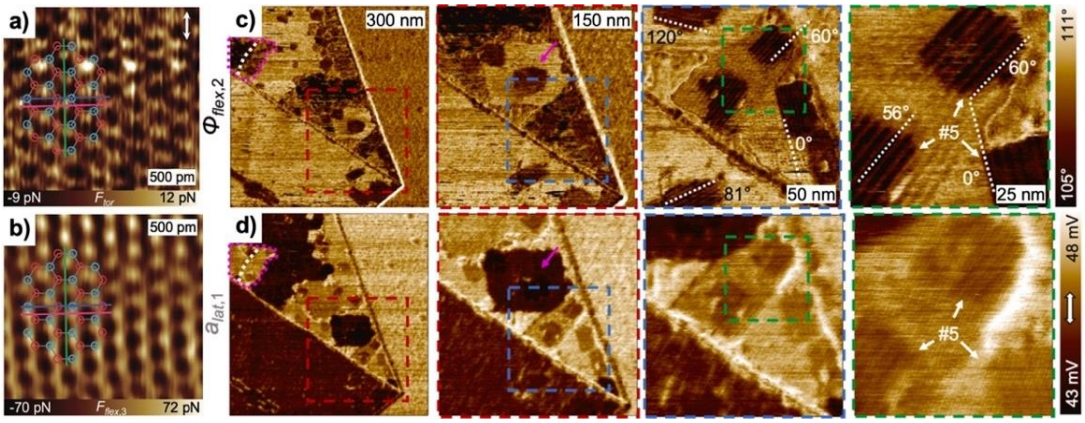

Figure 1: Trimodal AFM imaging of HOPG and adsorbates on few-layer graphene/graphite. a) Force deconvolution for in-plane and b) out-of-plane tip-sample interactions. c) Out-of-plane and d) in-plane tip-adsorbate interactions on different length scales. The surface reflects partially a graphene flake with two distinct layer thicknesses (left portion of the first images) on SiO_2 (right portion).

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Moiré modulation of van der Waals potential in twisted hexagonal boron nitride

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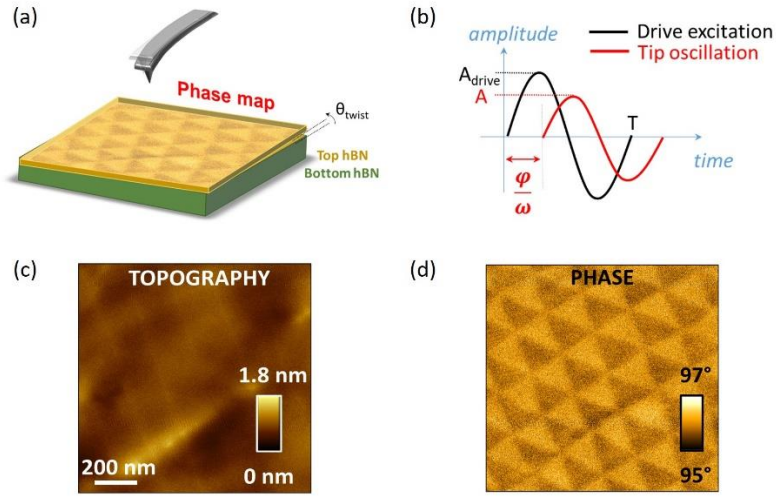
When a twist angle is applied between two layered materials (LMs), the registry of the layers and the associated change in their functional properties are spatially modulated by the so-called moiré superlattice. Several works have recently explored the optical,[1] electric,[2] and electro-mechanical[3] moiré-dependent properties of such twisted LMs, but, to the best of our knowledge, no direct visualization and quantification of van der Waals (vdW) interactions has been presented, so far. Here, we show the application of tapping mode atomic force microscopy (AFM) phase-imaging to probe the spatial modulation of vdW potential in twisted hexagonal boron nitride (t-hBN).[4] We find that a moiré superlattice is visualized in the phase channel only when non-contact (long-range) forces are probed, revealing the modulation of the vdW potential at the sample surface, following AB and BA stacking domains. Our results address tapping-mode phase imaging as a powerful technique for moiré superlattices characterizations. It is non-invasive, compatible with every environment and no sample perturbation is required.



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Insights into Dynamic Mechanical Properties of Nanoscale Interfaces in Polymer Nanocomposites using Multifrequency AFM

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The incorporation of a small fraction of hard nanoparticles into epoxy resins can simultaneously improve their multiple macroscopic properties, which is largely attributed to the formation of an interfacial polymer layer surrounding the nanoparticle with structural and mechanical properties different from the bulk matrix. Because of the nanoscale size of nanoparticle/polymer interfaces, it remains challenging to directly quantify the local mechanical properties of such a interfacial layer. In this study, we combined two recently developed atomic force microscopy (AFM)-based methods, namely bimodal amplitude- and frequency-modulated (AM-FM) and nano-dynamical mechanical analysis (nDMA) [1,2], to characterize the local mechanical properties of the interfacial layer formed surrounding nanosilica that are well dispersed in an epoxy-amine system. While the AM-FM method enables a simultaneous mapping of the elastic modulus and dissipated energy quantities at high spatial resolution [1,3], the nDMA mapping provides information about the dynamic mechanical responses of both epoxy matrix and interfacial layer over a broad temperature range [2]. As a result, both methods evidence the existence of an interfacial layer having the stiffness and adhesive properties significantly altered from the bulk matrix behavior. As shown in Figure 1 for AM-FM results, the glassy modulus of this layer reduced by several factors with respect to the matrix value, whereas the adhesive response of the epoxy network became weaker upon approaching close to the nanosilica interface [3]. In addition, the mechanical dynamics of the interfacial layer measured by the nDMA method demonstrates a significant deviation from the bulk behavior in both glassy and rubbery states.

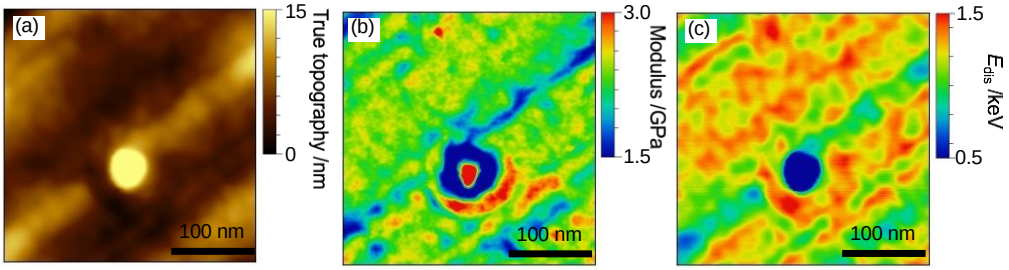

Figure 1: (a) True topography, (b) elastic modulus and (c) dissipated energy (E_{dis}) images captured surrounding a nanosilica in epoxy nanocomposites using AM-FM AFM method.

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High speed mapping of surface charge dynamics via Spiral Scanning Kelvin Probe Force Microscopy

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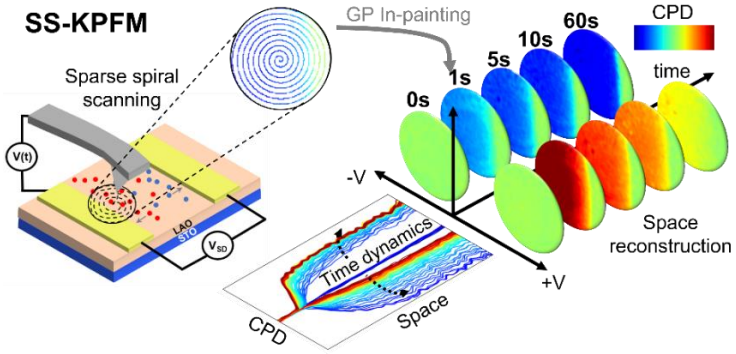
The understanding of dynamic charge processes is critical to the development of advanced materials and devices[1], such as batteries, fuel cells, and bioelectronics, to name but a few. However, analyzing electronic and ionic phenomena across different time and length scales is challenging due to the intrinsic heterogeneities of the materials, including grain boundaries, domain walls, and interfaces. While scanning probe microscopy techniques like Kelvin probe force microscopy (KPFM) provide high spatial resolution, their slow imaging speed, limits investigations of fast temporal dynamics. In this talk, two approaches will be discussed to address this issue: time resolved KPFM and high-speed imaging KPFM. Time-resolved KPFM includes G-Mode[2] and LIA-based open-loop methods, suitable for fast, reproducible events. Both imaging and spectroscopic modes of time resolved KPFM will be shown, which can be used to investigate ionic transport and electrochemical phenomena on length scales from microseconds to seconds. However, when samples cannot be excited by pulses of regular intervals or when investigating non-cyclo-stationary or irreversible events (such as irreversible structural changes or chemical reactions, e.g., the formation of a solid-electrolyte interface layer in a battery), a different approach is required. This led to the development of the high-speed Spiral-Scanning Kelvin Probe Force Microscopy (SS-KPFM)[3] approach, which combines sparse spiral scanning and image reconstruction through Gaussian processing. The full 2D Contact Potential Difference (CPD) maps can be captured at a rate of approximately 3-4 frames per second, providing real-space maps of the surface charge dynamics with automated and fast characterization. During the talk, spatiotemporal characterization of several relevant energy materials will be demonstrated, including photogenerated charge carriers in hybrid perovskite solar cells, spatiotemporal charge dynamics at a LaAlO3/SrTiO3 planar device, and charge diffusion dynamics in polycrystalline TiO_2 thin films.



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Nanoscale spatiotemporal transient dynamics quantification via Multifrequency Kelvin Probe Force Microscopy

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Probing and quantification of local electronic/electrochemical dynamics including transients arising from the charge-carrier diffusion/recombination and illumination-based photovoltage effects have been eagerly pursued since the advent of scanning probe microscopy (SPM) [1]. The past decades have seen SPM and its modalities become the de jure principle technique for nanoscale characterisation, imaging, and manipulation [2]. In particular, Kelvin Probe Force Microscopy (KPFM) based electrical measurements have provided unprecedented insight into charge transport phenomena across the materials domain [3]. However, the inherent measurement paradigm in classical feedback-based KPFM limits the information to static/quasi-static processes, while effectuating an irretrievable loss of information encoded in the transient response and higher-order harmonics [4]. In this work, we develop a new paradigm to detect, extract, and quantify the transient response arising from the dynamic charge transport whilst simultaneously enabling multi-parameter acquisition of physical properties. Our methodology uses Wavelet Transform as the underlying computation tool (ensuring high resolution across the frequency spectrum) and utilizes Principal Component Analysis (PCA)-based filtering, to extract spatiotemporal surface potential information from the acquired cantilever signal in a feedback-free implementation – termed as *Open-Loop Wavelet Transform Kelvin Probe Force Microscopy (OL-WT-KPFM).* We provide a comprehensive model with the new theory, simulation, and exacting experiments to support our case. Concurrently, using the proposed computational approach, the SP, capacitance gradient ($\partial C/\partial z$), and dielectric constant (ε) of materials can be simultaneously measured at the micro/nanoscale without the need for multiple LIAs. We undertook dynamic probing of a low bandgap, n-type semiconductor bismuth oxyiodide (BiOI) to capture surface photovoltage-induced µs surface diffusion of charge carriers, consistent with the bulk time-resolved measurements.

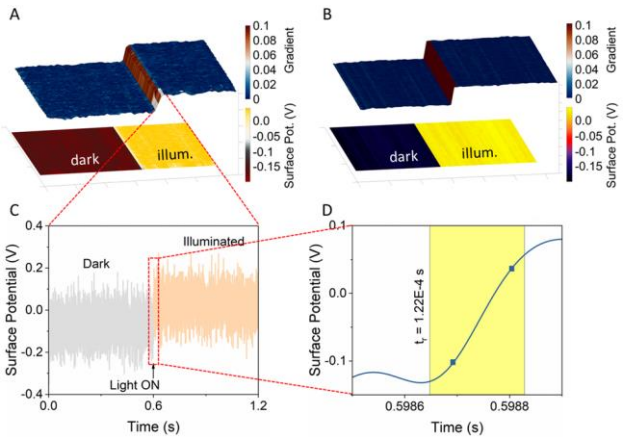

Fig. 1. Single-Pixel spectroscopic measurements on BiOI

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High—Low Frequency Kelvin Probe Force Spectroscopy for Measuring the Interface State Density

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With the recent miniaturization of semiconductor devices, understanding the physical and electrical properties of semiconductor devices, such as the dopant concentration, dopant distribution and defect level distribution, at the nanoscale has become important. Among the physical properties of semiconductors, information on semiconductor interface states is particularly important. For example, in semiconductor devices such as field-effect transistors, the presence of semiconductor interface states is known to significantly affect device operation characteristics. Therefore, direct observation of semiconductor surfaces with nanoscale spatial resolution will become even more important for understanding and controlling the effects of these properties on devices and for evaluating semiconductor device operation. Here, we propose high—low Kelvin probe force spectroscopy (high—low KPFS), an electrostatic force spectroscopy method using high- and low-frequency AC bias voltages to measure the interface state density inside semiconductors [1-3]. We derive an analytical expression for the electrostatic forces between a tip and a semiconductor sample in the accumulation, depletion, and inversion regions, taking into account the charge transfer between the bulk and interface states in semiconductors. We show that the analysis of electrostatic forces in the depletion region at high- and low-frequency AC bias voltages provides information about the interface state density in the semiconductor band gap. As a preliminary experiment, high-low KPFS measurements were performed on ion-implanted silicon surfaces to confirm the dependence of the electrostatic force on the frequency of the AC bias voltage and obtain the interface state density.

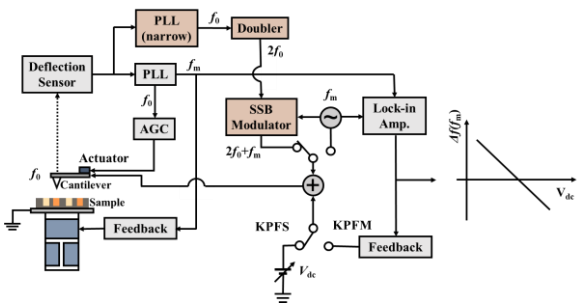

Figure 1: Block diagram of AFM and high—low KPFS. In the low KPFS, a signal $V_{ac}\cos 2\pi f_m t$ is generated by the oscillator. In the high KPFS, a signal $V_{ac}\cos 2\pi(2f_0 + f_m)t$ is generated by mixing the signal $\cos 2\pi(2f_0)t$ and the signal $\cos 2\pi f_m t$ in the SSB modulator.

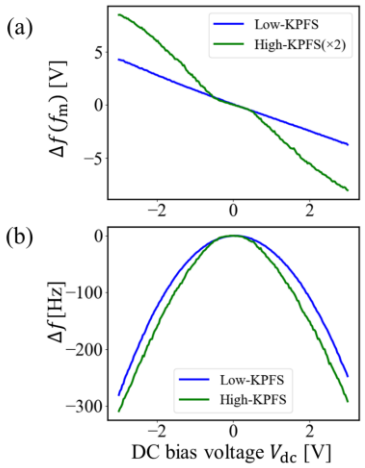

Figure 2: (a) $\Delta f(f_m)$—V_{dc} curves and (b) Δf—V_{dc} curves obtained on the n-type Si surface.

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ORAL PRESENTATIONS

2ND SYMPOSIUM ON SOLID-LIQUID INTERFACES

In Situ and Operando Characterization of Photocatalytically Active Faceted Semiconducting Nanoparticles

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Photo- and electrocatalytically active materials are expected to play an essential role in the transition towards sustainable processes for energy storage and chemical conversion. Performance and stability of the materials still need to be improved. Yet, the microscopic origin of their current limitations are often poorly understood. One key limitation is the lack of suitable techniques that allow for a detailed characterization of the structural and electrical properties of the interfaces on the nanometer scale. In this lecture, I describe our recent progress in establishing in situ and operando AFM spectroscopy for characterizing the surface charge and its response to illumination on photocatalytically active faceted nanoparticles of $SrTiO_3$ and $BiVO_4$ in ambient electrolytes of variable composition. Our measurements demonstrate the existence and pH-dependence of differences in surface potential between adjacent crystal facets, which are believed to drive the separation of photo-generated electron-hole pairs in photocatalysis. For visible light-driven $BiVO_4$, we monitor the variations of the local surface charge upon illumination, from which we extract the local surface photovoltage and thus the accumulation of charge carriers at the interfaces. The measurements suggest a strong influence of surface defects such as steps and disordered regions between adjacent facets for the accumulation of photo-excited charge carriers. I will conclude the lecture with an outlook on upcoming challenges in AFM-based characterization of catalytic materials for the energy transition.

Mapping nanoarchitecture of liquid-gel-solid interfaces: 3D nano-rheology microscopy nanoprobing from molecular layers to volume reconstruction

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One of the largest challenges for scanning probe microscopy (SPM) is exploiting its superior nanoscale to atomic resolution and SPM ability work in vacuum, air and liquid environments beyond the immediate surface of the sample.

A particular challenge is to study the soft matter surfaces (such as living cells or bacteria, polymer brushes or electrolyte-electrode interfaces in batteries) where the interfaces are not well defined and structures are truly three-dimensional. Here, we report novel 3D nano-rheology microscopy (3D-NRM) that uses a tiny (sub-nm to few nm) lateral dithering of the sharp SPM tip at kHz frequencies to probe the minute sample reaction forces. By mapping the increments of the real and imaginary components of these forces, while penetrating the soft interfacial layers, we obtain the true 3D nanoscale structure of sub–μm thick layers [1].

By combining 3D-NRM with the surface force-distance spectroscopy [2] (Fig 1d) in the *operando* electrochemical environment, we observed for the first time in real space the nanoscale dynamics of formation of the key solid electrolyte interphase (SEI) layer in Li-ion batteries starting from a few 0.1 nm thick electrical double layer to the full 3D nanostructured SEI (Fig 1e). We therefore were able to elucidate the key role of solvents in such formation and predict the conditions for building SEI for robust, safe and efficient Li-ion batteries.

The new 3D-NRM can provide unique opportunity for studies inorganic catalytic and separation processes, to explore biological interfaces probing bacterial and cell surfaces, and functional coating in the real space and appropriate operational environment.

