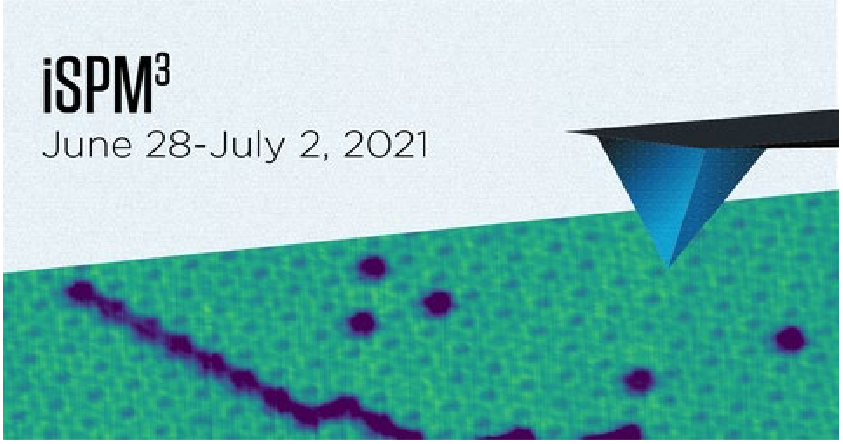
iSPM^3 Agenda

2021 International Scanning Probe Microscopy (ISPM) + Scanning Probe Microscopy on Soft & Polymeric Materials (SPMonSPM)



Foreword

Hello, and welcome to the 2021 Joint Conferences on *International Scanning Probe Microscopy* (iSPM) and *Scanning Probe Microscopy on Soft and Polymeric Materials* (SPMonSPM). This is the first time these long-running SPM meetings have been held concurrently, but it represents the 22nd ISPM and the 5th SPMonSPM. We originally hoped to host all of you last year in Breckenridge, CO, but as we know it's been a tough 18 months for our planet and many in our community are still in countries struggling to get ahead of the pandemic. Nonetheless, we have all learned about virtual meetings much faster than we could have imagined. We've been to numerous virtual conferences; some have been glowing successes while others were objective failures. We've done our best to combine the best technologies currently available to deliver a value-added conference experience. We know that we can't replace all the wonderful benefits of in person meetings such as coffee-break brainstorms and cocktail-hour new collaborations, but we are pleased as well by some of the benefits. We can bring together a diverse international audience, with lower costs and smaller carbon footprint. So, while we hope to be meeting again in person soon, we also hope that the best of the virtual meeting world sticks around and holds a place in the community.

The backbone of ISPM^3 is the CVENT virtual attendee hub. Within this hub you will find numerous conference experiences including plenary sessions, invited talks, contributed talks, posters, vendor exhibits and a topic we are very excited about, panel discussions. You can elect to engage with the talks in realtime using the "*simulive*" chat feature,or jump into the session break-out room to discuss the talks in person with your fellow attendees and speakers. If the talks of interest are outside your timezone, or conflict with your calendar schedule, please don't fret, all content (including contributed talks, posters, as well as live plenarys and panel discussions) will be available "on demand" for you to watch at your convenience. We strongly encourage speakers to be present for their talks/posters to answer questions related to their work.

Throughout the week we have scheduled free social events reflecting on the history of iSPM, SPMonSPM and a guided "*Whiskroscopy*" experience (details on how to participate below) which we welcome your participation in. Finally, we encourage your participation at the live panel discussions covering topical areas of interest to our community:

1. *Frontiers of Artificial Intelligence and Machine Learning in SPM*
2. *Quantifying Functional Properties: Can you really measure X*
3. *AFM for Soft and Bio: Latest advances and future prospects*
4. *Future opportunities and needs for SPM instrumentation, software, training and community*

The panels will involve a broad selection of domain experts to provide a diverse array of opinions, however, we really hope for active audience participation to allow a voice for the whole community.

On the final day of the conference, we will have a special awards presentation celebrating the best student posters and speakers from the conference.

We hope that you enjoy iSPM3 2021!

Sincerely

Jason Killgore and Liam Collins

Agenda

Monday - 6/28/2021

All times (ET)			
12.45 – 1.30 pm	Opening Remarks: Liam Collins and Jason Killgore		
	AFM of Cells and Related Media (I)	Dynamic AFM	Liquids and the Solid Liquid Interface (I)
1.30 – 2.00 pm	Igor Sokolov	David Haviland	Gabriel Gomilla
2.00 – 2.15 pm	Anhong Zhou	Philippe Leclere	Manuel Ralph Uhlig
2.15 – 2.30 pm	Delphine Sicard		Varun Vyas
2.30 – 2.45 pm	Pablo Dörig	Ryan Wagner	Elias Nakouzi
2.45 – 3.00 pm	Melanie Koehler	Jamie Colchero	Georg Gramse
3.00 – 3.15 pm	Albertus Viljoen	Arindam Phani	Lisa Almonte
3.15 – 3.30 pm	Petr Gorelkin	Gabriele Ferrini	Marcel Rost
3.30 – 3.45 pm	Alexander Erofeev	Shatruhan Singh Rajput	
3.45 – 4.00 pm	Michael Molinari	Vladimir Korolkov	
4.00 – 5:30 pm	Panel Discussion (I) : Frontiers of Artificial Intelligence and Machine Learning in SPM		
5:30- 7:00 pm	Poster Session		

Tuesday - 6/29/2021

All times (ET)			
11.00- 12.15 pm	Plenary (I): Ricardo Garcia		
12.15 – 1.45 pm	Panel Discussion (II) : Quantifying Functional Properties: Can you really measure X		
	Electromechanics	**Machine Learning and Artificial Intelligence**	**Advanced Instrumentation (I)**
1.45 – 2.15 pm	Neus Domingo	Adam Foster	Callie Higgins
2.15 – 2.30 pm	Kaiyang Zeng	Rama Vasudevan	Xiaoji Xu
2.30 – 2.45 pm	Loïc Musy		*(abstract withdrawn)*
2.45 – 3.00 pm	Ralph Bulanadi	Dalia Yablon	Pardis Biglarbeigi
3.00 – 3.15 pm	Christina Stefani	Javier Sotres	Ioan Ignat
3.15 – 3.30 pm	Kumara Cordero-Edwards	Iaroslav Gaponenko	Manouel Pichois
3.30 – 3.45 pm	Anthony Ferri		Rafiul Shihab
3.45 – 4.00 pm	Dalla Francesca Kevin		Francisco Espinosa
4.00- 5.30 pm	iSPM- Social Event		

Wednesday - 6/30/2021

All times (ET)			
10.30- 11.45 am	Plenary (II): Sergei Kalinin		
11.45 – 1.30 pm	Panel Discussion (III) : AFM for Soft and Bio: Latest advances and future prospects		
	AFM of Cells and Related Media (II)	**Polymer Characterization**	**Material Property Quantification**
1.30 – 2.00 pm	Sonia Contera	Bede Pittenger	Roger Proksch
2.00 – 2.15 pm	Georg Fantner	Ken Nakajima	Arnab Bhattacharjee
2.15 – 2.30 pm			Devon Jakob
2.30 – 2.45 pm	Thomas Le Neel	Hung Kim Nguyen	Kristen Hess
2.45 – 3.00 pm	(abstract withdrawn)	Amir Farokh Payam	Igor Sokolov
3.00 – 3.15 pm	Saanfor Hubert Suh	Muhammad Tariq	Lawrence Robbins
3.15 – 3.30 pm	Zeinab Al-Rekabi	Sakshi Yadav	
3.30 – 3.45 pm	Hannah Seferovic		
4.00- 5.30 pm	SPMonSPM- Social Event		

Thursday - 7/1/2021

All times (ET)			
11.00- 12.15 pm	Plenary (III): Simon Scheuring		
12.15 – 1.45 pm	Panel Discussion (IV) : Future opportunities and needs for SPM instrumentation, software, training and community		
	UHV and High Resolution	Liquids and the Solid Liquid Interface (II)	Photovoltaics
1.45 – 2.15 pm	Robert Wolkow	Takeshi Fukuma	Rajiv Giridharagopal Rachael Cohn
2.15 – 2.30 pm	Yuan Fang	Shuai Zhang	Samuel Berweger
2.30 – 2.45 pm	Harry Mönig		Justin Pothoof
2.45 – 3.00 pm	Sebastian Scherb	Victor Gisbert	Rosine Coq Germanicus
3.00 – 3.15 pm	Max Yuan	Miriam Jaafar	
3.15 – 3.30 pm	Stefania Moro	Shivprasad Patil	
3.30 – 3.45 pm	Furkan Altincicek		
3.45 – 4.00 pm	Jo Onoda		
4.00- 5.30 pm	Scanning Probe Whiskroscopy- Social Event		

Friday - 7/2/2021

All times (ET)			
	Advanced Instrumentation (II)		**Characterization of Biological Single Molecules**
1.30 – 2.00 pm	Steven De Feyter		Alice Pyne
2.00 – 2.15 pm	Joseph Kopanski		Peter Hinterdorfer
2.15 – 2.30 pm	Marco Zutter		
2.30 – 2.45 pm	Gheorghe Stan		Johanna Blass
2.45 – 3.00 pm	Jonathan Adams		Solène Lecot
3.00 – 3.15 pm	Ermes Scarano		Gijo Raj
3.15 – 3.30 pm	Charles Clifford		Rong Zhu
3.30 – 3.45 pm	Mathaeus Tschaikowsky		
4.00- 5.30 pm	**Award presentation & Closing Remarks**		

Scanning Probe Whiskroscopy

Learn about the Science and Art of Whisky making

Although its not entirely known if the Irish or the Scots were the first to give birth to *Uisce beatha* (water of life), it can be agreed that whisky has a long and distinguished history! Today many different forms of Whisky/Whiskey are enjoyed all over the world with the process of whisky making traversing both Science and Art. In this social hour we invite the connoisseur and the curious to come together to learn the differences between Irish, Scottish (Scotch) and American (Bourbon) whiskys. Join in a free whisky tasting experience by the world renowned whisky blender Dave McCabe, before sharing a glass amongst friends while exploring the nanoscale makeup of crystalized whisky.

The Whisky Expert

Dave McCabe, Whisky Blender

Dave first joined Irish Distillers in 2010, later helping establish and run the Irish Whiskey Academy from 2012 until 2016 in which he was the Academy Tutor as well as International Whiskey Ambassador. This involved educating people predominantly within the spirits industry about the production of Irish Whiskey produced in Midleton (county cork, Ireland) as well as travelling around the world attending whiskey events and seminars. Today, Dave is a Blender reporting directly to Billy Leighton, Master Blender of Redbreast. In this role Dave work's side by side with Billy to maintain the consistency of the Redbreast, source casks and bring to life new Redbreast innovations. A man of calm disposition, Dave knows that good things come to those who wait and when Redbreast 21 is your favourite whiskey this is an important point. Patience is key when this 21-Year-Old contains a range of casks from 21 to 32 years of age.

Suggested Libations

If you wish to join along at home with the whisky tasting, we have provided some suggested whisky types chosen due to their international availability. However, please note that these are suggestions and we welcome you to explore all whiskys available in your locality (or already in your cabinets) at home

Irish Whiskey	Scotch	Bourbon
Jameson (Original or black barrel) **Redbreast** (any of the range) **Powers –** (any of the range)	**Glenlivet or Glenfiddich** (single malt, non-peated) **Laphroaig, Ardbeg, or Bowmore** (single malt, peated) **Ballantines, Chivas Regal** (Blended Scotch)	**Four Roses** **Wild Turkey** **Jim Beam** **Makers Mark**

Disclaimer

Alcohol may be hazardous to health if consumed to excess, the operation of machinery or driving after the consumption of alcohol is not advisable. Please drink alcohol responsibly.

Abstracts

Approaches towards combining high-resolution and large-range AFM imaging

Jonathan D. Adams, Christian Bippes, Lukas Howald, Simon Fricker, Patrick Frederix and Dominik Ziegler

Nanosurf AG, Gräubernstrasse 12-14, CH-4410 Liestal, Switzerland

adams@nanosurf.com

Scanning probe microscopes are versatile tools because they can effectively resolve structures with length-scales from tens of micrometers down to the atomic-scale. Obtaining high quality images over scan sizes that may differ by more than five orders of magnitude presents a significant challenge in instrument design. In general, the stability of an instrument is improved by minimizing its size. However, for large sample systems, one must find novel approaches to maintain high stability which is required for high image quality down to the atomic level.

In this work, we present an overview of technological approaches used by our new instrument, the DriveAFM, which uses a novel optical design[1] that enables a fully-motorized tip scanner with photothermal excitation, and is capable of both high-resolution and large-range imaging (see Figure). The primary innovations involve scan head and controller designs that reduce the impact of noise sources, both electronic and mechanical, and mitigate environmental effects which cause drift.