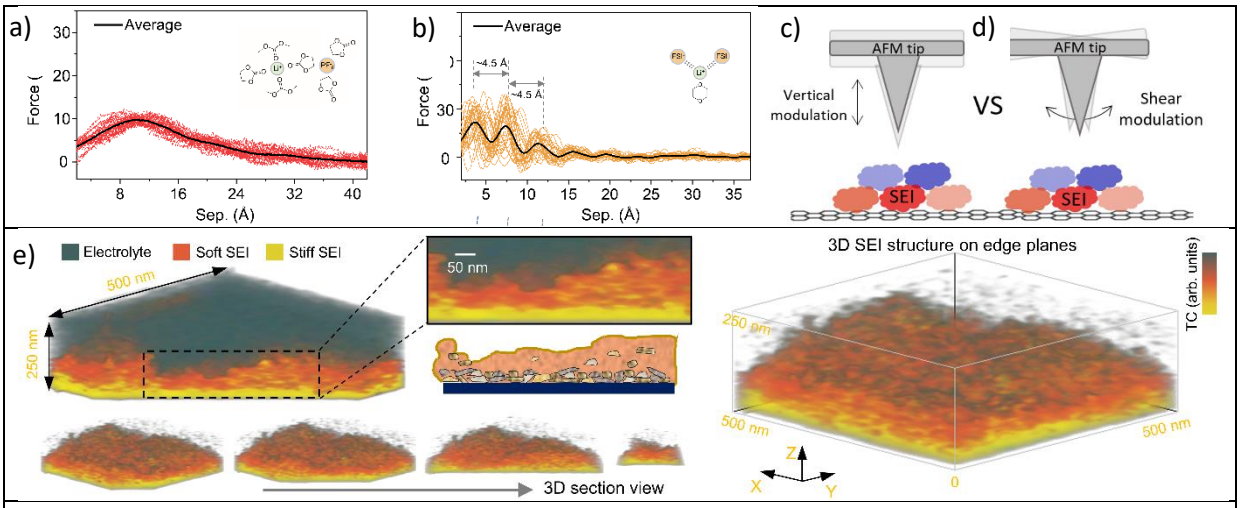

Figure1. a, b) Results of force-distance spectrocopy c) of the different electrolytes on the HOPG surface. e) 3D-NRM of the formed SEI in the Li-ion battery.

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Ion correlation increases hydration force and influences solution structure at interfaces

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Solution structure at mineral-aqueous interfaces creates inter-particle forces and chemical potential gradients that influence particle stability, reactivity, and aggregation. However, despite their relevance to a variety of research fields, the molecular details of interfacial solution structure remain poorly understood. In this study, we characterize the hydration layers at the boehmite-water and mica-water interfaces with sub-nanometer resolution using three-dimensional atomic force microscopy (3D AFM). For the boehmite case, the highest water densities correspond to hydrogen bonding interactions with the surface hydroxyl groups corresponding. The second layer of water molecules occurs above two adjacent crystallographic sites, with a third layer consisting of rows of water molecules along the [100] direction. These results are benchmarked against a suite of molecular dynamics simulations with different degrees of complexity which model the probe tip as a solvent molecule, a non-intrusive disk, a nanosized silica chip, or a spherical object that interacts with its surroundings according to a Lennard-Jones potential. Our investigations of both the boehmite and mica systems show that the solvent tip approximation is not valid in the presence of ions at high concentrations.

In addition to the local distribution of water molecules, we investigate the effect of solution structure on hydration forces. We observe that the hydration force increases at high ionic strength and for divalent versus monovalent ions. For the mica-water system, we find that the relationship between the energy required by the probe to displace the interfacial ions and water molecules is correlated with the *A* and *B* coefficients in the Jones-Dole equation, which are empirical proxies for ion-ion and ion-water interactions, respectively. This rationale is validated with 3D AFM measurements and atomistic simulations on the boehmite system, wherein ions with a stronger propensity for correlations resulted in stronger hydration forces. Our results offer an interesting perspective for controlling the hydration force – and hence the outcomes of particle interactions – by tuning solution chemistry. We anticipate that this work will spur further investigations into the molecular nature of solid-liquid interfaces with important implications on relevant problems in geochemistry, electrochemistry, and materials synthesis.

Tip-enhanced Raman spectroscopy of water under controlled nano-confinement

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Structural transformations originating from diverse rearrangements of the hydrogen bonding in water create various phases. Although most phases have been well investigated down to the molecular level, the molecular structure of the nanomeniscus, a ubiquitous form of nanoscale water in nature, still remains unresolved. For experimental study, we first demonstrated that the water nanomeniscus exhibits the stable, ice-VII-like molecular structure (Fig.) in ambient condition using surface-enhanced Raman spectroscopy (SERS) on trace amounts of water, confined in inter-nanoparticle gaps [1]. Recent first-principle molecular dynamic simulations showed water confined within nanoslits is expected to exhibit a bilayer ice-VII [2] at room temperature, but not the hexagonal ice structure. To address this structural dependence, we performed atomic force experiment of confinement-induced molecular structural change of water by employing tip-enhanced Raman spectroscopy (TERS) at room temperature. A novel DDAA molecular peak emerges in the OH-stretching band at sub-nanometer confinement, which exhibits the unit structure of ice-VII while the tetrahedral DDAA (ice-Ih) appears as the confinement lessens. Our results provide novel insights on water or ice that forms on Earth or in atmospheric clouds and help understand the peculiar sluggish dynamics of nano-confined water from molecular and biological aspects.

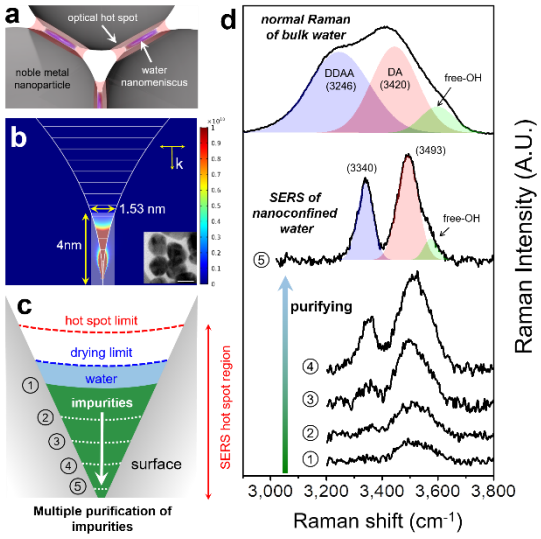

Fig. Surface enhanced Raman spectroscopy for ultra-purified nanoconfined water between gold particles.

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Atomic-resolution imaging of ice nucleating mineral-water interfaces

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Ice nucleation in clouds is triggered by the presence of mineral particles, where the specific arrangement of the water molecules at the interface is believed to induce nucleation at comparatively high temperatures [1]. In this context, feldspar minerals and silver iodide have attracted great attention, as they are known to be highly efficient in ice nucleation. Interestingly, despite many experimental efforts, the precise mechanism that explains the high efficiency remains elusive.
Obtaining insights into the ice nucleation process requires the knowledge of the atomic surface structure including the hydration at the mineral-water interface. In this respect, atomic force microscopy allows for gaining real-space, molecular-level information of the interfacial structure. In this presentation, atomic resolution imaging at the interface between water and (i) silver iodide [2] as well as (ii) feldspar mineral surfaces will be presented and discussed in the view of their ice nucleation ability.

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Electrochemical 3D-AFM Studies of Electrode-Electrolyte Interfaces

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Electrochemical solid-liquid interfaces are crucial for a large range of renewable energy systems, including batteries, supercapacitors, fuel cells, and various electrosynthesis processes. However, the molecular-scale structure of these interfaces, key for energy conversion, is still elusive. In this talk, I will discuss our recent efforts on combining 3D-AFM with a sealed electrochemical cell, which enables stable, operando, atomic-scale imaging of electrode-electrolyte interfaces. We have observed rich electrode morphology and electrical double layer (EDL) structures under controlled electrode potentials, in a series of electrolytes including ionic liquids, water-in-salt electrolytes, aqueous solutions, and organic electrolytes. Through experimental data analysis, atomistic simulations, as well as statistical mechanics modeling, we conclude that the EDL structures depend on both the atomic/molecular interactions as well as entropic effects.

Nanoscale Probing of Intercalated Water in Graphene with Torsional Resonance AFM

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Exfoliation of graphene in an ambient environment can trap moisture between graphene and a hydrophilic substrate [1]. This interfacial ice-like water layer significantly modifies graphene's tribological [2] and electronic properties [3]. The confined water also governs several other properties like adhesion, surface chemistry, conductance, and local strain in graphene. Visualizing micron-scale islands of trapped liquid is possible through topography in Atomic Force Microscopy (AFM). However, localized mapping of the sub-surface liquid regions remains challenging. This work shows Torsional Resonance AFM phase-contrast as a simple tool for nanoscale mapping of intercalated water in graphene. TR phase remains nearly the same on SiO_2 and graphene with intercalated water, while it is ∼1°-3° less on graphene without intercalated water. This technique probes the graphene-liquid interface in a non-invasive way with high sensitivity to near-field lateral forces due to a small torsional amplitude of 1-2 nm. The TR phase contrast shows the opacity of graphene to tip-SiO_2 van der Waals interaction while partial to full transparency to tip-intercalated water interaction. We also investigate the implications of this transparency effect on the role of interfacial water in friction on graphene. Furthermore, we utilize this TR phase mapping technique to study the effect of moisture intrusion and thermal processing on the distribution of intercalated water in graphene.

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Unveiling the Nanotribological Behavior of Two-Dimensional Materials through Atomic Force Microscopy

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Friction is a ubiquitous phenomenon with profound implications for resource utilization. This talk presents a comprehensive exploration of the frictional properties of two-dimensional (2D) layered materials, and their interactions with small molecules in different environments. The goal is to enhance our understanding of friction reduction and the origin of friction, enabling the development of novel technologies. Using atomic force microscopy (AFM), we investigate the dynamic behavior of small molecules during sliding friction processes in both air and liquid environments, shedding light on their influence on the frictional properties of 2D materials. The results reveal the impact of adsorbed small molecules on friction hysteresis in single-layer graphene, affecting its ability to recover from deformation. Additionally, we explore the influence of surface-adsorbed small molecules on the nanoscale frictional properties of four transition metal dichalcogenide materials, highlighting changes in lattice patterns and friction forces. This study highlights the influence of surface-adsorbed small molecules on the frictional properties of 2D materials and underscores the importance of surface cleanliness. Moreover, it emphasizes the significant impact of third-party molecules on the friction between sliding objects. The findings presented in this talk contribute to the fundamental understanding of friction and pave the way for the development of advanced materials and lubrication technologies.

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Structure of solvation layers on 2D materials revealed by molecular simulation and comparison to AFM results

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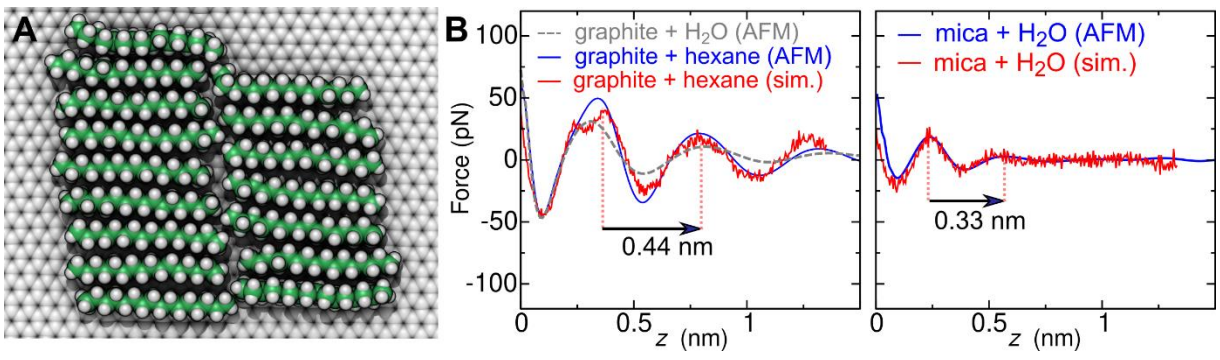
Atomic force microscopy (AFM) has revealed unexpected structural features at aqueous interfaces of hydrophobic 2D materials including graphene, hexagonal boron nitride, and transition metal dichalcogenides. In this talk, I will describe molecular dynamics simulations performed by my group to help interpret these experimental results and understand the structure of solvation layers on solid surfaces at the atomic level. Notably, AFM images have shown nanometer-scale striped patterns at aqueous interfaces of graphite, graphene, and hexagonal boron nitride [1], which have been observed by multiple groups and controversially attributed to various causes, including dense layers of atmospheric nitrogen or self-assembled organic contaminants. Our simulations show that nitrogen does not exhibit behavior that would explain the observed stripes, but that typical concentrations of heavy alkanes in air (on the order of micrograms per cubic meter) are sufficient for spontaneous formation of ordered monolayers that cover the graphene–water interface (Figure A, below) [2]. Furthermore, our simulations demonstrate that solvation layers of different compositions should lead to distinct characteristic lengths of oscillations in force–distance curves measured by AFM: ≈0.3 nm for water, ≈0.35 nm for aromatics, and 0.44–0.50 nm for alkanes. As shown in Figure B, these calculated force–distance curves agree well between simulation and AFM for graphite in alkane solvent and mica in water, while the situation for graphite (and other relatively hydrophobic materials) in water is more complex. The Garcia group recently measured changes in force–distance curve wavelengths from ≈0.3 nm to 0.44–0.50 nm for freshly cleaved graphite in water, which together with our simulation results, strongly suggests accumulation of alkane-like contaminants at this interface over the course of several minutes [3]. Finally, I will discuss recent computational results suggesting near-universal contamination of aqueous interfaces of hydrophobic materials by such heavy alkanes.



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Interfacial layering of hydrocarbons on pristine graphite surfaces immersed in water.

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Interfacial water participates in a wide range of phenomena involving graphite, graphite-like and 2D material interfaces. Recently, several high-spatial resolution experiments have questioned the existence of hydration layers on graphite, graphite-like and 2D material surfaces. Three dimensional AFM (3D AFM) is an advanced Atomic Force Microscopy technique which allows to image the organization of liquid molecules above a solid surface with sub-nm resolutions[1,2]. In this work, 3D AFM was applied to follow in real-time the evolution of graphite-water interfaces. Pristine graphite surfaces upon immersion in water showed the presence of several hydration layers separated by a distance of 0.3 nm, which is a value characteristic of hydration layers, close to the van der Waals diameter of a water molecule[3,4]. Those layers were short-lived. After several minutes, the interlayer distance increased to 0.45 nm. At longer immersion times (~50 min) we observed the formation of a third layer. An interlayer distance of 0.45 nm characterizes the layering of predominantly alkane-like hydrocarbons and it is comparable to the values that have been measured on graphite surfaces immersed in organic solvents such as hexane or pentadecane[4]. Molecular dynamics calculations supported the experimental observations. Free-energy considerations show that the replacement of water by alkanes is a spontaneous process[5].

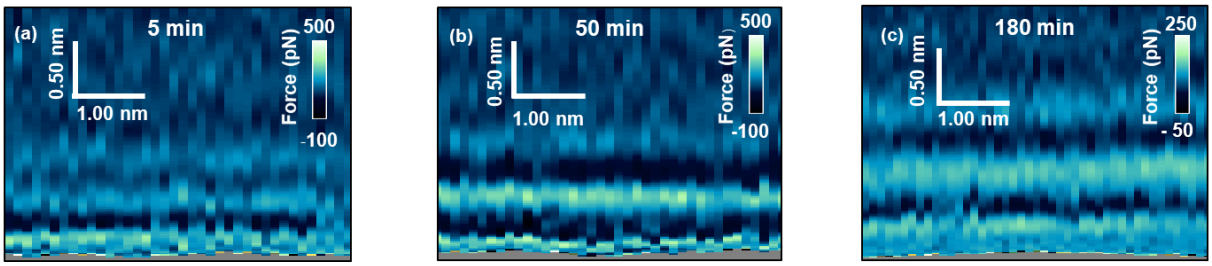

Figure 4. Time evolution of 2D force maps (x,z) of graphite-water interfaces extracted from 3D AFM volume images.

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Ion adsorption and Hydration Forces: a comparison of crystalline mica vs. amorphous silica surfaces

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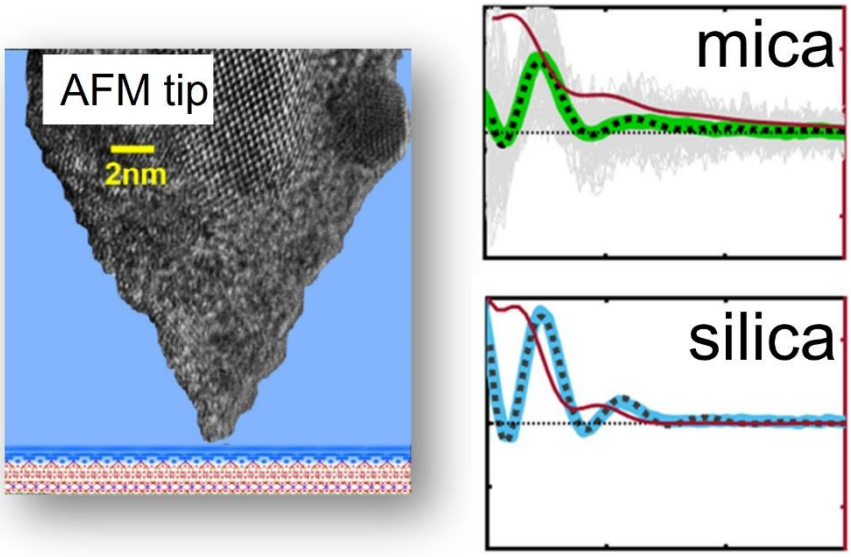
Hydration forces are ubiquitous in both nature and technology. Yet, the characterization of interfacial hydration structures and their dependence on the nature of the substrate and the presence of ions have remained challenging and controversial. We present a systematic study using dynamic Atomic Force Microscopy of hydration forces on mica surfaces and amorphous silica surfaces in aqueous electrolytes containing chloride salts of various alkali and earth alkaline cations of variable concentrations at pH values between 3 and 9. Our measurements with ultra-sharp AFM tips demonstrate the presence of both oscillatory and monotonically decaying hydration forces of very similar strength on both atomically smooth mica and amorphous silica surfaces with a roughness comparable to the size of a water molecule. The characteristic range of the forces is approximately 1nm, independent of the fluid composition. Force oscillations are consistent with the size of water molecules for all conditions investigated. Weakly hydrated Cs^+ ions are the only exception: they disrupt the oscillatory hydration structure and induce attractive monotonic hydration forces. On silica, force oscillations are also smeared out if the size of the AFM tip exceeds the characteristic lateral scale of the surface roughness. The observation of attractive monotonic hydration forces for asymmetric systems suggests opportunities to probe water polarization.