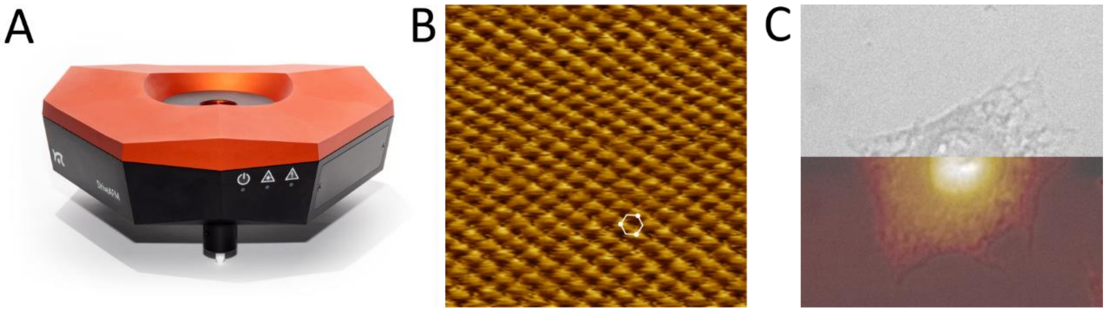


Figure 1: A) Photograph of the DriveAFM scan head. B: HOPG topography recorded in contact mode in air. Image size is 4×4 nm^2. No Fourier or gaussian filtering was applied to the image. C: Overlay of DIC optical image (top) and dynamic mode AFM topography image (bottom) of a live fibroblast cell in culture medium at 37°C. Image size is 50×50 μm^2.

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

Characterizing nanomechanical properties of comedones after treatment with sodium salicylate using atomic force microscopy

Zeinab Al-Rekabi[1*], Anthony V. Rawlings[2], Robert A. Lucas[3], Nidhin Raj[3] and Charles A. Clifford[1]

[1] Department of Chemical and Biological Sciences, National Physical Laboratory, Teddington, TW11 0LW, United Kingdom.
[2] AVR Consulting Ltd, Northwich, CW9 8FH, United Kingdom.
[3] Research and Development, GlaxoSmithKline Unlimited, Weybridge, KT13 0DE, United Kingdom.

* Corresponding author: zeinab.al-rekabi@npl.co.uk

Excessively oily skin can often cause unwanted skin traits in patients, such as excessive shine, enlarged pores, frequent outbreaks or acne. Investigating the biophysical properties of precursor lesions as microcomedones may prove beneficial in understanding their dissolution and prevention. Therefore, to support this exploration, sodium salicylate (NaSal), a common ingredient employed in skin care products, is applied *ex vivo* to micocomedone-associated nose strips and their nanomechanical properties are assessed using atomic force microscopy (AFM). Although the exact mechanism of NaSal on comedones is not fully understood at present, it appears to exhibit a significant exfoliation effect on the skin after repeated use. Herein, we investigated the physical properties of comedones pre- and post-treatment using 2% NaSal under ambient temperature and humidity. When treating comedones with 2% NaSal, samples appeared significantly softer ((1.3 ± 0.62) MPa) when compared to their pre-treated measurements ((7.2 ± 3.6) MPa; $P = 0.03826$). Furthermore, the elastic modulus maps generated, showed that after NaSal treatment, areas in the comedone appeared softer and swollen in some, but not in all areas, further proving the valuable impact of the 2% NaSal solution in altering the biomechanics and morphology in the microcomedones. Our results provide evidence that NaSal is indeed beneficial as an active ingredient in topical creams aimed at targeting eruptive skin conditions.

A Study of Silicon Dangling Bond Pairs in Search of a True Random Number Generator

Furkan Altincicek[1*], Lucian Livadaru[2], Roshan Achal[1], Jeremiah Croshaw[1], Samuel Ng[3], Taras Chutora[1], Max Yuan[1], Jason Pitters[4], Robert Wolkow[1,2,4]

[1]Department of Physics, University of Alberta, Edmonton, Alberta, T6G 2J1, Canada
[2]Quantum Silicon Inc., Edmonton, Alberta, T6G 2M9, Canada
[3]Department of Electrical and Computer Engineering, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada
[4]National Institute for Nanotechnology, National Research Council of Canada, Edmonton, Alberta, T6G 2M9, Canada
[*]altincic@ualberta.ca

Hydrogen terminated silicon has seen a recent resurgence in popularity due to several works demonstrating its use for ultra-dense memory, atomic electronics, and quantum devices. On this surface, individual hydrogen atoms can be removed with atomic precision through STM pulses, leaving a dangling bond (DB) behind. DBs are quantum dot-like entities that can hold either 0, 1, or 2 electrons, with their discrete energy levels in the bandgap. We are studying DBs to achieve true random number generators (RNG) on the atomic scale. Two DBs patterned in close proximity to each other on a highly n-doped crystal host a net extra electron, which can quantum mechanically tunnel to reside on either side. Through measurement of the extra electrons' spatial location in the pair, it is expected that the electron will be found on either side with equal probability in the absence of any biasing effects. This opens the possibility of using the system as a true RNG, where the bits are generated by probing the electrons' spatial location. Quantum processes are most desired for RNG because the randomness can be such that no common naturally occurring or imposed noise will alter the number generation. We study these DB pairs by DFT methods to establish the effect of geometry, external fields, and nearby dopants on charge localization and consequent RNG bit generation.

References:

[1] Achal, R et al. Lithography for robust and editable atomic-scale silicon devices and memories. Nature Communications 9, 2778 (2018). https://doi.org/10.1038/s41467-018-05171-y
[2] Huff, T. et al. Binary atomic silicon logic. Nature Electronics 1, 636–643 (2018). https://doi.org/10.1038/s41928-018-0180-3
[3] Wyrick, J et al. Atom-by-Atom Construction of a Cyclic Artificial Molecule in Silicon. Nano Letters 18, 12, 7502-7508 (2018). https://doi.org/10.1021/acs.nanolett.8b02919

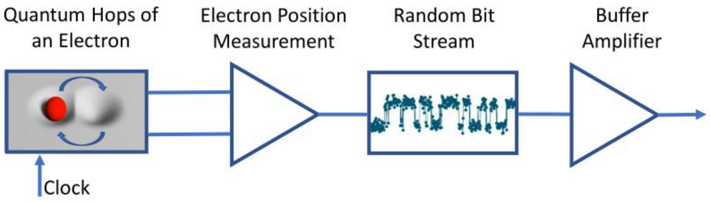


Figure 1. Measuring the position of an electron on a DB pair over a time range generates random bits, which will be used as true random number generator.

Atomic Force Microscopy Data acquisition and imaging using wavelet transforms

Pardis Biglarbeigi, Alessio Morelli, Patrick Lemoine, James McLaughlin, Dewar Finlay and Amir Farokh Payam
Nanotechnology and Integrated Bioengineering Centre (NIBEC), School of Engineering, Ulster University,
Jordanstown Shore Road, Northern Ireland BT37 0QB, UK.

The unique ability of Atomic Force Microscopy (AFM) to image, manipulate and characterize materials at the nanoscale has made it a remarkable tool in nanotechnology [1]. In dynamic AFM, acquisition and processing of the photodetector signal originating from probe–sample interaction is a critical step in data analysis and measurements. However, details of such interaction including its nonlinearity and dynamics of the sample surface are limited due to the ultimately bounded bandwidth and limited time scales of data processing electronics of standard AFM [2]. Similarly, transient details of the AFM probe's cantilever signal are lost due to averaging of data by techniques which correlate the frequency spectrum of the captured data with a temporally invariant physical system. Here, we introduce a fundamentally new approach for dynamic AFM data acquisition and imaging based on applying the wavelet transform on the data stream from the photodetector [3]. This approach provides the opportunity for exploration of the transient response of the cantilever, analysis and imaging of the dynamics of amplitude and phase of the signals captured from the photodetector. Furthermore, it can be used for the control of AFM which would yield increased imaging speed. Hence the proposed method opens a pathway for high-speed transient force microscopy.

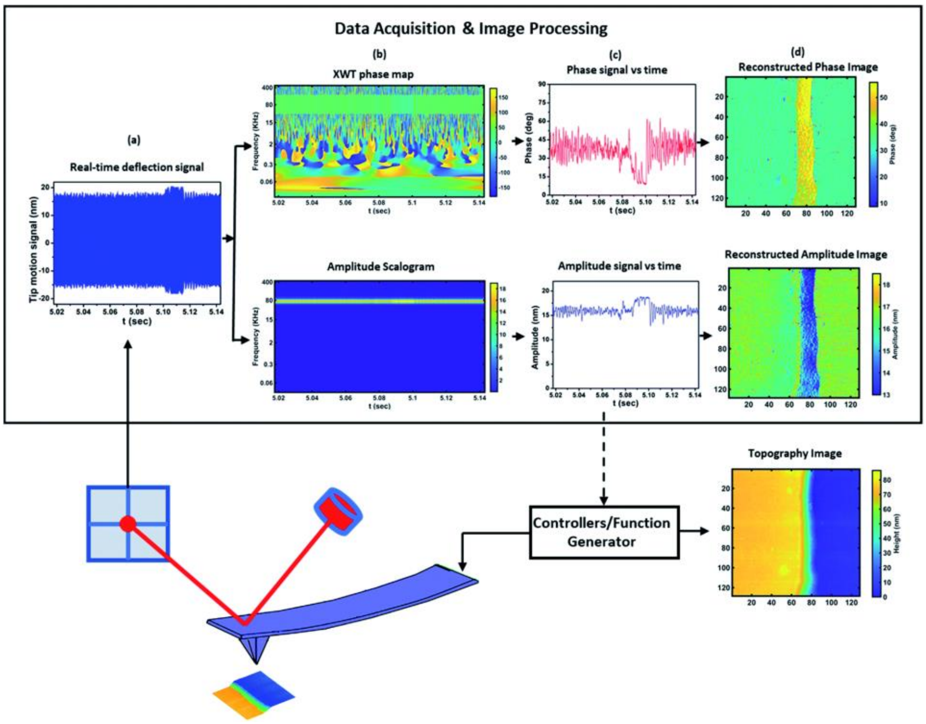


Fig. 1 Schematic of the proposed method. Images collected over a single tapping mode scan with the feedback maintaining a set-point amplitude. (a) The real-time signal acquired by the photodetector. (b) Scalogram and time–frequency phase map obtained via CWT using the raw signal from the photodetector. (c) Temporal amplitude and phase versus time at each frequency extracted from the CWT maps. (d) The amplitude and phase images reconstructed from the temporal signals of the cantilever.

References

1- Y. F. Dufrêne, *et al. Nat. Nanotechnol*ogy, 2017, 12(4), 295–307.
2- A. Belianinov, *et al. Nat. Communication*, 2015, 6, 1–7.
3- A. F. Payam, *et al.*, *Nanoscale Adv*., 2021, 3, 383-398.

Nanomechanics of DNA self-assemblies and light-driven molecular motors

Michael Penth[1,2], Yijun Zheng[1], Arzu Çolak[1], Kordula Schellnhuber[1,2], Mitchell K.L. Han[1], Aránzazu del Campo[1,2], Roland Bennewitz[1,2] and Johanna Blass[1*]

[1.]INM - Leibniz for New Materials, Campus D22, 66123 Saarbrücken
[2.]Saarland University, Faculty of Natural Sciences and Technology, 66123 Saarbrücken
* johanna.blass@leibniz-inm.de

Single-molecule force spectroscopy has become an essential tool to unravel the structural and nanomechanical properties of biomolecules. In this study, we present Flow Force Microscopy (FlowFM) as a massively parallel approach to study the nanomechanics of hundreds of molecules in parallel. The high-throughput experiments performed in a simple microfluidic channel enable statistically meaningful studies with nanometer scale precision in a time frame of several minutes. A surprisingly high flexibility was observed for a self-assembled DNA construct typically used in DNA origami. The persistence length was determined to be 10.2 nm, a factor of five smaller than for native DNA. The enhanced flexibility is attributed to the discontinuous backbone of DNA self-assemblies.

We also quantified the forces actuated by a unique molecular machine that can apply forces at cell-matrix and cell-cell junctions using light as an energy source. Micrometer-sized beads tethered to the surface via entangled rotary motors were retracted against drag forces from 1 pN to 5 pN within the first minute of UV-irradiation.

[1] M. Penth, K. Schellnhuber, R. Bennewitz, J. Blass under review in Nanoscale, 2021.
[2] Y. Zheng, et al. Optoregulated force application to cellular receptors using molecular motors, bioRxiv 2020.03.31.015198., accepted in *NatureCommun 2021*.

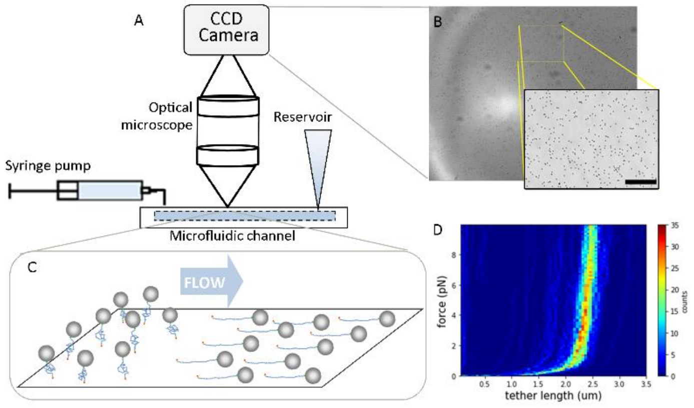


Figure 1. (A) Sketch of experimental setup for the observation of tethered particle motion in a microfluidic flow channel. (B) Optical microscopy t of thousands of surface-tethered beads (scalebar: 100 µm). (C) Sketch of the molecular arrangement with and without flow force. (D) 2D histogram of 302 force-distance curves recorded in one optical field of view, color scale reflects the number of datapoints in one bin.