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ORAL PRESENTATIONS

3RD SYMPOSIUM ON CELL AND SOFT MATTER NANOMECHANICS

Building mechanically compliant responsive scaffolds for spinal cord injury

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Progress in the last decades is proving that mechanotransduction, understood as the capacity of living cells and tissues to feel and respond to mechanical forces, have pivotal implications in both physiology and pathology [1]. In our laboratory, we are working on the design of novel biomaterials able to more accurately mimic the mechanical properties of soft neural tissues for neural regeneration at the injured spinal cord. By using strategies integrated into the recently defined field of regenerative rehabilitation [2,3], we are specifically exploring the regenerative ability of reduced graphene oxide (rGO) scaffolds and natural magnetic hydrogels to support pivotal features of neural repair. 3D randomly porous rGO foams with Young's modulus values around 1 kPa, in close mechanical compliance with native spinal tissues [4], have proved positive regenerative outcomes when chronically combined with treadmill training routines in chronically hemisected rats. More recently, we have also immersed into the examination of magnetically responsive collagen hydrogels containing iron oxide nanoparticles as promising 3D scaffolds to support neural repair. Promising results with primary neural cells *in vitro* anticipate an enormous potential of these natural hydrogels for regenerative rehabilitation strategies in neural tissue engineering approaches.

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Titin Advanced Glycation End Products Induce Cardiomyocyte Stiffening

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Protein glycation is a hallmark of diseases like diabetes and aging. A common feature of these conditions is cardiac tissue stiffening, partly by accumulation of extracellular crosslinking advanced glycation end products (AGEs). However, modification of extracellular proteins fails to explain why cardiomyocytes themselves become stiffer. Here, we demonstrate that methylglyoxal, a major contributor to protein glycation, induces cardiomyocyte stiffening (**Figure 1**). Based on the observation that titin is glycated in aged myocardium, we have examined the mechanistic link between titin glycation and cardiomyocyte stiffening using high-resolution, single-molecule protein nanomechanics profiling by atomic force microscopy (AFM). Our single-molecule data show that methylglyoxal induces substantial formation of intramolecular crosslinks in titin domains, which become remarkably stiffer as a result of reduced contour length, and, unexpectedly, enhanced mechanical folding. We speculate that intramolecular crosslinks in intracellular proteins with mechanical roles can contribute to altered mechanical properties of tissues beyond the myocardium.

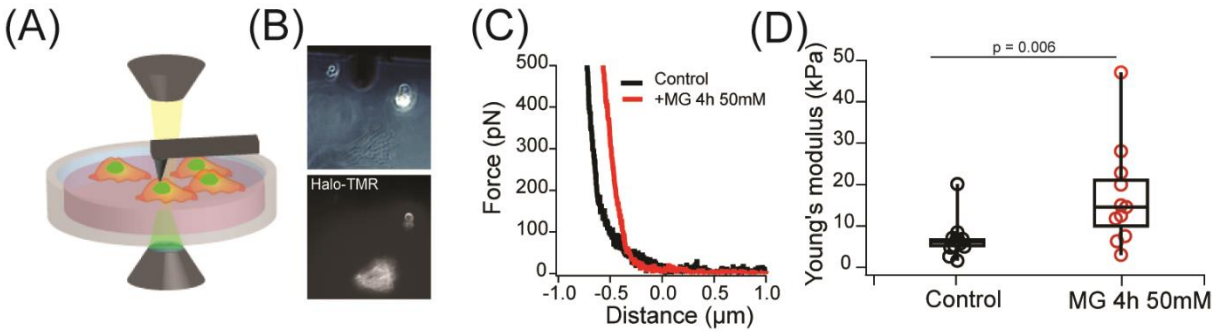

Figure 1. Methylglyoxal treatment induces cardiomyocyte stiffening. (A) Schematic cartoon of the experimental setup displaying a combined AFM-epifluorescence microscope. **(B)** Bright-field image of a skinned neonatal cardiomyocyte adhered on Matrigel (upper panel) and the corresponding fluorescence image showing titin labelled with Halo-TMR (bottom panel). **(C)** Examples of the approach segment of force-distance curves recorded on control (black trace) and skinned cardiomyocytes incubated with 50 mM MG for 4h at 37°C (red trace). **(D)** Young's modulus values of control and MG-treated skinned cardiomyocytes. Each dot represents the median value obtained for a single cell. Horizontal lines represent median values. Statistical significance calculated using the two sample Mann-Whitney U test.

Using the fast-QI mode to dynamically map the nanoscale topography and stiffness of lamellipodia and lamella

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Cell migration affects cellular function and can have detrimental effects in many pathologies such as cancer metastasis. To directly probe and quantify the nanoscale dynamics of the structure and mechanics of living cells is a challenging task. At the leading cell edge, the lamellipodium and the lamellum are flat actin modules that interact to drive cell migration [1]. The lamellipodium projects itself from the lamellum and exhibits rapid changes on the timescale of seconds, hence measuring its stiffness has remained difficult. Here we describe the fast-quantitative imaging (fast-QI) mode, and we show that fast-QI is able to map both the lamellipodium and the lamellum at the same time (Figure 1), and with increased spatiotemporal resolution compared to the classic quantitative imaging™(QI) mode [2]. Especially, we demonstrate that, at the leading edge, the lamellipodium is both slightly thinner and much softer than the lamellum. Moreover, we demonstrate that the fast-QI mode produces accurate maps of the height and of the apparent Young's modulus, through simple and efficient processing of the force-distance curves (Figure 2). The lamellipodium is a mechanosensing machine that can sense substrate stiffness, through the regulation of focal adhesion dynamics by both substrate stiffness and membrane tension [3]. Therefore, our results highlight the potential of the fast-QI mode to study the role that the nanoscale structure and stiffness of motile cell modules might play in mechanosensing.

Figure 1:

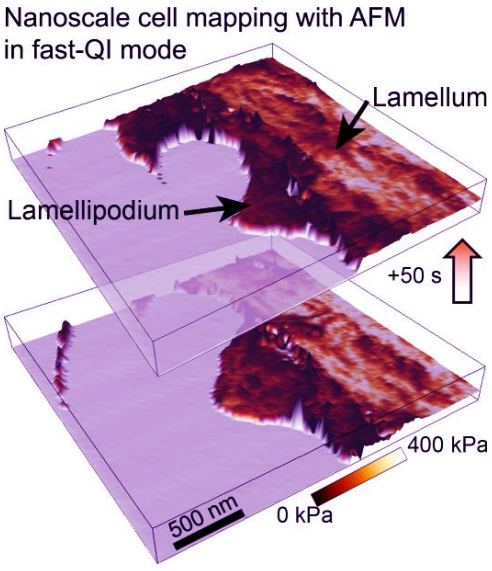
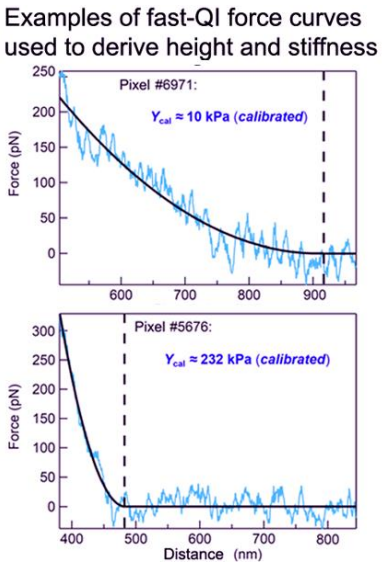


Figure 2:



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Structural and Viscoelastic Properties Profiling to Elucidate Glycocalyx Composition and Function in Pancreatic Cancer Cells

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Cell surface viscoelastic properties, often pertaining to cell shape and chemistry, have been investigated *in vitro* as putative biomarkers of disease. However, cells are highly heterogeneous across their individual topographies and subcellular components, especially when compared to one another. Atomic Force Microscopy (AFM) mapping methods can visualize these heterogeneities in a multi-dimensional (spatiotemporal) context, however processing such large datasets using a time-dependent viscoelastic approach represents a significant data science problem. Here, we introduce a method that leverages recent advancements in viscoelastic analysis via discrete modified Fourier transform (Z-transform). Our approach allows for the viscoelastic inversion of high-resolution spatiotemporal data at rates which are orders of magnitude faster (more than 1000 times) than optimizing a traditional rheological model for each pixel. In addition, the method utilized model-free viscoelastic quantities, such as the material retardance and relaxance. Our nanoscale multi-timescale viscoelastic measurements revealed that 2D adherent human pancreatic cancer cells exhibit reduction in elastic storage modulus and viscous loss modulus compared to healthy counterpart pancreatic ductal epithelial cells. Moreover, we observed a progressive reduction in both storage and loss moduli with metastatic progression - a hallmark of cancer metastasis. Then, we investigated the biophysical effects of glycocalyx architectural modulation in pancreatic cancer cells. We observed distinct architectural remodeling in the glycocalyx with perturbations of hyaluronic acid (HA), sialic acid (SA), mucins, and N-glycans via enzymatic digestive treatments and by addition of aggrecan. Interestingly, removal of SA and N-glycans significantly soften and increase the fluidity of the glycocalyx, whereas removal of HA and mucins appears to soften and fluidize some cell lines but not others. Together, our results suggest a fundamental role for the glycocalyx of pancreatic cancer cells in regulating the extracellular surface architecture, material properties, composition, and function that promotes tumor progression and metastasis.

Atomic force microscopy and fluidic force microscopy to probe cell mechanics in response to physical cues

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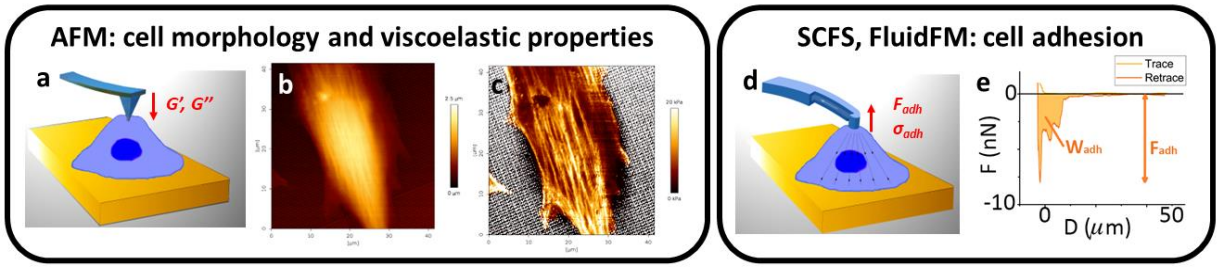

Figure 5 – Force spectroscopy imaging (a) and fluidic force microscopy (d) techniques for the characterization of the morphology (b), elastic modulus (c), and adhesion (e) of live cells.

Understanding the causal relationships between cell mechanics and biological function is emerging as an essential need in the biomaterials field. Using physical cues, *e.g.*, surface topography and mechanical properties, to tune the functional cell behavior is a potentially powerful strategy for designing novel cell instructive medical implants. [1] However, the cell's mechanical response to physical stimuli has been poorly studied, and the mechanotransduction mechanisms that elicit the cell's biological function are still largely unknown, limiting the rational design of such cell instructive implants.

In this work, we present two relevant case studies using atomic force microscopy (AFM) and fluidic force microscopy (FluidFM) techniques to quantify the mechanical parameters of live cells interacting with biomaterials with different physical cues, intending to elucidate the mechanical mechanisms governing the cell response to the physical environment. The first case study is on the influence of topography on the mechanics and osteogenic differentiation of preosteoblasts. We characterized the morphology, the elastic modulus, the adhesion force, and the adhesion strength of the cells interacting with different topographies at different contact times (from 2 s to 24 h) by combining Quantitative Imaging (QI) mode, single-cell force spectroscopy (SCFS), and fluidFM. Our results showed a correlation between the osteogenic differentiation after 21 days and the cell biophysical properties detected at the early stage of cell-surface adhesion (from 2 s to 24 h) [1-2]. The second case study is on the influence of substrate stiffness on the mechanics and polarization of macrophages, innate immune cells that orchestrate the foreign body response and play a significant role in implants' outcomes. Here, we present our preliminary results using AFM to characterize the morphology and viscoelastic properties (loss and storage modulus) of human monocyte-derived macrophages responding to substrates with different stiffness. Our results show how AFM techniques can reveal mechanical insights into cell-materials interactions and link these to key cellular functions, enabling advancements in the rational design of cell-instructive biomaterials.

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Nanoscale quantification of the molecular mobility in fluid biomembranes with high-frequency AFM

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Biological membranes are self-assembled nanoscale structures that comprise a wide variety of lipids, proteins and other biomolecule. They form the barrier separating the inside from the outside of the cell and can dynamically adjust their shape and local composition in response to changes in the environment. This is partly achieved through nanoscale control of the molecular mobilities within the membrane, with diffusion being often non-Brownian [1]. Diffusion typically occurs freely over nanoscale regions [2], but the global dynamics is influenced by possible lipid nanodomains [3], locally crowded protein regions [4] and contact with cytoskeletal and external structures.

Tracking this complex and multi-level molecular dynamics is challenging, with experimental and theoretical techniques often at odds [1]. Part of the problem comes from the need to obtain local information that combines fast dynamics, high spatial resolution, and minimal interference or modification of the sample.

To tackle this issue, we develop a fast nano-actuator that can function in conjunction with a standard AFM [5] and enable highly localized rheological measurements of supported bio-membranes. The area probed is typically only a few square nanometers, but the oscillation frequency is fast enough to capture the local mobility of diffusing molecules in synthetic and native fluid membranes. The approach has the added advantage that it is compatible with high nanometer resolution imaging in solution, allows to pick any point of interest of the membrane for conducting measurements, and does not require any marker or membrane modification. We first benchmark the technique with measurements on model fluid bilayers (DOPC: 1,2-Dioleoyl-sn-glycero-3-phosphocholine) with increasing concentration of cholesterol. The results quantify the interplay between confinement, local relaxation timescales and lateral mobilities within the membrane. We then apply our nano-rheological measurements to native bovine lens membranes, distinguishing between lipid-rich fluid regions, protein-crowded regions as well as intermediate regions where isolated proteins self-assemble in clusters. The results show a clear dependence of the local molecular mobility on composition and nanoscale molecular arrangement.

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AFM automation allows high throughput nanomechanical characterization of vesicles

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Extracellular Vesicles (EVs) are unique, heterogeneous, lipid bilayer-based nanoparticles secreted by cells. Their subpopulations differ in size, charge, biogenesis and vesicle lamellarity. As a potential class of cell-free diagnostic and therapeutic vehicles, their physicochemical characterization, in particular their mechanical properties, is an issue of recent investigations [1], [2]. AFM presents itself as a striking technique for the characterization of these nanometric objects, which has previously been used in imaging [3] or force spectroscopy modes. The latter have used simple parameters such as linear stiffness, a more standardized Young's modulus to evaluate elasticity, thin-shell theory, or an ad hoc model based on a modification of the Calham-Helfrich theory[2].

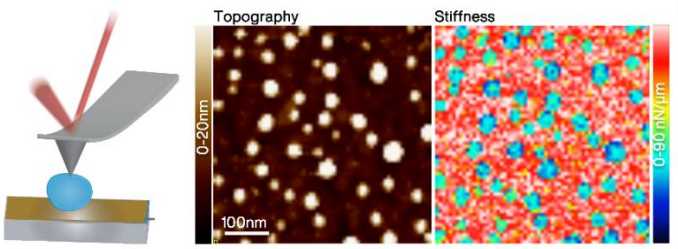

Fig. 1. Left) representation of the AFM nanomechanical mapping on vesicles adsorbed to the surface. Vesicles deform according to the affinity for the substrate. Middle) Topography image of DPPC:Chol (3:1, mol:mol) 50 nm extruded vesicles adsorbed on $Si0_2$ substrate in 150 mM KCl, 10 mM Tris, pH =7.4. Right) Its corresponding stiffness map.

Here, we adapted an automated procedure developed in-house for application to prokaryotic cells [4]. Low-resolution force maps were acquired and automatically processed to detect regions of interest (ROIs), where high-resolution maps were continuously collected until exhaustion of the defined ROIs. During the analysis, automatic masking was applied, from which pixels were selected for analysis using specific criteria for the vesicle. This approach allowed us to automatically map each vesicle, increasing the throughput of vesicle measurements to hundreds per preparation. The procedure includes data processing, which allows us to compare different existing mechanical models and derived parameters. As a proof-of-concept, we used synthetic vesicles, as shown in **Fig. 1**.

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Low-stress Young's Modulus Mapping with Scanning Ion-conductance Microscope for studying β-amyloid aggregate formation on living cell surfaces

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Alzheimer's disease (AD) is the most common form of dementia, a progressive neurological disorder characterized by short and long-term memory loss, including cognitive and functional impairment, which is refractory to current therapy. It is suggested that the aggregation of β-amyloid (Aβ) peptide on neuronal cell surface leads to various deviations of its vital function due to myriad pathways defined by internalization of calcium ions, apoptosis promotion, reduction of membrane potential, synaptic activity loss etc. These are associated with structural reorganizations and pathologies of the cell cytoskeleton mainly involving actin filaments and microtubules, and consequently – alterations of cell mechanical properties. Thus, the effect of amyloid oligomers on cells' Young's modulus has been observed in a variety of studies. However, the precise connection between the formation of amyloid aggregates on cell membranes and their effects on local mechanical properties of living cells is still unresolved. In this work, we have used correlative scanning ion-conductance microscopy (SICM) to study cell topography, Young's modulus mapping and confocal imaging of Aβ aggregates formation on living cell surfaces with subsequent assessment of the reactive oxygen species levels inside single cells using platinum nanoelectrodes. SICM for quantitative nanomechanical mapping (QNM) is based on intrinsic force interactions between nanopipettes and samples and has been previously suggested as a promising alternative to conventional techniques [1 – 5]. We showed that correlative SICM technique, in conjunction with topography mapping and confocal imaging, can be used for Patch-Clamp recordings from living cells with evidently formed FAM-labeled Aβ aggregates on its surface. As we demonstrated, SICM can be successfully applied to studying cytotoxicity mechanisms of Aβ aggregates on living cell surface.