Artificial Neural Networks Measure Fast Dynamic Carrier Motion *via* EFM on Energy Materials

Madeleine D. Breshears[1], Rajiv Giridharagopal[1] and David S. Ginger[1*]

[1.] University of Washington, Department of Chemistry, Seattle, WA.
* Corresponding author: dginger@uw.edu

Advances in scanning probe microscopy (SPM) have necessitated parallel development in big data handling and data science. One of the emerging methods for analyzing large datasets is to use artificial neural networks (ANNs). ANNs can be trained to correlate a specific input with an expected result. [1] Here, we use an ANN to discover the otherwise hidden time constant in fast free time-resolved electrostatic force microscopy (FFtrEFM) data. FFtrEFM measures the time-dependent electrostatic force gradient between the cantilever and the sample in response to an excitation source such as a transient LED or voltage pulse down to approximately 10 ns resolution. [2] A limitation of conventional FFtrEFM is that the characteristic time constant, which describes the carrier dynamics, is obfuscated by the cantilever physics. Our feedforward ANN efficiently and accurately enables direct extraction of the time constant from simulated FFtrEFM data. We further discuss the challenges of machine learning when applied to target values that span multiple orders of magnitude and how joint classification and regression through our ANNs addresses this issue. Finally, we present results on photoinduced carrier dynamics in lead halide perovskites that support earlier work showing that grain centers display faster photocharging behavior than grain boundaries. [3]

References:

[1] SV Kalinin, et al., ACS Nano **10** (2016) p. 9068-9086.
[2] DU Karatay, et al., Rev. Sci. Instrum. **87** (2016), p. 053702-1-11.
[3] R Giridharagopal, et al., ACS Nano **13** (2019) p. 2811-2821.

Photoexcited Carrier Dynamics on the Nanoscale in Perovskite Thin Films using Microwave Near-Field Microscopy

Samuel Berweger[1]*, Fei Zhang[2], Bryon W. Larson[2], Andrew J. Ferguson[2], Axel F. Palmstrom[2], Obadiah G. Reid[2], Thomas M. Wallis[1], Kai Zhu[2], Joseph J. Berry[2], Sanjini U. Nanayakkara[2], and Pavel Kabos[1]

[1.] National Institute of Standards and Technology, Boulder, CO, 80305
[2.] National Renewable Energy Laboratory, Golden, CO, 80401
* samuel.berweger@nist.gov

Spatially resolved studies of photoexcited charge carrier dynamics in photovoltaic systems remain challenging on their characteristic nanosecond time scales. Here we present a new experimental approach that allows for spectrally resolved spatio-temporal studies of optically excited free carriers in hybrid organic-inorganic perovskite thin films. Our approach is based on a near-field microwave impedance microscope (MIM, often called scanning microwave microscopy, SMM), which we combine with a spectrally continuously tunable pulsed optical excitation source based on a supercontinuum laser. Leveraging the inherent GHz bandwidth of our microwave instrument, we obtain temporal resolution as high as 5 ns.

Using our new approach, we study the effect of composition, annealing temperature, and surface treatment, on local lifetimes and the spectral response. We find that a C60 surface treatment significantly enhances the local carrier lifetime, and that deterioration under ambient conditions leads to increased spatial inhomogeneity in the photoconductive response and an overall increase in carrier lifetimes. We validate our time-domain MIM using sample-averaged time-resolved microwave conductivity, which we use together with time-resolved photoluminescence to further elucidate the nature of surface vs. bulk carrier lifetimes.

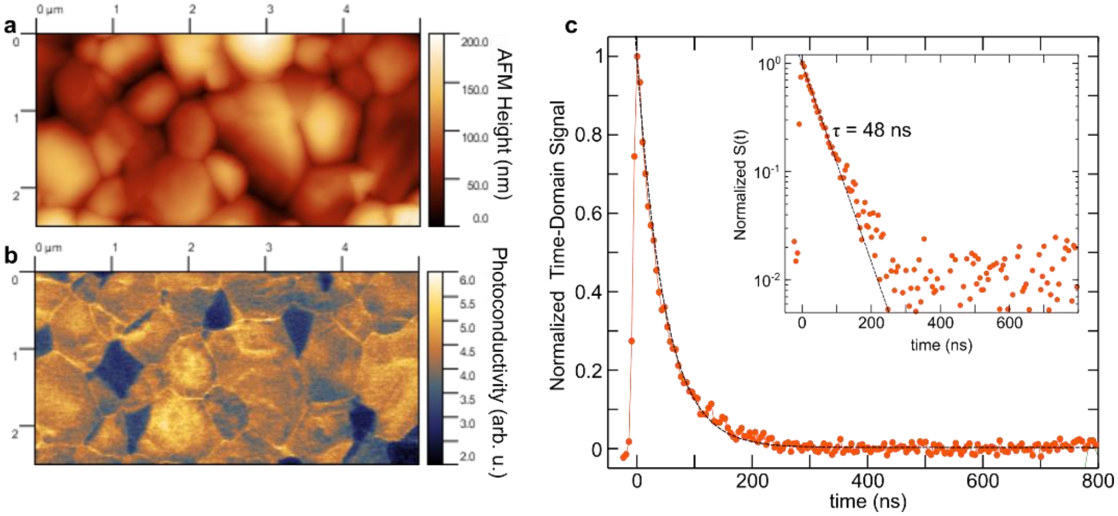


Figure 1. AFM topography (a) reveals the characteristic granular structure of a Formamidinium-Cesium Lead Iodide perovskite thin films, while the corresponding MIM photoconductivity map reveals the presence of low-conductivity regions not otherwise discernible. The time-domain MIM trace (c) shows the clearly resolved lifetime τ = 48 ns obtained by single-exponential fitting to the photoconductivity decay transient (inset: semi-log plot of the same data).

Quantifying the Impact of Varying Defect Landscapes on Domain Wall Motion

Ralph Bulanadi[1,†], Kumara Cordero-Edwards[1], Philippe Tückmantel[1], Sahar Saremi[2],
Giacomo Morpurgo[1], Lane W. Martin[2,3], Patrycja Paruch[1]

[1] *Department of Quantum Matter Physics, University of Geneva, 1211, Geneva 4, Switzerland,* † ralph.bulanadi@unige.ch
[2] *Department of Materials Science and Engineering, University of California, Berkeley, CA 94720, USA*
[3] *Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA94720, USA*

Ferroelectric materials, such as lead titanate, spontaneously retain electrical dipoles via the displacement of their central ion. The walls between domains have been modelled as a disordered elastic system, both statically and during motion as their adjacent domains expand and contract [1,2], while their motion has also been observed to follow avalanche statistics at larger scales via the detection of Barkhausen noise [3]. The universality of these classifications allow for modelling of domain walls at larger scales than typically possible via *ab initio* methods.

Here we report scanning probe microscopy studies into the switching dynamics of lead titanate thin films with varied and controlled defect disorder, in order to enhance our understanding of ferroelectric domain walls as a disordered elastic system. The films, grown on strontium titanate substrates, present strain-relieving *a*-domains that extend through the film, in-plane, along the crystallographic axes. Varying point defect densities have also been introduced via bombardment with He^{2+} ions. The gradual motion of 180° domain walls in these films under sequential bias scans were imaged using piezoresponse force microscopy. Computational tools were also developed to access information pertaining to the rate of switching at the nanoscale, as a function of both point defect density and *a*-domain proximity.

We observe that increasing point defect density significantly increases the average voltage required for switching, as well as the voltage range over which domain wall motion is observed. Domain nucleation also appears preferentially in more heavily bombarded samples. The *a*-domains appear to act as strong extended pinning sites, imposing directional constraints on the domain wall motion along the film crystallographic axes. In the non-irradiated sample, the effect of these *a*-domains appear to dominate, leading to large scale domain wall jumps to configurations determined by *a*-domain position. In contrast, all samples have been observed to express Barkhausen behaviour with similar critical exponents, regardless of defect implantation. In this complex disorder landscape, preliminary observations suggest point defects may therefore apply a screening effect to the impact of *a*-domains, yielding anomalous behaviour that is locally constrained around *a*-domains in the non-bombarded sample, while being statistically similar on a larger scale to ion-bombarded datasets.

[1] Giamarchi, T., et al., *Jamming, Yielding and Irreversible Deformation in Condensed Matter*, Springer (2006): p91.
[2] Paruch, P., Guyonnet, J., *Comptes Rendus Physique*, 14 (2013): p667.
[3] Salje, E.K.H., et al. *Physical Review Materials*. 3 (2019): p014415.

A method to measure AFM probe tip wear and lifetime

Charles Clifford[1*],Zeinab Al-Rekabi [1], Héctor Corte-León[1] and James Vicary[2]

[1.] National Physical Laboratory, Hampton Road, Teddington, TW11 0LW, United Kingdom
[2.] NuNano Ltd, Unit DX, Albert Road, Bristol, BS2 0XJ, United Kingdom
* Corresponding author: charles.clifford@npl.co.uk

We develop and example a simple method to measure probe tip lifetime and wear of atomic force microscopy (AFM) tips. AFM is used extensively in the semiconductor industry for measurement and quality control of nanoscale features such as lines, trenches and holes for example in CMOS structures, micro lenses, etc. where there is an inevitable drive to smaller and more cost-effective devices [1,2]. AFM probe tip sharpness, durability and lifetime are key issues where accurate measurement is paramount and down-time of the AFM tool minimized. However, there are not yet standard protocols to assess this.

We present a simple method to assess probe lifetime and wear. The method is based on imaging two representative samples as a function of time. The samples studied are a highly topographic porous aluminum sample, and a typical silicon step-height sample. Our results show that continuous scans of the samples show reproducible behaviour on the probe degradation (Fig. 1(a)). The measurements are complemented with SEM images showing different stages of degradation of the samples (Fig. 1(b)). These results represent a first step towards a standard protocol to assess probe longevity, and guide both users and probe manufacturers.

References:

[1] N. G. Orji et al., Nat. Electron., **1(10)** (2018) p. 532
[2] F. Hui and M. Lanza Nat. Electron, 2(6) (2019) p. 221

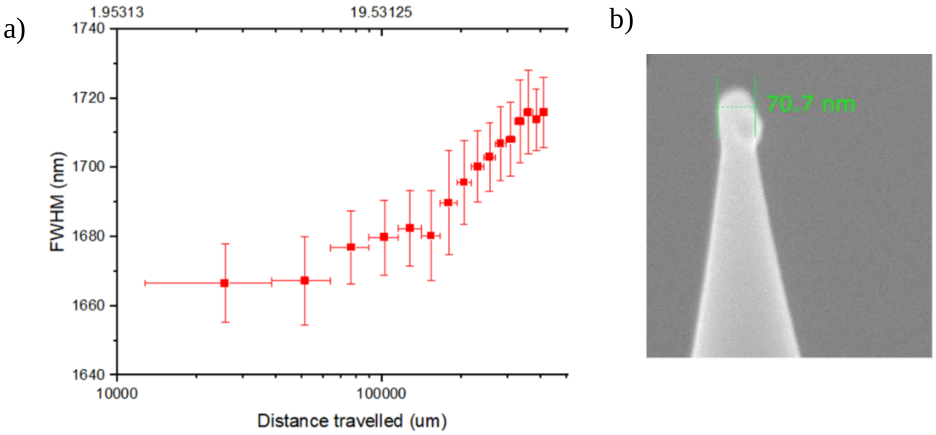


Figure 1. (a) Example results showing the evolution of the FWHM of the measured image as a function of time for one probe and (e) SEM image of a different probe after imaging.

Photo-Induced Resistance and Capacitance Measurements in Organic Solar Cell Materials using Broadband Local Dielectric Spectroscopy and Phase-Kick Electric Force Microscopy with Nanosecond Time Resolution

Rachael Cohn[1] and John Marohn[1*]

[1.] Cornell University, Department of Chemistry & Chemical Biology, Ithaca NY, USA.

* Corresponding author: jam99@cornell.edu

We use electric force microscopy (EFM) to understand the temporal dynamics and spatial distribution of charge generation and recombination in organic solar cells (OSCs). Here we report an EFM study of a film of PBDB-T/ITIC (Fig. 1a) [1]. We begin by using broadband local dielectric spectroscopy (BLDS) [2] to determine the film's charge response time, $\tau = R_s(C_{tip} + C_s)$, where R_s is sample resistance, C_{tip} is tip capacitance, and C_s is sample capacitance. In BLDS measurements, the tip voltage is amplitude modulated at a fixed frequency of 45 Hz and sinusoidally modulated at frequencies ranging from 200 Hz to 1.5 MHz. We report the first BLDS measurement on an OSC material. We find that $\tau \simeq R_s C_{tip}$ and conclude that light changes R_s, and not C_s, as is universally assumed in EFM experiments done on OSCs.

We are working to measure temporal changes in the film's photoinduced capacitance and resistance on the nanosecond timescale using scanned-probe microscopy. Achieving nanosecond time resolution is required to observe photogenerated charges before they recombine. Phase-kick EFM (pk-EFM) [3] measures the photocapacitance and photoresistance charging time, τ_R, by measuring the change in cantilever frequency and phase as a function of the delay times, t_p, between light and voltage pulses (Fig. 1b). The pk-EFM method is capable of achieving sub-cantilever-period, nanosecond time resolution. I will describe our efforts to push pk-EFM's temporal resolution from μs to ns in an organic photovoltaic film.