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Viscoelastic properties of epithelia in 2D and 3D

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Viscoelastic properties of epithelial cells subject to shape changes were monitored by indentation-retraction/relaxation experiments. MDCK II cells cultured on extensible polydimethylsiloxane substrates were laterally stretched and, in response, displayed increased cortex contractility and loss of excess surface area. Thereby, the cells preserve their fluidity but inevitably become stiffer. We found similar behavior in demixed cell monolayers of ZO-1/2 double knock down (dKD) cells, cells exposed to different temperatures and after removal of cholesterol from the plasma membrane. Conversely, the mechanical response of single cells adhered onto differently sized patches displays no visible rheological change. Sacrificing excess surface area allows the cells to respond to mechanical challenges without losing their ability to flow. They gain a new degree of freedom that permits resolving the interdependence of fluidity on stiffness. MDCK cells also form multicellular cysts (acini or spheroids) comprising a closed monolayer of polarized cells that encloses liquid. The tissue tension of these cysts is typically assessed through Laplace's law by measuring the internal pressure. Here, we present force-relaxation experiments performed on MDCK II cysts and describe the response to external deformation by a theoretical framework accounting for possible superviscoelasticity of the spheroids. It was found that the cells provide excess tissue area by thinning of the cell monolayer to reduce tension upon deformation. This mechanism that also protects single cells from lysis is used on larger length scales as a universal mechanism to withstand external stress.

Viscoelastic relaxation of living cells with atomic force microscopy

Jeanlex Soares de Sousa

Universidade Federal do Ceará, Brazil

The viscoelasticity of living cells is emerging as an important biomarker to study their internal structure as a consequence of diseases, and their interaction with therapeutic drugs and the extracellular environment. The elasticity moduli of single cells obey a power-law relaxation function $E(t) \propto t^{-n}$, where the exponent *n* carries information about the fluidity of the cells and cytoskeleton organization. In this presentation, I will briefly present methods to characterize the viscoelasticity of cells with atomic force microscopy in both time and frequency domains, and present two study cases: (i) how the viscoelasticity of stomach cancer matches clinical observations, and (ii) how the rigidity of stiff polyacrylamide gels affects the viscoelasticity of L929 fibroblasts.

Viscoelastic and rheological AFM measurements on living cells

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Live cells are fascinating mechanical systems with complex viscoelastic properties, which are governed by active and passive mechanics of the cytoskeleton. However, the underlying biological processes and pathways and their role in health and disease are by far not completely understood. As the atomic force microscope (AFM) is a unique powerful tool for probing mechanical properties at the micro- and nanoscale, it is widely used to measure the viscoelastic properties of living cells *in vitro*. In my talk I will review recent advances in AFM-based methods for measuring viscoelastic properties in both the time and frequency domain with their respective benefits and challenges as well as different modelling approaches to quantify physical quantities from them. I will then present recent examples for viscoelastic AFM measurements on living cells and their combination with other techniques. For example, we found that cell stiffness and viscosity are widely distributed within live cells, but collapse onto a cell type-specific correlation curve, which is directly linked to active cellular prestress in the cell. This connection is, for example, affected in cancer cells resulting them to respond entirely differently to their cellular environment than normal cells. In summary, viscoelastic and rheological AFM techniques for measuring material properties of living cells provide new insights into the basics of cellular viscoelasticity and help to establish a comprehensive framework for the description of cell mechanics in health and disease.

Nanorheology and Nanoindentation Revealed a Softening and an Increased Viscous Fluidity of Adherent Mammalian Cells upon Increasing the Frequency

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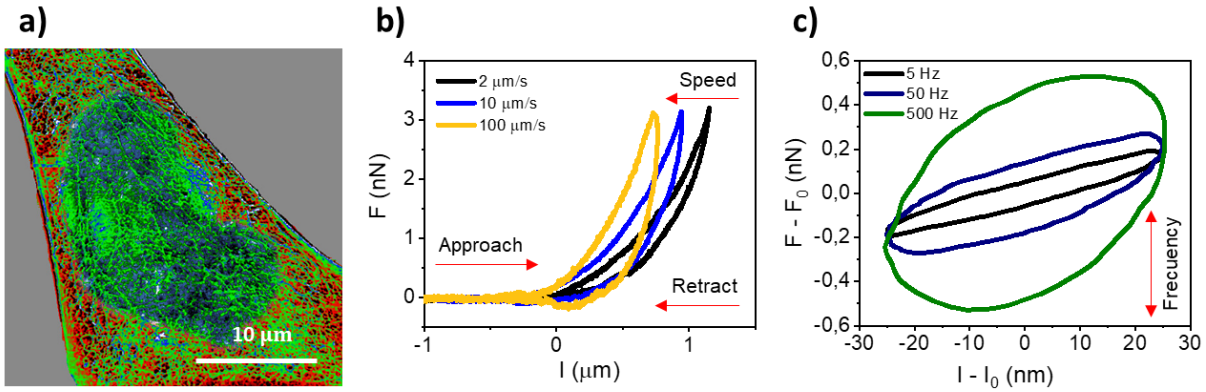
The mechanical response of a cell depends on the frequency or velocity at which the cell is probed [1,2]. The components of the cell which contribute to this property and their interplay are not well understood. Here, we integrated local deformation methods and theory[3,4] to develop a force microscopy approach to characterize frequency and velocity-dependent properties of living cells. We show that mammalian cells soften and fluidize upon increasing the frequency or velocity of the deformation. This behavior was independent of the method applied to deform the cell and the indentation values (25 or 1000 nm). At low frequencies (2-10 Hz) or velocities (1-10 μm/s), the response was dominated by the mechanical properties of the cell surface. At higher frequencies (> 10 Hz) or velocities (> 10 μm/s), the response became dominated by the hydrodynamic drag of the cytosol. Softening and fluidization did not involve any structural remodeling. It reflected a redistribution of the applied stress between the solid and liquid-like elements of the cell response as the frequency or the velocity was changed.



a) AFM nanomechanical map. b) Force-distance curve (nanoindentation) on a HeLa cell at different velocities. c) Oscillatory force-distance curves (nanorheology) on a HeLa cell at different frequencies.

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Deciphering the role of glycans as attachment factors in viral infection using AFM

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During the last three decades, a series of key technological improvements turned atomic force microscopy (AFM) into a nanoscopic laboratory to directly observe and chemically characterize molecular and cellular biological systems under physiological conditions. I will present the key technological improvements that enable us to apply AFM as analytical laboratory to observe and quantify living biological systems at the nanoscale. I will report the use of advanced FD-based technology combined with chemically functionalized tips to probe the localization and interactions of chemical and biological sites on single native proteins and on living cells at high-resolution. I will present how an atomic force and confocal microscopy set-up allows the surface receptor landscape of cells to be imaged and the virus binding events within the first millisecond of contact with the cell to be mapped at high resolution (<50 nm). I will also highlight theoretical approaches to contour the free-energy landscape of early binding events between virus and cell surface receptors.

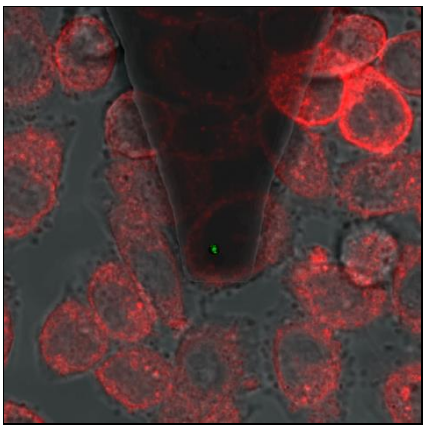

Figure. Combination of AFM and fluorescence microscopy image showing an AFM tip functionalized with a single virus while mapping virus binding sites on living mammalian cells

Key publications:

- R. Natividade et al., PNAS (2023)
- M. Koehler et al. , Nat. Commun. 10 (2019) 4460
- M. Delguste et al., Sci. Adv. 4 (2018) eaat1273
- R. Newton et al., Nat. Protoc. 11 (2017) 2275-2292
- D. Alsteens et al. Nat. Nanotechnol. 12 (2017) 177-183

When a molecular tug-of-war goes wrong: uncovering the biological consequences of titin mechanical loss of function

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The focus of my laboratory is to understand how mechanical proteins generate and sense mechanical forces in the context of a living cell. One of our main interests is the giant protein titin, a fundamental component of sarcomeres, the contractile units of skeletal and cardiac muscle cells. In sarcomeres, titin is subject to end-to-end pulling forces and, as a consequence, is a major determinant of the passive mechanical properties of (cardiac) muscle tissue. Over the years, single-molecule force spectroscopy methods have provided a wealth of information on how titin mechanics is built and modulated by the environment, posttranslational modifications and mutations. These biophysical results have generated hypotheses on how titin mechanics integrates with other important aspects of muscle physiology, for instance to provide much needed mechanosensing. In my presentation, I will introduce a new strategy to manipulate titin mechanics in living cells and animals, which we are currently exploiting to understand how titin mechanics determines myocyte function in the heart and in skeletal muscle. Our results indicate that loss of titin mechanical integrity in the heart triggers unexpected fibrosis at the level of the extracellular matrix, while in skeletal muscle, a regenerative response is induced. Our strategy, which we have termed mechanical loss-of-function, can be applied to other mechanical proteins to shed light on how mechanical signals are translated into metabolic and biochemical words, the language of the cell.

[1] Alegre-Cebollada J (2021). Protein nanomechanics in biological context. Biophysical reviews *13*, 435-454. https://doi.org/10.1007/s12551-021-00822-9

POSTERS

Comparative Study of Image Contrast in Contact Mode Scanning Probe Microscopy

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True sample topography cannot be obtained when imaging with scanning probe microscopy (SPM) without considering the effects of scan angle and the cantilever orientation relative to the sample topography. Here, we investigate the image quality for different scan angles and cantilever orientations and quantify it using the contrast metric given by Forchheimer et al. The topography of a compact disc (CD) and a digital versatile disc (DVD) is known and standard, and hence they have been employed to compare images obtained using different scan parameters. It is seen that the highest value of the metric was obtained when both the cantilever's longitudinal axis and the fast scan direction were normal to the tracks of the CD or DVD. The lowest value of the metric was obtained when the cantilever's longitudinal axis was normal to the tracks, and the fast scan direction was along the tracks. The image with the highest metric is closest to the true sample topography, and therefore for general samples, scanning must be performed repeatedly at varying scan parameters until a high metric is obtained.

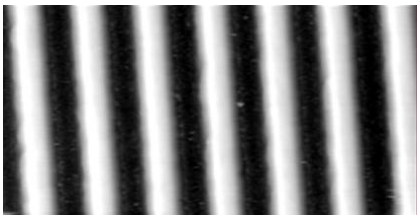

Fig. 1: Tracks on the DVD sample. The image was taken when both the cantilever's longitudinal axis and fast scan direction were normal to the tracks.

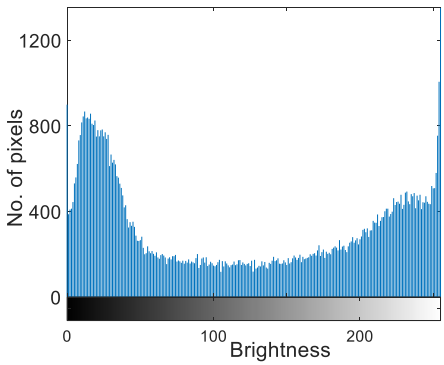

Fig. 2: Histogram of the imaged DVD sample which is a mixture of two normal distributions.

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Characterization of the mechanical properties of individual bacteria in air by multimode tracking of squared nanomechanical resonators

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Nanomechanical resonators have emerged as exceptional tools in biosensing, finding a variety of applications in biomedicine and clinical diagnosis. Remarkably, researchers have demonstrated the characterization of the mass and mechanical properties of individual human cells in liquid environment, as well as single bacteria, viruses and proteins in vacuum [1].
In this work we demonstrate the characterization of the mass and mechanical properties single bacteria cells in ambient conditions. As sensors, we use squared shaped nanomechanical resonators (40 µm length and width, 100 nm thickness). Two distinct bacteria kind (*Staphylococcus Epidermidis and Escherichia Coli*) are deposited individually at specific positions of the sensor with micrometric precision, using an injection micropippette system. Before and after the adsorption of the analyte, we simultaneously monitor the resonance frequencies of multiple mechanical modes of the sensor, in particular, the 1st and 2nd flexural modes, and the 1st and 2nd torsional modes (Figure 1). Monitoring multiple modes enables disentangle the mass and stiffness effects induced by the analyte, as well as accessing information about its morphology [2]. Finally, we probe that this technique enables discerning in between the two bacteria kind, mainly thanks to accessing information about their stiffness and morphology.
In the near future we plan to extend this technique for liquid operation, the natural medium for biology, as well as to many other bacteria kind, and even other microbiological entities such as human cells and viruses. The developed technique will bring new knowledge about biological processes, strongly impacting the biomedicine and biophysics fields.

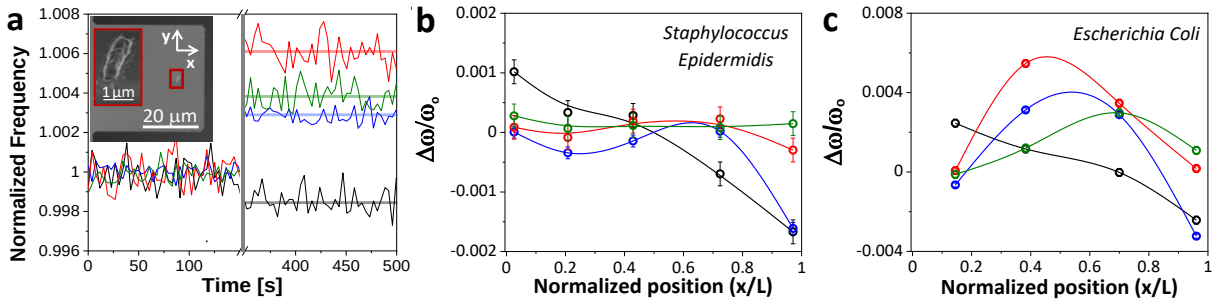

Figure 1.a. Normalized frequencies (ω/ω_o) of the sensor 1st flexural (black), 1st torsional (red), 2nd flexural (blue) and 2nd torsional (green) before and after the adsorption of an *Escherichia Coli* cell. Insets show scanning electron microscope images of our sensor with the adsorbed bacteria cell. Solid lines indicate the averaged normalized frequencies after the bacterium adsorption. **b-c.** Relative frequency shift of the mechanical modes of the sensors induced by the adsorption of individual bacteria cells, respectively, versus the normalized longitudinal position (x-axis). All the cells are adsorbed on the sensors central position respect to the y-axis. Solid lines are guides to the eye.

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Monitoring Membrane Insertion of SARS-CoV-2 Fusion Peptide by AFM

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A critical stage in the infection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the membrane fusion both of its envelope and the membranes of a host cell. Membrane fusion is initiated by the insertion of a fusion peptide (FP) on the SARS-CoV-2 spike (S) protein into the host membrane. A thorough understanding of the molecular mechanisms of membrane insertion of SARS-CoV-2 FP is therefore of high importance. To probe the molecular mechanisms of SARS-CoV-2 FP binding and insertion to the host membrane at the single-molecule level, we implemented the kinetics and thermodynamics analysis of the interaction of SARS-CoV-2 FP with the host membrane using biolayer interferometry and atomic force microscopy-based single-molecule force spectroscopy. We found that the cholesterol improved the binding and insertion of both SARS-CoV-2 FP and host membrane with significant affinity. This enhanced effect of cholesterol was verified through the TMPRSS2-cleavage S2 under physiologically relevant conditions. More importantly, we demonstrate that SARS-CoV-2 infectivity was significantly decreased after eliminating cholesterol from the host membrane based on the pseudovirus infection assay. Our findings shed light on the binding and insertion mechanisms of the cholesterol-promoted SARS-CoV-2 FP to the host membrane and may also have implications in the design of broadly effective vaccines and therapies against SARS-CoV-2.

Study of the local conductivity of LCO cathodes for Li-ion batteries by C-AFM

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Climate change and environmental degradation represent a global challenge that will mark future generations, leading to radical changes both at the economic and social level. The EU has set the highly ambitious target of reducing greenhouse gas emissions by 55 % by 2030 and becoming carbon neutral by 2050.[1] Batteries are a key element in the transition to a sustainable carbon-neutral future to supply power on a continuous basis from the storage of renewable energy obtained from sources, which are mostly intermittent, highly seasonal and of reduced mobility. Research on materials and components used in Li-ion batteries (LIBs) is crucial to reach the EU goal.

In this work, we study the local conductivity of epitaxial $LiCoO_2$ electrodes (a well-known Li-intercalation cathode) prepared by Pulsed Laser Deposition. The local conductivity is measured and mapped by conductive atomic force microscopy (C-AFM). Small changes in the magnitude and nature (from rectifying to ohmic) of the conductivity allow us to determine the local concentration of Li according to the dependency proposed by Milewska *et al.* [2] This characterization lets to correlate the concentration of Li with different morphological features (e.g., facets, grain size…) which in turn depend on the electrode preparation parameters. In summary, given that the cathodes are the source of Li in LIBs, this study seeks to optimize the Li content (capacity) in the LCO electrodes through the deposition parameters.

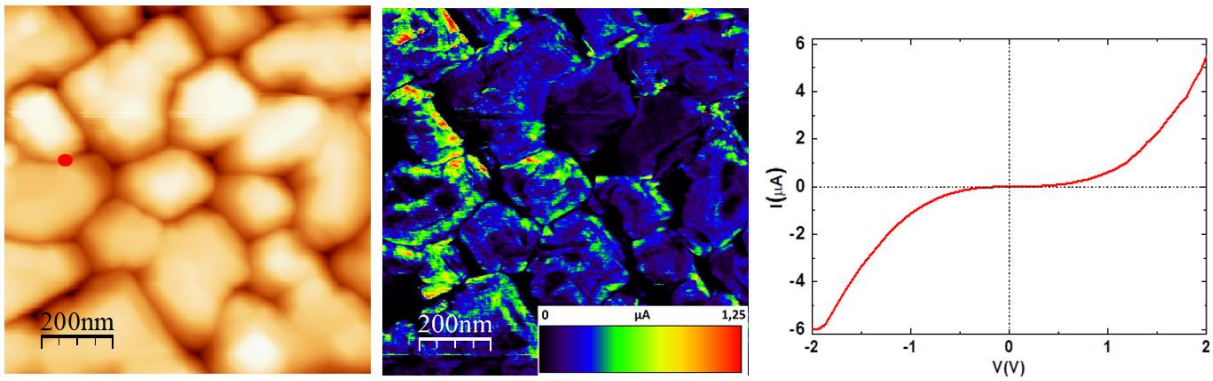

Figure: Topography (left) and current map (center) obtained by C-AFM in epitaxial (104) LCO/(100)SRO/(100)STO. IV curve (right) measured in the red dot on the topography image.