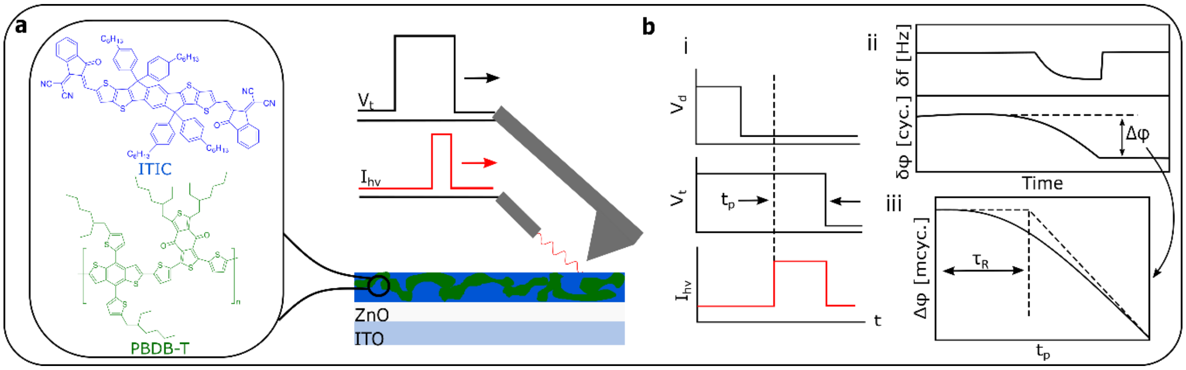


Figure 1. a. Experimental setup, with structures of donor (PBDB-T) and acceptor (ITIC) shown. **b.** Diagram of the phase-kick electric force microscopy measurement. (i) Timing diagram of applied voltage and light pulses, with V_d the cantilever drive voltage, V_t the tip voltage, and $I_{h\nu}$ the light intensity. (ii) Cantilever frequency and phase shifts. (iii) Total phase shift, $\Delta\varphi$, as a function of pulse time, t_p.

References:

[1] W. Zhao et al., Adv. Mater. **28** (2016) p. 4734 – 4739.
[2] A. M. Tirmzi, R. P. Dwyer, T. Hanrath, and J. A. Marohn, ACS Energy Lett. **2** (2017) p. 488 – 496.
[3] R. P. Dwyer, S. R. Nathan, and J. A. Marohn. Sci. Adv. **3** (2017) e1602951.

High performance AFM - Optical microscope, constructed using carbon fiber based materials.

Alejandro Colchero Truniger[1,2], Javier Sotres Pieto[3], Juan Francisco Gonzalez[3], Lisa Almonte-Garcia[2]
and Jaime Colchero Paetz[2*]
[1.] University of Barcelona, Condensed Matter Physics Department, Barcelona, Spain.
[2.] University of Murcia, Murcia, Spain.
[3.] University of Malmö, Malmö, Sweden.
* Corresponding author: colchero@um.es

A new AFM-optical microscope setup has been designed with the goal of very high stability, low thermal drift, modular design, easy access and easy integration into other experimental setups, in particular Optical Microscopy and/or Synchrotron Radiation facilities. The fundamental design criterion has been to separate the AFM system into three different (and modular) parts, each having different requirements with respect to stability and size. First, the smallest stage integrating the AFM tip holder and sample scanner having (sub-) nanometer stability; second, the optical stage with the components for the optical lever detection scheme; and third a stage having all additional techniques to be combined with AFM measurements, which are "looking" at the same sample spot as the AFM tip. This third stage may conceptually as large as needed. In the simplest case it will only have the components needed for optical microscopy. However, it may also be a whole optical table or even a Synchrotron radiation source.

Another important design criterion has been to use an optimized material for critical parts. Compared to aluminium carbon fibre based materials have similar elastic properties, half the density and ~20 times smaller thermal expansion coefficient. Hence, the AFM components will have a higher resonant frequency –and therefore higher mechanical stability- as well as much lower thermal drift. Following these general guidelines, a home-made optical microscope combining machined carbon fibre parts with –wherever possible- standard opto-mechanical components has been constructed. AFM beam deflection is implemented through the microscope objective. This allows for shorter focal lengths of the objective and high numerical apertures up to NA=0.6, giving optimised access for optical techniques, in particular for high resolution images, and improves the light gathering capacity of the microscope, which is crucial for low light techniques as for example Raman spectroscopy.

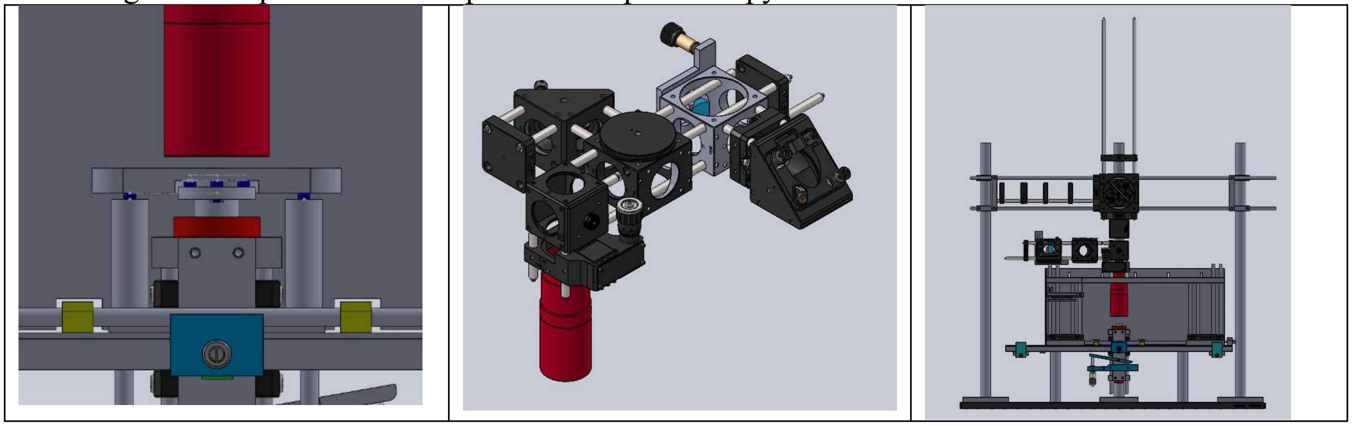


Figure 1. AFM part of the microscope (left). AFM beam deflection and blue drive optics (center). Complete AFM-optical microscope (right).

Circles—A Scheme to interpret linear and non-linear interactions in Dynamic AFM

Jaime Colchero[1*], Pablo Contreras Vélez[1], Jesus Sánchez Lacasa[1,2] and Juan Francisco Gonzalez[1,3]

[1.] CIOyN, Dep. of Physics. Campus Espinardo, University of Murcia, Spain.
[2.] Department of Chemical and Biological Physics, Weizmann Institute of Science, Rehovot, Israel.
[3.] University of Malmö, Malmö, Sweden.
* Corresponding author: colchero@um.es

Dynamic Scanning Force Microscopy (DSFM) is in most cases a (highly) non-linear oscillator. However, it is mostly modelled as a (multifrequency) harmonic oscillator. This very simple approximation works surprisingly well. A new scheme to interpret and unify linear and nonlinear interactions is proposed. Using the classical model for the driven damped harmonic oscillator, its response is described by means of a complex number. We explicitly calculate how the time dependence of the deflection is processed by a typical Lock-In setup to obtain a point in the complex plane representing the oscillation state.