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Effect of functionalization and loading on the mechanical properties of soft polymeric nanoparticles

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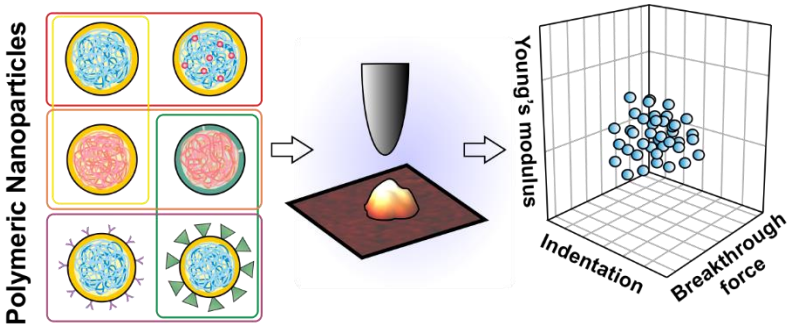
Drug and gene delivery systems based on polymeric nanoparticles offer a greater efficacy and a reduced toxicity compared to traditional formulations. Recent studies have evidenced that their internalization, biodistribution and efficacy can be affected, among other factors, by their mechanical properties. Here, we analyze by means of Atomic Force Microscopy force spectroscopy how composition, surface functionalization and loading affect the mechanics of nanoparticles. For this purpose, nanoparticles made of Poly(lactic-co-glycolic) (PLGA) and Ethyl cellulose (EC) with different functionalizations (dendrons, antibodies) and loading (fluorophores) were prepared by nano-emulsion templating using the Phase Inversion Composition method (PIC) to form the nano-emulsions. This process possesses the appropriate versatility to create NPs of similar sizes with different polymers, and surfactants, incorporating a fluorophore at the inner core or attaching surface functionalization.

The obtained results showed that composition, surface functionalization and loading affect the nanomechanical properties in a different way, thus requiring, in general, to consider the overall mechanical properties after the addition of a functionalization or loading. A graphical representation method has been proposed enabling to easily identify mechanically equivalent formulations, which is expected to be useful in the development of soft polymeric nanoparticles for pre-clinical and clinical use.

These results evidence the need to carefully consider these properties to optimize and enhance cell uptake or bioavailability before their preclinical and clinical use in addition to the commonly studied non-mechanical parameters such as the stability of the preparation or its binding efficiency.[1]



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Anomalous Stiffness Dependent Positive Shift in Natural Frequencies in a 2D-MoS_2 Coated Microcantilevers

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A microcantilever with a functional 2D material coating has shown an excellent future in biosensing and atomic force microscopy [1]. In general, adding materials to a cantilever reduces its natural frequency. However, it has been reported that along with the mass dependency, its stiffness plays a crucial role in determining the natural frequency of the modified cantilever [2,3]. Motivated by this finding, in our study, we have coated a cantilever with a controlled 2D-MoS_2 via a gas-phase transfer process and have monitored different natural eigenmodes as a function of laser position. Concerning the original cantilevers, the eigenmode frequencies of the coated-cantilever increase in all the laser spot positions (Figure 1). We correlated this anomalous positive frequency shift with the stiffness of the adsorbate MoS_2 layer. The increment in the frequency indicates that the stiffness effect is higher than the mass effect in our case. Further, we propose a better analytical model, and the results match nicely with the experimental findings implying the validity of the simulation. Finally, the combination of the experiment with the theoretical simulation provided insight into the origin of this anomaly and the cantilever dynamics.

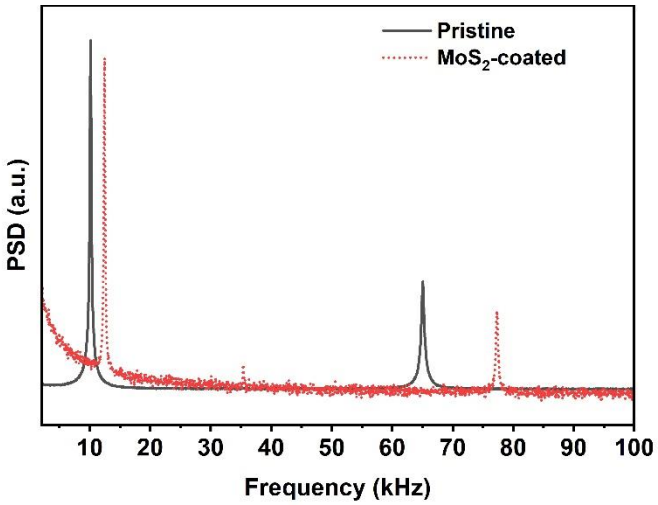

Figure 1. Thermal noise spectra of pristine and MoS_2- coated cantilevers.

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Studying the anisotropic in-plane nanomechanical properties of cellulose nanocrystals

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Cellulose nanocrystals (CNCs) exhibit remarkable mechanical and chemical properties, making them promising eco-friendly nanomaterials for a wide range of applications, including composites, biomedicine, electronics, energy, and packaging. These crystals have a rod-like shape, are composed of highly ordered cellulose molecules and exhibit an anisotropic nature. Understanding their anisotropic mechanical properties is crucial for determining how their structure and orientation affect their mechanical behavior in different directions. However, understanding the anisotropic mechanical behavior of CNCs at the nanoscale remains challenging. To address this issue, we adopted a bimodal approach based on the AMFlex-FMTor method to simultaneously excite flexural and torsional eigenmodes and to obtain out-of-plain as well as in-plane nanomechanical properties of the crystal structure [1]. We hypothesize that in-plane properties vary depending on the orientation of the crystal relative to the direction of applied shear stress. The first results show that when the crystals are aligned perpendicular to the direction of shearing, the structural arrangement and orientation of the CNCs become visible. We attribute the observed differences in shear stress to the variation in force between elementary nanocrystals and their intermolecular forces. The long-term goal would be to correlate these results with the mechanical properties of cellulosic fibres of our previous research [2].

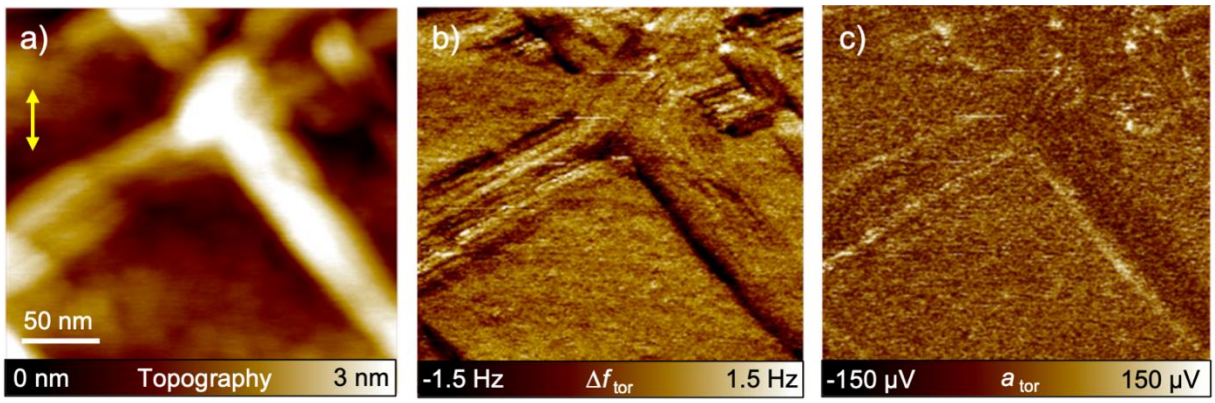

Fig. 1. Multifrequency AFM imaging of CNCs using the AMFlex-FMTor mode. a) Topography, b) torsional frequency shift and c) torsional drive amplitude images of two crystals crossing each other, thus representing different spatial orientations. The yellow arrow shows the direction of the shear motion of the AFM tip.

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Nanomechanical Mapping of Ultrathin Interfaces with Bimodal Atomic Force Microscopy

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Accurate nanoscale measurement of the mechanical properties of interfaces and thin layers is of great importance in materials science and biology. Bimodal AFM [1] is one of the most advanced nanoscale techniques for the measurement of the elastic modulus of interfaces. In nanometer-thin materials, the bottom effect [2-4], this is the influence of the rigid support, produces an overestimation up to a 10-fold factor during the determination of the nanomechanical properties. This makes semi-infinite models inappropriate to describe the mechanical response of thin samples such as lipids and proteins when the indentation is comparable to the thickness of the material. Here we develop a bottom-effect correction for bimodal AFM that measures the real Young's modulus value of thin films independent of its thickness [5-6].

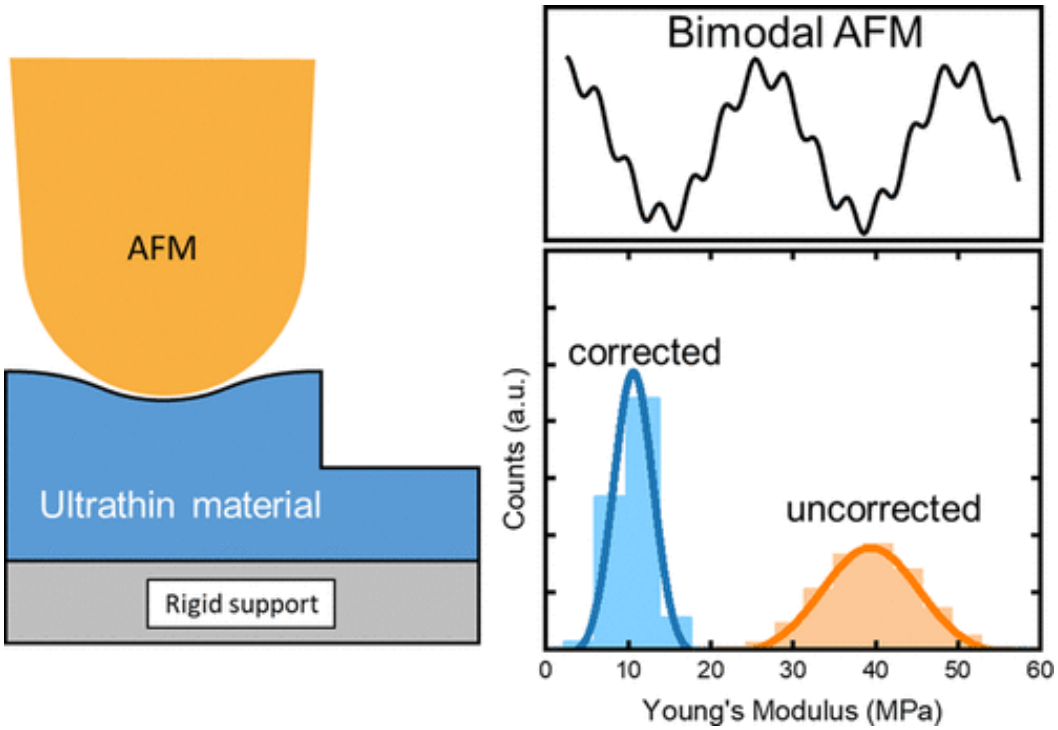

Figure - Scheme of a tip, ultrathin-layer and solid support interface. The bimodal AFM measurement of the nanomechanical properties of such a system yields an overestimation of the Young's modulus due to the bottom effect. With the bottom effect correction, the corrected value of the Young's modulus is measured.

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Revealing AC Cu-ion transport at the nanoscale in $CuInP_2S_6$ - $In_{4/3}$ P_2S_6 flakes during ferrielectric to paraelectric phase transition

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Van der Waals layered materials exhibiting robust room temperature ferroelectricity offer a versatile platform for miniaturization of ferroelectric device technology. Within this class of materials, $CuInP_2S_6$ has received a significant degree of interest due to the revelation of ionically mediated ferroelectric polarization switching pathways, that result in multistate ferroelectricity, ionic conduction, and complex interfacial chemistry. Moreover, it is possible to form stable self-assembled heterostructures of ferroelectric $CuInP_2S_6$ (CIPS) and non-ferroelectric (i.e., lacking Cu) $In_{4/3}$ P_2S_6 (IPS), by controlling the targeted composition and kinetics of synthesis. Besides, the properties of CIPS/IPS interfaces naturally forming by in-plane epitaxy have so far received little attention. In this work[1], we use an advanced scanning probe microscopy approach to explore in detail the nanoscale variability of the AC ion-transport dependent functional properties (electromechanical, dielectric, and conductive) in $CuInP_2S_6$ - $In_{4/3}$ P_2S_6 flakes during the ferrielectric-paraelectric transition. First, we show evidence of a kHz ionically mediated electromechanical response of $CuInP_2S_6$ in the paraelectric phase (above T_C). Second, we reveal the local dielectric constant and ionic conductivity changes across the CIPS-IPS heterostructure, imaging the dielectric peak of the ferroelectric phase transition at the nanoscale. Finally, we find an enhanced dielectric loss/ionic conductivity at the CIPS/IPS boundary that we assume it is caused by an enhanced Cu movement at the CIPS/IPS interface, which is supported by DFT calculations, indicating the possibility of engineering CIPS/IPS interfacial properties. Our results expose a new insight into polar properties of CIPS and position CIPS/IPS interfaces as new platform for tunning ferroelectric properties in van der Waals ferroelectrics.

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Nanomechanical Mapping of Thermally Evolved Cu-MOF Mediated Porous Copper Oxide Nanoparticles

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Recently, porous nanoparticles have garnered significant attention due to their high surface area, tunable pore size, and myriad active sites [1]. They are extensively utilized in diverse applications such as energy harvesting and storage, catalysis, drug delivery, etc. [2]. Among different synthesis methods, metal-organic framework (MOF) mediated porous nanomaterials synthesis is fast and cost-effective. Furthermore, the as-produced metal oxides are crystalline and stable [3]. Owing to this vast range of applications, exploring the mechanical properties of such nanomaterials is extremely important. In the present study, we examine and quantify the temperature-dependent evolution of a copper-based MOF into porous copper oxide (CuO) using bimodal Amplitude Modulation- Frequency Modulation (AM-FM) and bimodal Amplitude Modulation (AM-AM) atomic force microscopy techniques. The evolution of Young's modulus as a function of temperature is mapped (Fig.1) with the reported value of 6-10 GPa for the pristine Cu-MOF (Fig.1a) which matches with the literature values [4]. Furthermore, the transformation of MOF into porous copper oxide nanoparticles and their subsequent Young's modulus mapping (Fig. 1b and 1c) shows an enhancement in the elastic modulus. The nanomechanical and viscoelastic properties are then extracted from the AM-AM measurements and compared with the data obtained from the AM-FM studies.

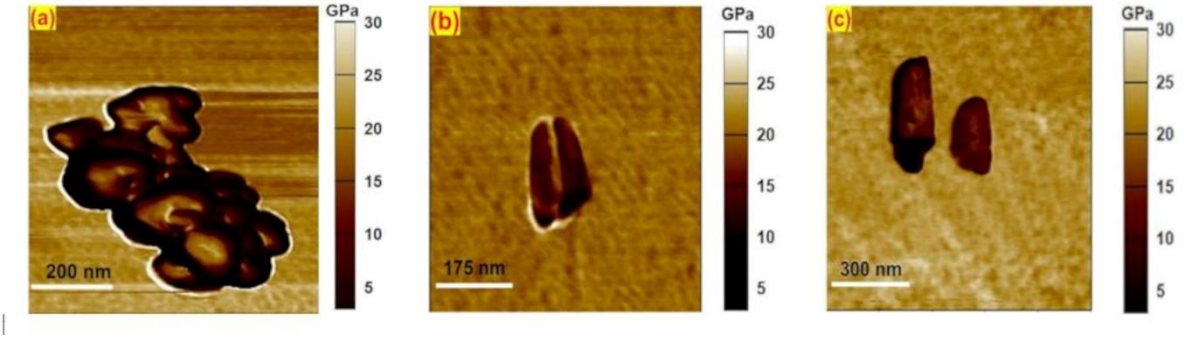

Figure 1. Young's modulus mapping of Cu-MOF (a) at room temperature, (b) at 300^0 C and (c) 500^0 C.

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Confocal Atomic Force Microscopy

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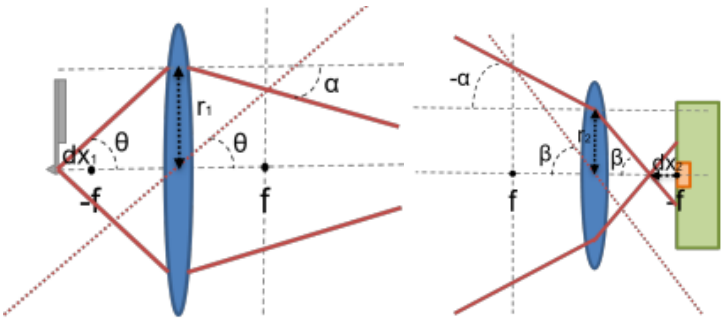
Atomic Force Microscopy (AFM) has revolutionized our ability to image and manipulate matter at the nanoscale, and study the mechanical and physical properties of materials and systems at the molecular level playing a crucial role in numerous scientific fields, including materials science, biophysics, and nanotechnology. Fast and accurate measurements of small displacements in the nanometer range of AFM cantilevers is essential for such applications.
Optical detection methods have emerged as powerful tools for measuring the displacements of AFM cantilevers with high sensitivity and resolution. To measure the deflection of AFM cantilevers, usually an optical beam deflection system is used. However, optical beam deflection systems have a limited detection bandwidth of ~10MHz, because of the weighted sum taken of a 4-quadrant photodetector [1]. Another way to detect these displacements is using an interferometer [2]. However, to accurately measure quasi-static displacements with an interferometer, the position of the reference mirror and the laser wavelength should be kept extremely stable. Therefore, this method is rarely used in AFM.
To overcome the limitations of optical beam deflection systems and interferometers, we propose to use confocal displacement sensing to detect the cantilever deflection in AFM (see Figure). This method is based on purely geometrical optics. Moreover, the deflection signal can be captured on a single photodetector, hence the speed of detection is set by the photodetector bandwidth. Since confocal displacement sensing only needs a single photodetector, its detection bandwidth theoretically can go up to the GHz regime. This shows the potential for using confocal displacement sensing in high-speed AFMs as well. In our work, we show and experimentally verify a model for the sensitivity of such a confocal system. Sensitivities similar to an optical beam deflection system or an interferometer can be reached [3]. Finally we use confocal displacement sensing in an AFM and show that it can be used to make a regular contact mode AFM image of a calibration grating.



Schematic of the working principle

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Exploring Focal Adhesion Dynamics in Living Cells with Nanoendoscopy-AFM

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Nanoendoscopy-AFM (Atomic Force Microscopy) has been developed as a non-invasive, label-free imaging technique for visualizing intracellular structures in living cells without disrupting their structural integrity. This technique involves the insertion of a long, ultrathin nanoprobe into living cells for intracellular imaging. It enables the visualization of a wide range of intracellular structures, including suspended actin stress fibers. Notably, our experiments demonstrate that the use of ultrathin nanoprobes for imaging purposes does not negatively impact cell viability [1]. In this study, our focus is on the examination of focal adhesions (FAs) in living cells, which are integral to the process of cell migration. FAs contain multiple adapter proteins such as paxillin, talin, and vinculin, which together create a dynamic nanostructure that mechanically links actin bundles to the extracellular matrix. While previous microscopic approaches have been used to study FAs, but there is still much to learn about how actin filaments bind to FA adapter proteins or the dynamic behavior of FAs in living cells. We have used 3D nanoendoscopy with sharp nanoprobes (Fig. 1a), to image FA ultrastructure directly within living cells, as confirmed by the confocal microscopy (Fig. 1b,c). Using this approach we are able to monitor FA growth over time, as well as the dynamic rearrangement of stress fibers connecting to individual FAs (Fig. 1d,e). Our results provide a new nanoscale look at FA ultrastructure inside living cells, and at the dynamic processes regulating stress fiber attachment to these structures. We are currently optimizing our measurement conditions to further enhance the spatial and temporal resolution of our images with the aim of obtaining additional insight into the dynamic molecular process regulating FA and stress fiber structure and function.

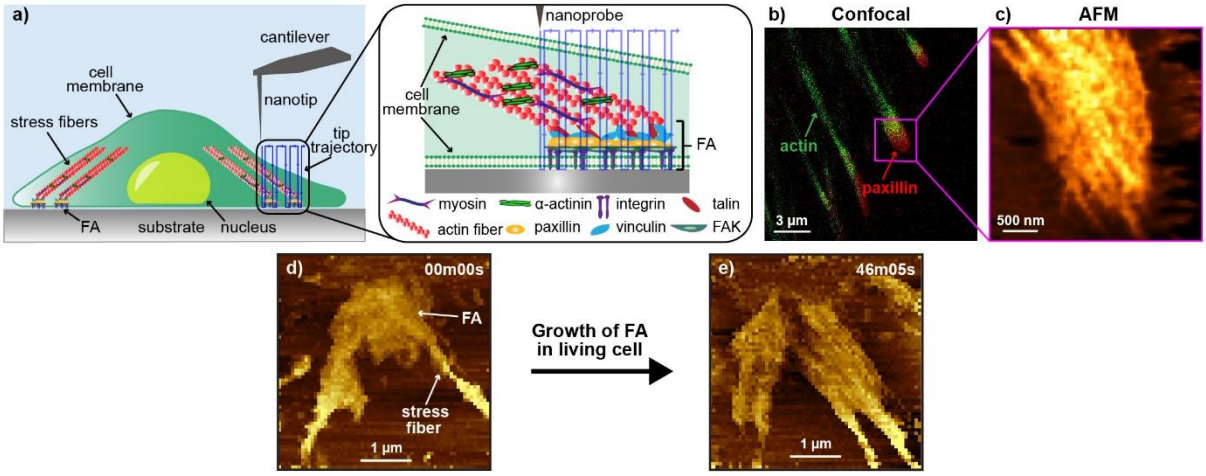

Fig. 1: (a) Schematic of 3D nanoendoscopy measurement of FAs. (b) Confocal image of actin stress fibers connected to FAs (paxillin and actin staining) and (c) a corresponding AFM image of an individual highlighted FA. (d) and (e) represent contact point mapping images of the same FA at 0 and 46 minutes of imaging, indicating the maturation of the FA.

Reference

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Molecular-Scale 3D-SFM Imaging of Ionic Liquid/Au Electrode Interface Structures and Its Tip/Sample Bias Dependence

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In recent years, electric double layer transistors (EDLTs) using ionic liquid (IL) as a gate insulator have received great attention[1]. EDLTs allow to accumulate charges with a much higher density than conventional field-effect transistors. Elucidation of the nanostructures of IL/solid interfaces is required to achieve better charge storage and carrier mobility. 3D scanning force microscopy (3D-SFM)[2] has the potential to satisfy these requirements because it allows direct observation of solid-liquid interfacial structures with subnanometer-scale resolution. Meanwhile, it has been reported that the tip charge may have a significant influence on the force distribution at the electrolyte/solid interface obtained by 3D-AFM[3] including 3D-SFM. Therefore, a 3D-SFM measurement method and an analysis method that takes this effect into account are needed.

In this study, to clarify the influence of the tip charge (potential) on the 3D-SFM images, we performed 3D-SFM measurements of the DEME-TFSI/Au(111) electrode interface structure using the setup shown in Fig. 1a. We controlled the V_s and the tip potential (V_t) while 3D-SFM imaging. As the result, we found that the layer contrast resulting from the electric double layer was clearly observed when V_t was positive (Fig. 1c) compared to when V_t was negative (Fig. 1b). The cause of this contrast was investigated by MD calculation, and we found that the layer contrast mainly reflects the distribution of large molecular weight $TFSI^-$. Furthermore, a stable TFSI- adsorption layer is formed on the tip surface when V_t is positive. The electrostatic repulsion/attraction between it and the TFSI-/DEME+ emphasises the layer contrast. This result clarifies the influence of the tip potential (charge) on the measurement of the IL interface structure

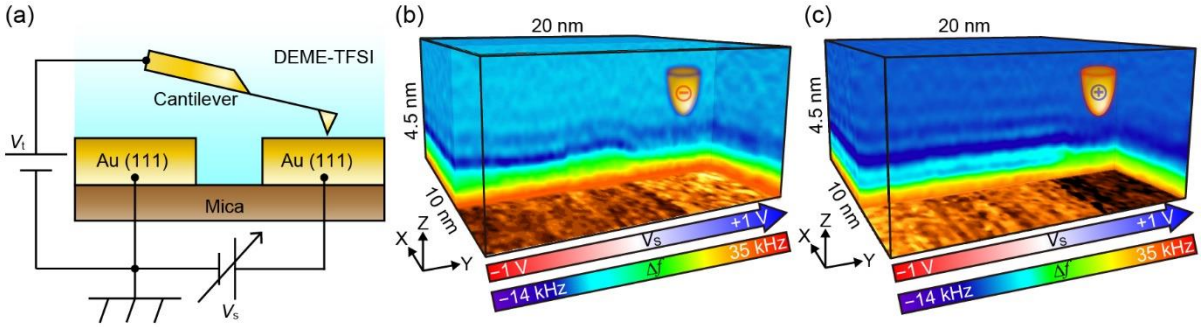

Fig.1: (a) Schematic of the measurement setup. (b, c) 3D-SFM images of DEME-TFSI /Au(111) interface with variable tip/electrode bias. Tip bias voltages were (b) −0.5 V and (c) +0.5 V. Sample bias was swept from −1 V to +1 V during the 3D-SFM imaging.

and the need to control it. In the future, this found should lead to more accurate IL research methods using 3D-AFM including 3D-SFM.

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Corrosion Mechanism of Aluminum Alloy Investigated by in-Liquid Nanoscale Potential Measurement Technique

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Corrosion have destroyed 3-4% of GNP[1]. To prevent this, we need to understand the corrosion mechanism. Generally, the origin of corrosion is considered to be a pair of oxidation reaction sites (anodes) and reduction reaction sites (cathodes), refer to as corrosion cells, which are known to be distributed at the nanoscale. However, in situ imaging of local corrosion cells has not been achieved, limiting the study of corrosion mechanisms. To solve this problem, we developed in-liquid potential measurement technique called open-loop electric potential microscopy (OL-EPM) (Fig. 1)[2]. Previously, OL-EPM has visualized local corrosion cells through the potential distribution.[3] In this study, we applied OL-EPM for the first time to analyze unknown corrosion mechanisms of Al alloys (Al-Zn-Mg and Al-Mg alloy) in pH 2.5 H_2SO_4 solution. Al alloys have a complex microstructure such as grain boundaries (GBs) and intermetallic particles (IMPs), leading to form the local corrosion cells. We directly observed corrosion behavior by OL-EPM (Fig. 2). In Al-Zn-Mg alloys, MgZn2 precipitates at the GBs have been considered to cause the corrosion at GBs. The topographic image obtained at 28 min after we started to replace pure water with H_2SO_4 solution showed the depression corresponding to the GB as indicated by the blue arrow (Fig. 2a(i)). The potential shows bright spots as indicated by the green arrows (Fig. 2a(i)). At 97 min, these spots disappeared (Fig. 2a(ii)). OL-EPM visualizes anode sites with relatively high potential. Thus, this disappearance of these spots suggests dissolution of the $MgZn_2$ precipitates. Meanwhile, we also observed corrosion behavior around Al-Fe IMPs in Al-Mg alloys (Fig. 2b). At 6 min, the Al-Fe IMP showed a higher potential than the matrix. At 40 min, the potential at the boundary between Al-Fe IMP and the matrix was significantly enhanced (green arrows in Fig. 2b(ii)). Meanwhile, the Al-Fe/matrix boundary was topographically depressed as indicated the green arrows in Fig. 2b(ii). These results suggest that the corrosion at the Al-Fe/matrix boundary is due to the anodic dissolution. These examples demonstrate that direct visualization of local corrosion cells by OL-EPM can help understand nanoscale corrosion mechanisms.

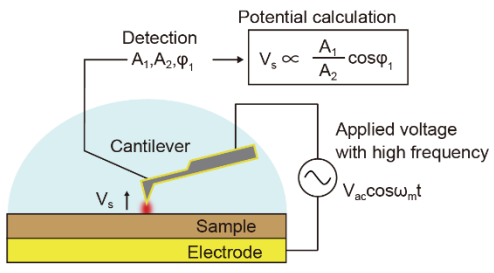

Figure 1: OL-EPM setup.

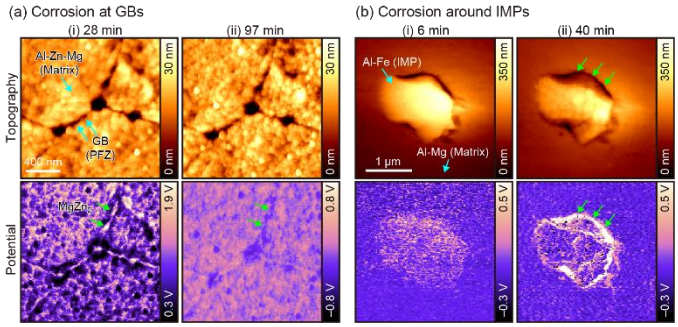

Figure 2: Topographic and potential images obtained by OL-EPM (a) at GBs and (b) around IMPs on the Al alloy surfaces.

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Mechanical properties of hygroscopic polymeric nanofibers through AM-AFM semi-empirical models

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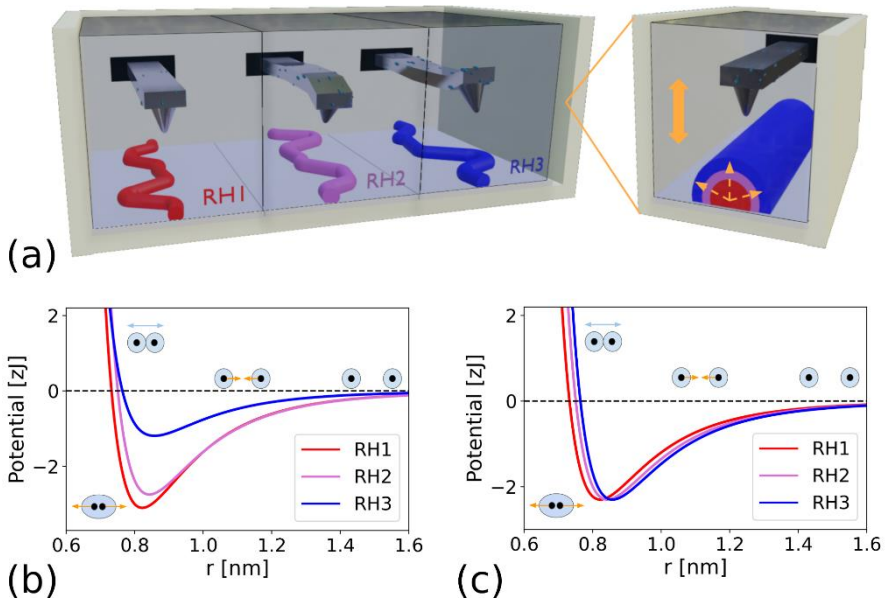
Nanomechanical properties of bio-compatible polymers play a very important role in the design of tissue scaffolds for implants and filtering devices. The mechanical and hygroscopic properties of those fibers have been studied mostly from a very coarse perspective reaching a micrometer scale. However, at the molecular (nanoscale) the mechanical response of polymeric fibers becomes more challenging due to both experimental and theoretical limitations. In particular, the environment-mediated mechanical response of polymeric fibers demand advanced models that consider sub-nanometric changes in the local structure of single polymer chains. Here, we combine Atomic Force Microscopy (AFM) experiments and numerical simulations to determine the elastic properties of the nanofibers as a function of relative humidity. We explore the effect of purely morphological changes, and also an ensemble of inter-chain interaction strength and morphological changes on the maximum force exerted from the AFM's Tip. Whereby, we report considerable differences spotted from a simple molecular model at a polymer chain level. In this manner, the interpretation of experiments can be performed by evaluating only a few observables, namely, the nanofiber's height, and the force-distance curves. Moreover, this semi-empirical approach provides the AFM experimental community with a feature that enables it to rapidly (on-the-fly) adjust operational parameters based on certain measurement criteria, like, the maximum exerted force, or the dissipated energy.



(a) Illustration of the PVA fibers at different relative humidities, namely, RH1=29.5, RH2=39.9 and RH3=81.5, (b) Model 1 considers topology and elasticity from the measurements AFM measurements to fit the molecular force field, (c) Model 2: employs only the topology of AM-AFM measurements. The polymer chains, sketched as beads inside the nanofiber, are shown in the inset (b) and (c) to illustrate attractive and repulsive regimes.

Contactless Sensitivity of Micro-Channeled Cantilevers Calibrated by Nanofluidics

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Keywords: Atomic Force Microscopy, Fluidic Force Microscopy, Contactless Calibration of Optical Lever Sensitivity, Nanofluidics

Atomic force microscopy (AFM) is a very powerful tool to image the surface topography but also to determine, in a direct manner, interaction and adhesion forces between various samples, such as surfaces, colloidal particles, liquid droplets, and living cells.[1][2]

For a quantitative evaluation of these forces, an accurate calibration of the cantilever spring constant and the (inverse) optical lever sensitivity (InvOLS) is essential.[3] The latter is normally determined by ramping the tip against a hard, non-deformable substrate and evaluation of the cantilever's response in the so-called constant compliant region.[4]

However, for soft samples, which are often encountered in biological applications, determining the optical sensitivity is impossible due to a lack of this constant compliance region. The Fluidic Force Microscopy (FluidFM) technique is based on micro-channeled cantilevers, allowing aspiration or injection at the cantilever's aperture. This technique is mostly used in biological or medical applications, often involving soft surfaces like hydrogels.[2]

Here, we present a new method that has been developed specifically for calibrating the InvOLS of cantilevers used for FluidFM. The principle is based on hydrodynamics and the thrust equation: When an overpressure is applied to the internal microfluidic channel, a defined mass of the fluid is ejected. The expulsion leads to a recoil force in the opposite direction based on Newton's 3rd law. This recoil effect was used to calibrate the InvOLS. The obtained values are in very good agreement with the classical calibration on a hard substrate, and the results agree with simulations by Finite Element Analysis via COMSOL.

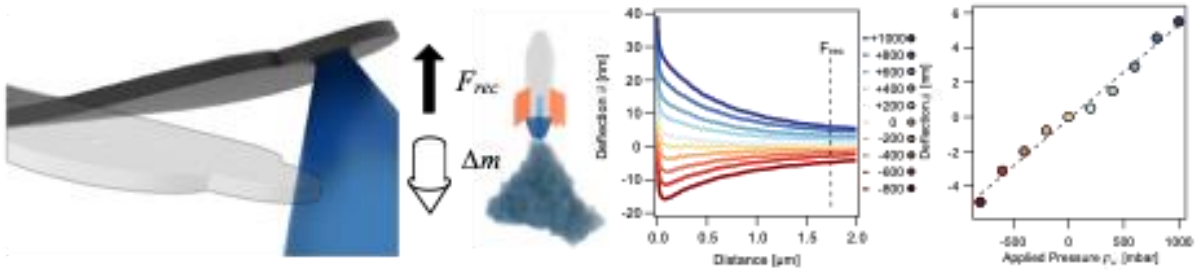

Figure 6 The principle of the hydrodynamic sensitivity, a cantilever is deflected by a recoil produced by ejecting masse from its aperture, similar to a classical rocket. From the linear dependency of the deflection on the applied pressure, the sensitivity is then calculated.

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Simultaneous quantification of stiffness and dispersion forces of materials with nanoscale precision

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Advances in nanotechnology rely on characterizing, with sub-nanometric precision, the properties of materials and functional interfaces [1]-[2]. Studying local variations in elasticity and dispersion forces is however challenging with existing methods which tend to be either time consuming and destructive or inaccurate as averaging over multiple locations [3]-[4]. Here, we present a non-invasive, fast and quantitative method based on frequency-modulation atomic force microscopy (AFM). The frequency shift of the AFM cantilever is used to reconstruct the effective Young's modulus and the Hamaker constant describing the material elasticity and the dispersion forces between the AFM probe and the substrate, respectively. Using the calculated Young's modulus and Hamaker constant, the proposed method further allows analytically reconstructing the interaction forces between the tip and the sample. The accuracy of the method is validated by simulations and experiments on a wide spectrum of soft and hard systems in both air and liquid, ranging from 2D materials including HBN, MoT_2, WSe_2 and graphite to polymers and supported lipid bilayers. The method offers a robust analytical approach to characterizing both rigid and compliant materials for a broad range of technological applications from artificial cartilages to rechargeable lithium-ion batteries.

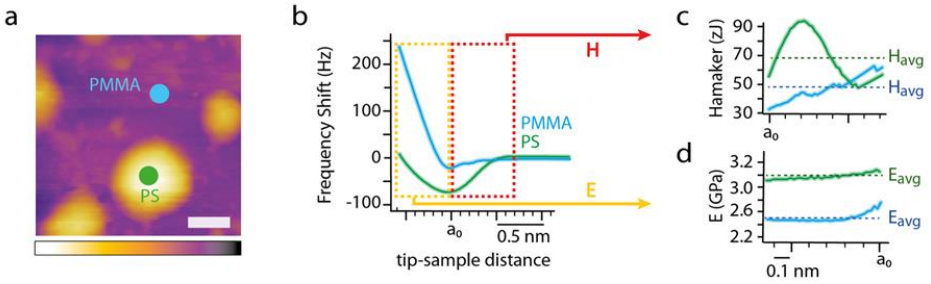

Fig. 1. Hamaker constant and Young's modulus derived from FM-AFM spectroscopy conducted at specific locations of a polymeric blend sample.

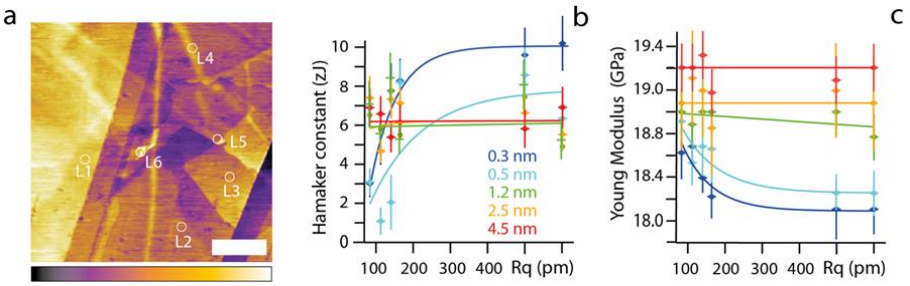

Fig. 2. Impact of nanoscale features on the effective Hamaker constant and Young's modulus at the HOPG-water interface.

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Anomalous underscreening in concentrated aqueous electrolytes: myth or reality?

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Concentrated electrolytes play an important role in many natural and technological settings ranging from saline aquifers to battery technology. While Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal interactions is not applicable in these situations, standard liquid state theory predicts a transition from the DLVO regime of monotonically decaying correlations and forces to a regime of oscillatory correlations when the Debye screening length becomes comparable to the ion size that decay within a few ionic diameters. In contrast to these expectations, recent measurements with surface forces apparatus report a re-entrant behavior with monotonically decaying electrostatic forces with a range that exceeds the Debye screening length up 100 times [1]. Similar trends for both for ionic liquids and aqueous electrolytes and qualitative support by colloidal stability measurements [2] suggest universal validity of the phenomenon. Yet, theory and simulations fail to reproduce the observations.

We report systematic Atomic Force Spectroscopy measurements for various alkali chloride solutions in a concentration range from 1 mM to 5 M at pH 6 and 9 and temperatures of 25°C and 45°C [3]. Experiments were carried out using flat substrates and submicrometer-sized colloidal probes both made of smooth oxidized silicon. While strong repulsive forces were observed for the smallest tip-sample separations, none of the conditions explored displayed any indication of anomalous long range electrostatic forces as reported for mica surfaces. Instead, forces are universally dominated by attractive van der Waals interactions at tip-sample separations beyond approximately 2nm for salt concentrations of 1M and higher. Complementary DFT calculations based on classical density functional theory for the primitive model support these experimental observations and display a consistent decrease in screening length with increasing ion concentration. Possible origins of the discrepancies will be discussed.

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3. **Absence of anomalous underscreening in highly concentrated aqueous electrolytes confined between smooth silica surfaces.** Kumar, S., P. Cats, M.B. Alotaibi, S.C. Ayirala, A.A. Yousef, R. van Roij, I. Siretanu, and F. Mugele, Journal of Colloid and Interface Science **622** (2022) 819-827.

Light-induced modulation of visco-elastic properties in azobenzene polymers

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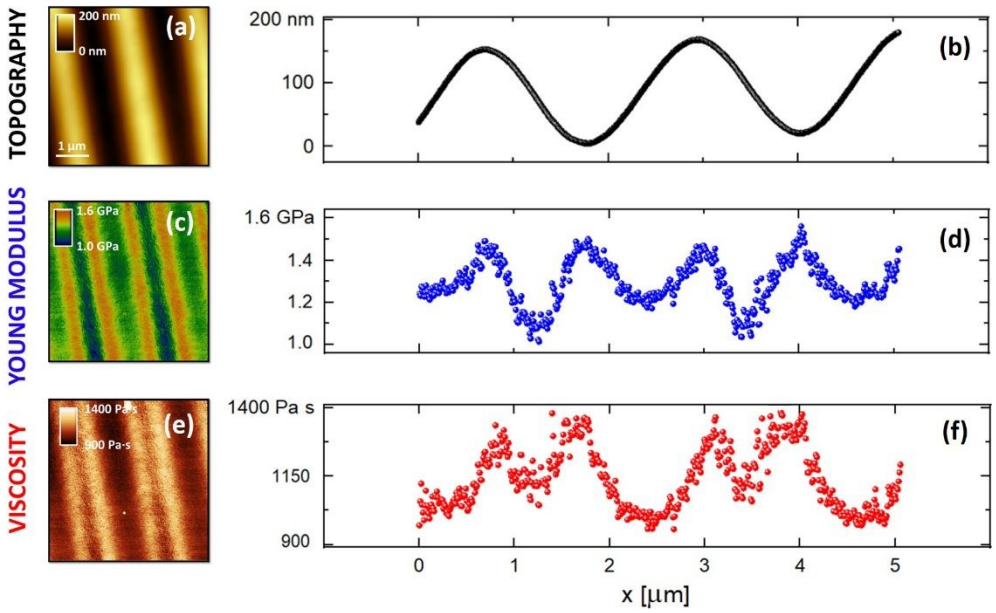
Photoinduced isomerization of azobenzene molecules can induce mass-migrations in azopolymers.[1] The resulting macroscopic material photo-deformation has found many applications in literature, however the precise mechanisms behind the mass-transfer is still under debate.[2] In this regard, azopolymer mechanical properties have been intensively studied, but the lack of a nanoscale technique capable of accurate and precise quantitative visco-elastic measurements has possibly hindered the field evolution. Here, we propose bimodal atomic force microscopy (AFM) as a powerful technique for full nanomechanical characterizations of azopolymers. With this multifrequency AFM approach, we are able to map in a fast, quantitative and non-destructive way, both elastic and viscous contributions of the azopolymer after photo-induced surface relief grating formation. Our findings address a light-induced spatial modulation of both the azopolymer Young modulus and shear viscosity which we explain through a model based on two main microscopic mechanisms: photo-softening and photo-alignment (or hole-burning effect). Due to a non-flat azopolymer morphology, proper care is taken to provide bimodal nanomechanical results free from topographical cross-talks and non-linearity effects.



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Towards a real-time imaging of the assembly and disassembly of collagen nanofibers:

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Collagen type I is the most abundant protein in mammals. It is constituted by molecules known as tropocollagens. Tropocollagen molecules are the building blocks to form collagen nanoribbons and microfibrils. Those structures have a periodic structure known as the D-band [1]. This contribution aims to image in real-time the aggregation of single tropocollagens into collagen microfibrils [2]. High-speed AFM (HS-AFM) was applied to imaging the collagen self-assembly with a spatial resolution of 10 nm and time resolution of 0.3 s. The formation of the microfibril is driven by electrostatic forces between charged aminoacid residues therefore a change in pH leads to the binding and unbinding of the microfibril [3]. By tuning the pH of the buffer solution, it was possible to image either the self-assembly of tropocollagens (pH > 7) or the disassembly of the collagen nanoribbons and microfibrils (pH < 7).

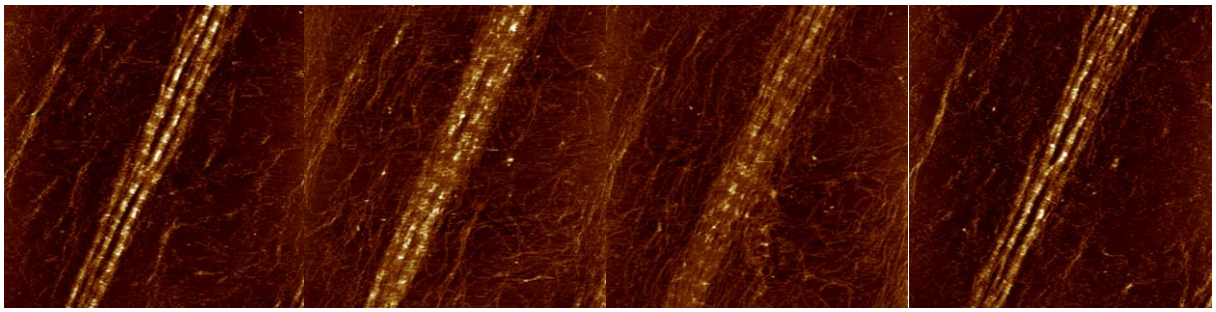

Figure 1. HS-AFM images of the reversible assembly and disassembly of collagen nanoribbons.

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Nanoendoscopy-AFM Measurements of Live Cells: Impact on Proliferation and Stress Response

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Nanoendoscopy AFM is an innovative technique that allows researchers to study the internal structure, dynamics, and mechanics of live cells. An ultrathin nanoprobe nanoendoscopy-AFM technique successfully investigated intracellular structures and mechanical properties [1]. However, the insertion of ultrathin probes into cells has the potential to damage the cell membrane, which can affect the cell's morphology, functionality, and viability. To investigate this issue, we conducted a study to evaluate the impact of nanoendoscopy AFM on live cells by examining their proliferation and intracellular Ca^{2+} response.

Our study involved performing 3D and 2D nanoendoscopic measurements on multiple cells while monitoring their proliferation under optical microscopy. We found that, within a reasonable timeframe, the cells were able to divide multiple times, indicating that nanoendoscopy AFM does not significantly impair cell proliferation.

In addition, we optimized the scanning conditions and investigated the cells' intracellular calcium ion response to the mechanical stresses generated by the nanoendoscopic measurements. Our results showed that the calcium ion response was more strongly associated with the size of the scan than the location of the scan. Specifically, we observed that cells showed a relatively low calcium ion response to scan sizes below 1.5 micrometers.

Overall, our study suggests that nanoendoscopy AFM measurements can be performed without inducing fatal damage to live cells, and they are compatible with cell proliferation. These findings represent an important step forward in understanding the impact of nanoendoscopy AFM on live cells and pave the way for further research in this area.

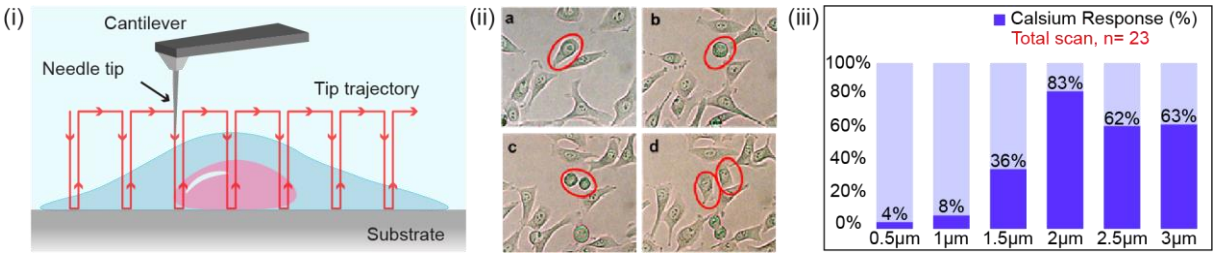

Figure 1: (a) Schematic of the setup used for 3D nanoendoscopy-AFM measurement of a live cell. (b) Cells undergoing division after 3D nanoendoscopy-AFM measurements. (c) Ca^{2+} response percentage at different scan sizes.

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In-plane and out-of-plane analysis of adsorbate formation, removal, and plasma-induced evolution of defects in multilayer graphene and graphite by multifrequency atomic force microscopy

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The preparation of adsorbate-free graphene and graphite with well-defined layer numbers is a current challenge in materials and surface science. One strategy to tailor the number of layers is oxygen-plasma treatment of few-layer graphene/graphite flakes. However, when graphitic materials are stored in air under ambient conditions, it is almost inevitable that airborne adsorbates deposit onto their surfaces. When precisely removing individual graphene layers from graphite and graphene flakes by oxygen-plasma treatment, the amount and type of adsorbates strongly affect the required plasma-treatment process and duration. To examine the removal/etching mechanism involved in removing such layers, we stored few-layer graphene/graphite flakes, with areas of different layer numbers, in ambient air and stepwise exposed them to oxygen plasma in a shielded configuration. The flakes were then successively analyzed by our developed multifrequency atomic force microscopy approach (AMFlex2-OLTor1-FMLat1) [1] in combination with Raman spectroscopy, focusing on the etching rate as well as adsorbate and defect evolution. Combined in-plane and out-of-plane tip-adsorbate-substrate interaction analysis facilitated discrimination of different types of adsorbates (water, polycyclic aromatic hydrocarbons, and linear alkanes) (see Figure 1) and their progression with time [2].

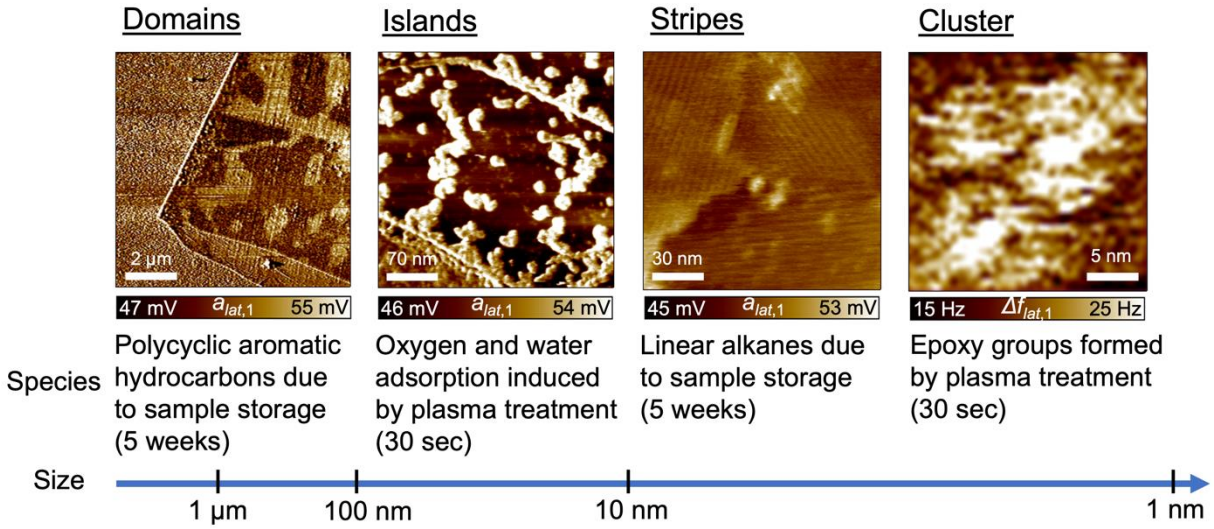

Figure 1: Different adsorbate structures visualized by multifrequency atomic force microscopy that were formed upon ambient storage and oxygen-plasma treatment of multilayer graphene. The three first images show the drive amplitude for the first lateral cantilever eigenmode to maintain the oscillation amplitude and the last one shows the lateral frequency shift of the first lateral eigenmode.

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Observation of Non-linear Elasticity of a Hygroscopic Biological Material using Atomic Force Microscopy

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Hygroscopic biological materials are a subclass of materials that exhibit a myriad of unique, water-responsive properties, however, there have been no established theories to describe the underlying mechanics of such materials. Recently our group proposed a simple model that predicts both equilibrium and non-equilibrium mechanical properties of hygroscopic biological materials[1]. Building on this work, here we conduct atomic force microscopy-based force-indentation experiments to study the elastic modulus of a regenerated cellulose film, cellophane. From the analysis of these force curves with the commonly used Hertz model, we observe trends that support strong non-linear elastic behavior at large depths of indentation. This approach can be used to further test the theory that predicts the unusual equilibrium and non-equilibrium mechanical properties of hygroscopic biological materials.

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A new modular polyprotein system compatible with single-molecule force spectroscopy by atomic force microscopy and magnetic tweezers.

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Single-molecule force spectroscopy (SMFS) has provided multiple advancements in the understanding of the free energy landscape of proteins under force[1]. Specific covalent tethering of proteins to surfaces has improved both the yield and the quality of data acquisition in Atomic Force Spectroscopy (AFS) [1,2] and Magnetic Tweezers (MT) [3]. Given the complementary information obtained by AFS and MT, we propose a modular polyprotein system with HaloTag specific covalent tethering to surfaces, in order to enable testing of the same protein preparation by both techniques. The modular design allows to easily include the proteins of interest in the system via SnoopTag/SnoopCatcher and SpyTag/SpyCatcher orthogonal spontaneous isopeptide bonding[4,5]. The system also includes elastin-like polypeptides to reduce non-specific interactions with the surface[5] and fingerprinting protein markers to ensure the identification of successful single molecule events that can exploit improved accuracy by concurrent experiments[6]. Our preliminary results show an increase in efficiency of 14 times for our double modular covalent strategy and of 5 times for our full modular covalent strategy in AFM experiments. The full modular covalent strategy also allows long-duration MT experiments at forces >60 pN. In summary, the proposed modular polyprotein strategy provide an accessible and versatile setup, easily adaptable to different proteins of interest, and includes recent improvements in sample preparation to increase the yield, quality and accuracy of SMFS experiments.

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Probing relative humidity-dependent stiffness of *Bacillus subtilis* spores

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Bacterial spores are dormant bodies containing genetic material that are resistant to high temperatures, radiation, low humidity and chemical agents[1]. Although the mechanisms underlying the fundamental aspects of resistance are not fully understood, bacterial spores have a great capacity to regulate water content and show water-responsive mechanical behavior. These properties confer spores a diverse range of applications such as high work density actuation[2], as stimuli-responsive materials and nanogenerators[3]. Bacterial spores are known to go through alterations in size and shape depending on the relative humidity. Moreover, their mechanical properties are heavily determined by the amount of water molecules confined in nanopores within their structures. In this work we describe the design of an atomic force microscopy-based experimental setup to monitor the gradual changes of contact stiffness of single *Bacillus subtilis* spores as a function of relative humidity (RH). This type of measurement can help test a recently developed theory[4] by our group in relation to the mechanics of hygroscopic materials. To this end, we make use of an automated feedback-based humidity control system coupled with force-distance curves acquisition. Using this set-up, performing a sweep from 3% RH to 90% RH while acquiring force-distance curves continuously over the course of about 10 minutes is feasible.

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Femtolitre Volume Blot-free Electron Cryo-Microscopy Sample Preparation Using Fluid Force Microscopy

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Transmission electron cryo-microscopy (cryo-EM) enables high-resolution imaging of biological structures with atomic resolution [1]. Although promising, the limitations of the current sample preparation methods are hindering the full potential usage of cryo-EM [2]. Damage to the native environment of the biological structure and the need for excessive sample volume are the primary roadblocks. To address these issues, we have developed a sample preparation method based on fluid force microscopy [3] for targeted subcellular objects. The samples are prepared using an automated process of stage movement, local climate control, cantilever force-feedback, aspiration, dispensing, and blot-free plunge freezing of the electron microscopy grid. A custom design cylindrical hollow probe tip (Fig 1A) capable of cell membrane penetration is used for single-cell biopsy. Using the setup, a record-breaking 25 femto-litre volume (Fig 1B) tobacco mosaic virus solution is dispensed on the grid. The sample is vitrified by plunge-freezing in liquid ethane (about -188^{o}C) without blotting. A high-resolution cryo-EM image of the virus obtained is shown in Fig 1C. Our next steps are to prepare single-cell biopsy samples for cryo-EM.

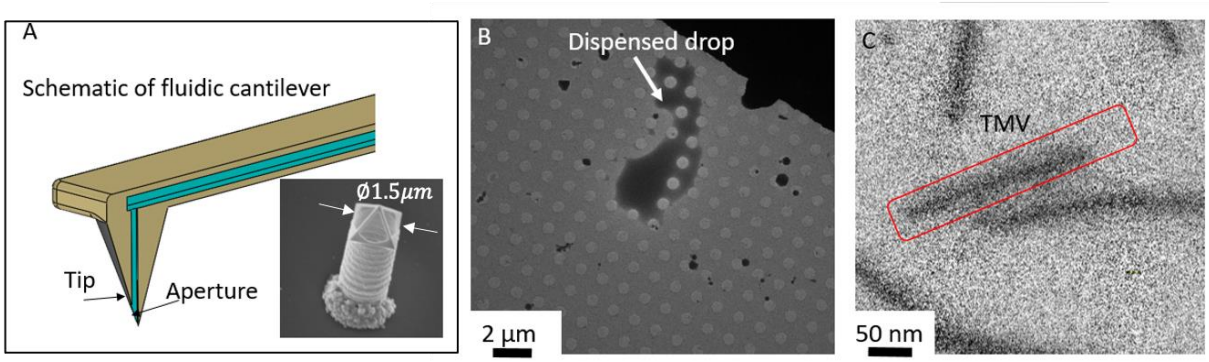

Figure 1: A blot-free cryo-microscopy sample preparation. (A) Schematic of the fluid-force microscopy probe. The inset shows a scanning electron microscopy image of the hollow cylindrical probe tip with a 1.5 μm diameter aperture. (B) cryo-EM image of vitrified 25 femtolitres of TMV solution on a quantifoil grid. (C) High-resolution cryo-EM image of the TMV virus from the dispensed sample.

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Inferring Hydration Structure Using Multi-Channel Analysis of FM-AFM Data

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It has been shown by multiple experiments that frequency-modulation atomic force microscopy (FM-AFM) may reflect molecular scale ordering of water molecules near surfaces and macromolecules. However, conversion of measured force maps into a detailed, coherent picture of the hydrogen bond network remains challenging. As a step in this direction, we present a novel approach, that combines height measurements ("z-channel") with measurements of the oscillatory driving force needed to maintain a constant oscillation amplitude ("excitation-channel"). Z-channel measurements yield the actual AFM tip height, tracing the hydration structure beneath the AFM cantilever apex. At the same time, the excitation channel data disclose the work done in removing water molecules in the volume occupied by the oscillating tip. Analysis of the correlation between the two channels (often, anti-correlation) discloses the exact position of removable water molecules at that location (Figure 1). Specifically, the bright spots seen in the excitation channel image (top right-hand panel) are found above cavities in the z-image (inset b), indicating the position of hydrating water molecules removed by the scanning tip. Furthermore, the asymmetric shape of bright excitation spots (marked by white lines in inset c) indicates the orientation of the adsorbed water molecules (see molecule drawing). The novel approach presented here lends itself to the extraction of three-dimensional hydration geometries from two-dimensional AFM scans that are typically characterized by higher resolution compared with 3d force maps.

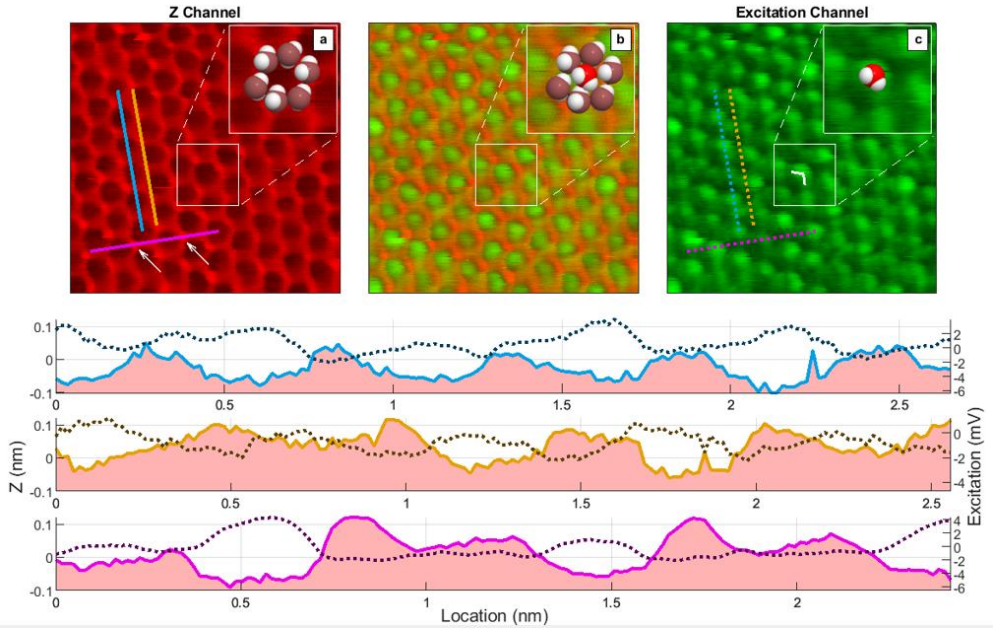
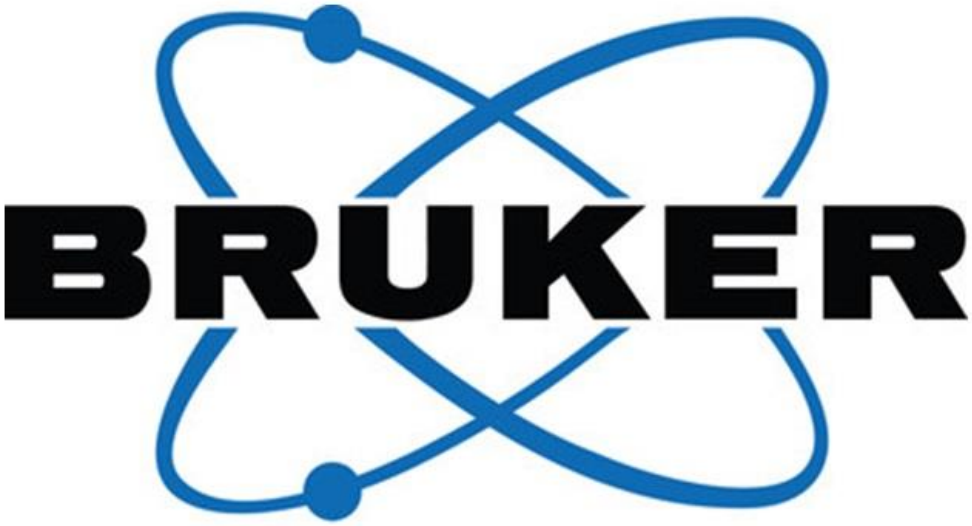


Figure 1: Extraction of hydration structure from a high-resolution scan of Muscovite mica surface immersed in a 100mM KCl and 1mM $MgCl_2$ solution. The top-left panel discloses the height map (Z-channel) plotted in red. The top-right panel depicts the corresponding excitation channel encoded in green. The top-center panel depicts superposition of the two signals. A clear overlap between bright excitation spots and Z-channel cavities is evident. The bottom series of three plots indicates one-dimensional profiles along the blue, yellow and purple lines. The Z and excitation signals are plotted using solid and dotted curves, respectively (also in the top panels). The two signals are evidently anti-correlated, indicating removal of a single water molecule from each cavity. The orientation of these molecules can be inferred from the non-circular shape of the bright spots in the excitation map (see white markings in inset c). As seen, the honeycomb pattern is made of six hydrating water molecules (inset a) adsorbed to the mica. Three of the water molecules are oriented slightly upwards (bright red spots), creating ~0.1 nm protrusions (see arrows along the purple line and the corresponding peaks in the bottom line-profile at 0.8 nm and 1.7 nm).

EXHIBITORS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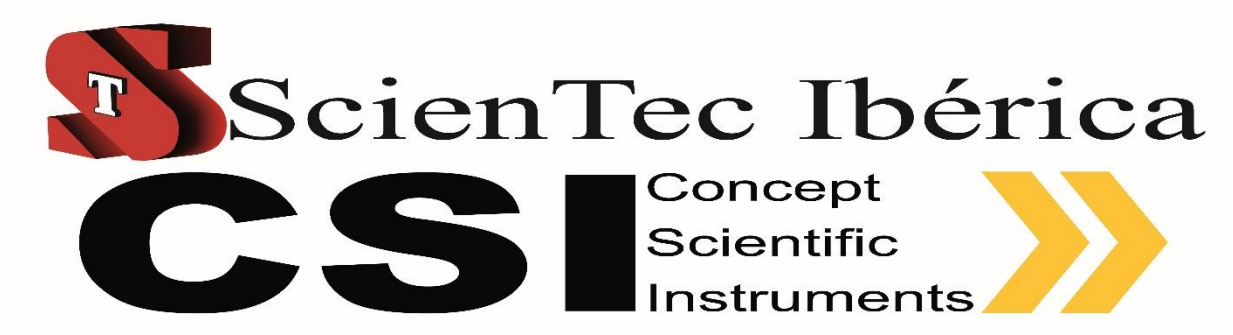
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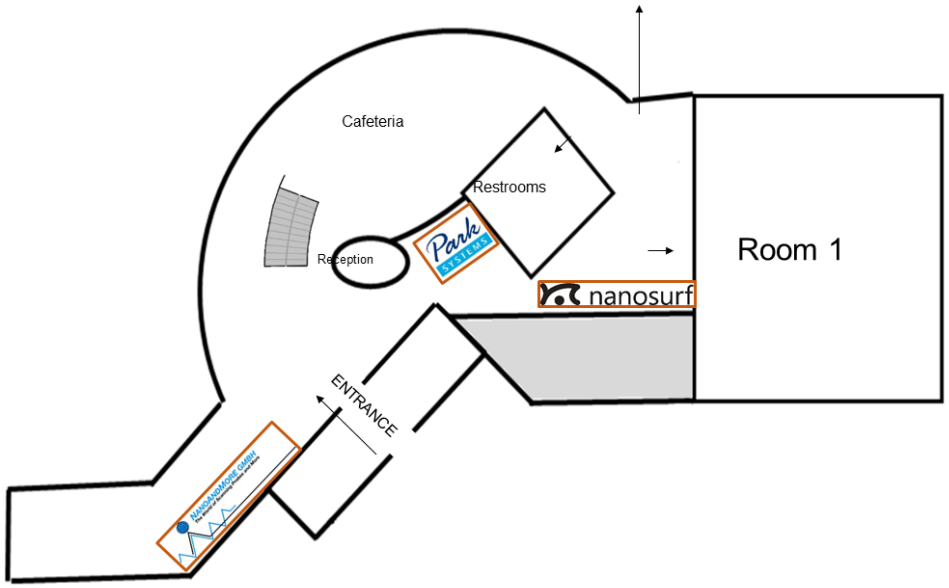

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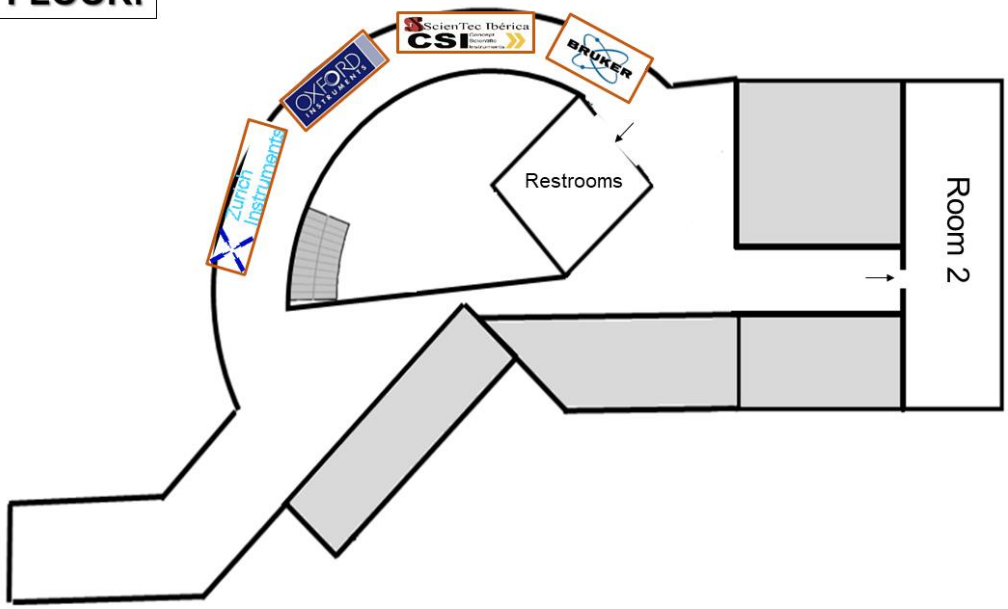

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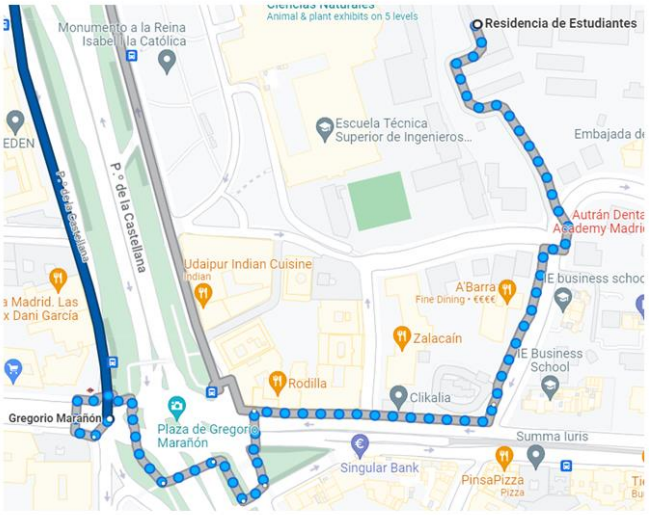

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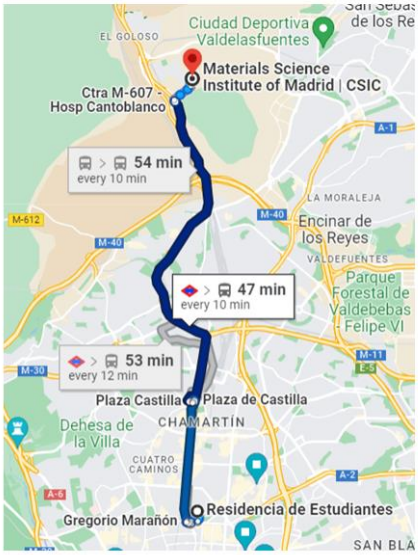

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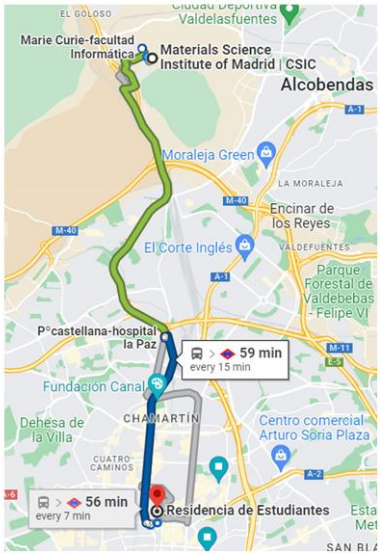

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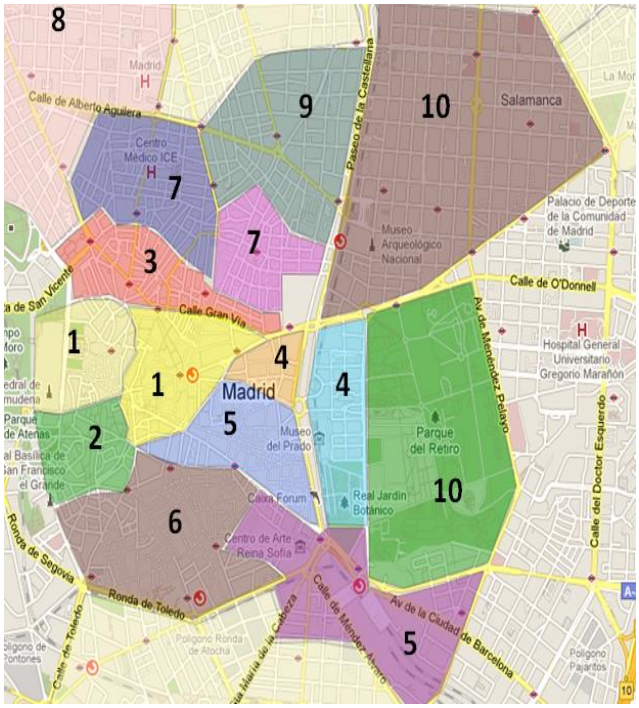

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