Using the Virial Theorem and work of San Paulo et al. [1] as starting point, we show how a linear DSFM system can be easily interpreted in terms of "Circles". Essentially, as the drive frequency is tuned through the resonance frequency the outputs of a typical DSFM electronics describe to a very good approximation a "Circle" when visualized in xy-mode on an oscilloscope. Interestingly, our model shows that this "Circle" remains essentially invariant when including the non-linearity of typical tip-sample interactions. These "Circles" are distorted only by dissipation which varies with tip-sample distance. We therefore propose that this representation scheme allows a very sensitive method to separate the effect of conservative interactions (linear as well as non-linear) from the effect of dissipative interactions.

[1] A. San Paulo and R. García. PRB 66:041406 (2002).

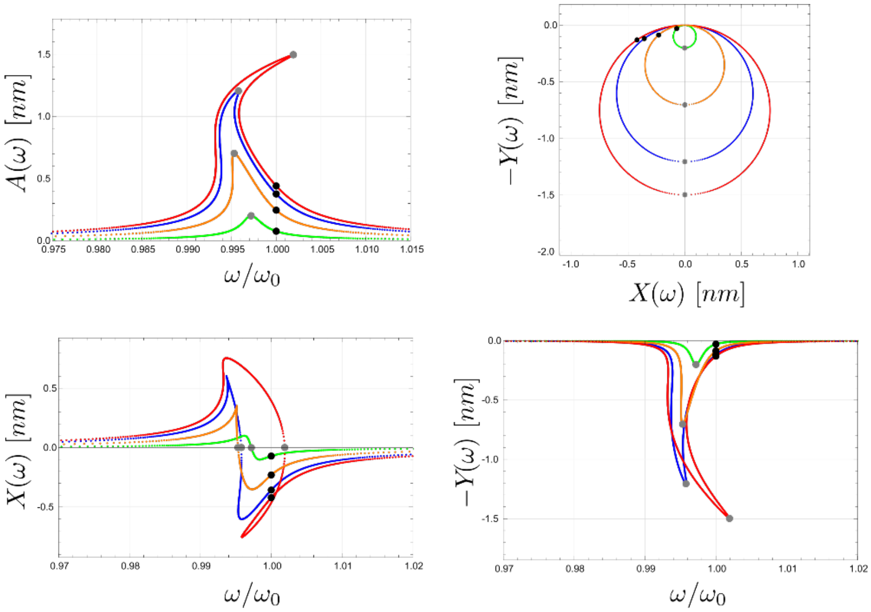


Figure 1. Top: non-linear response of the tip-sample system to a typical attractive and repulsive force curve; left: amplitude vs. frequency plot, and right: bode plot representation of Real and Imaginary part of the complex amplitude as a function of frequency. Bottom: individual Real and Imaginary parts.

Nanoscale viscoelasticity of living tissues with AFM: physics of biological growth and shape across temporal and spatial scales

Jacob Seifert, Casey Adam, Alba Piacenti, Sonia Contera*

Clarendon Laboratory, Physics Department, University of Oxoford, Parks Road, OX1 3PU Oxford, UK

*corresponding author: sonia.antoranzcontera@physics.ox.ac.uk

The dynamic shapes of biologial tissues emerge from a complex interplay of physics, chemistry and genetics, which determines--at each temporal and spatial scale--the mechanical properties that eventually form the structures of living organisms. Shape and mechanical stability of living organisms rely on precise control in time and space of growth, which is achieved by dynamically tuning the mechanical (viscous and elastic) properties of their hierarchically built structures from the nanometer up. It is now well-established that cellular behaviour (including stem cell differentiation) crucially depends on the mechanical properties of the cells' environment. Attention has been directed towards the importance of the *stiffness* of the natural (extracellular matrix, ECM) or artificial matrices where cells grow, with the purpose of either understanding mechanotransduction, or controlling the behaviour of cells in tissue engineering. While stiffness (i.e. the capacity of a material to elastically store mechanical energy) has been the focus of most experimental research, neither cells or matrices are elastic. Biological systems dissipate energy (i.e. they are viscous) and hence they do not respond to mechanical deformations instantaneously (like an ideal Hookean spring), but present different time responses at different spatial scales that characterise their responses to external stimuli. Measuring viscoelasticity (especially at the nanoscale) has remained experimentally challenging[1,2]. In my talk I will present atomic force microscopy (AFM)- based techniques to measure and map the viscoelasticity of living tissues, cells, membranes, collagen, ECMs, and tissue engineering matrices across the spatial and temporal (from Hz to 100s of kHz) scales, and chirp-based spectroscopic techniques to assess viscoelasticity from Hz to 100s kHz at the nano and micro scale developed in my lab. I will also present tests for assessing which viscoelasic model better fits the experimental AFM results. Our results have uncovered that extracellular matrices of both plants [3] and tumours present an almost perfect linear viscoelastic behaviour

References:

[1] "Multifrequency AFM reveals lipid membrane mechanical properties and the effect of cholesterol in modulating viscoelasticity" 2019. Z Al-Rekabi, S Contera; Proceedings of the National Academy of Sciences 115 (11), 2658-2663.

[2] "Mapping nanomechanical properties of live cells using multi-harmonic atomic force microscopy" 2011 A Raman, S Trigueros, A Cartagena, APZ Stevenson, M Susilo, E Nauman, S Contera. Nature Nanotechnology 6 (12), 809.

[3] "Mapping cellular nanoscale viscoelasticity and relaxation times relevant to growth of living Arabidopsis thaliana plants using multifrequency AFM" 2021 J Seifert, C Kirchhelle, I Moore, S Contera. Acta Biomaterialia 121, 371-382

Local conductivity of transparent nanostructured $SrVO_3$ perovskite film grown on nanosheets by SSRM

Rosine Coq Germanicus[1*], A. Boileau[1], M. Dallocchio[1], F. Baudouin[2], A. David[1], W. Prellier[1], V. Demange[2], Ulrike Lüders[1] and Arnaud Fouchet[1]

[1.] NORMANDIE UNIV, ENSICAEN, UNICAEN, CNRS, CRISMAT, 14000 CAEN, France.
[2.]Univ Rennes, CNRS, ISCR – UMR 6226, 35042 Rennes, France

Corresponding author: rosine.germanicus@unicaen.fr

Thin $SrVO_3$ (SVO) films crystallized in the perovskite structure show functional properties comparable to what is achieved in the standard indium-tin oxide (ITO) [1]. By using $[Ca_2Nb_3O_{10}]^-$ nanosheets (NS) [2] as germination layers, growth of 40 nm thick SVO film by pulsed laser deposition (PLD) on covered glass results in crystalline perovskite with the preferential (001) orientation with a room temperature resistivity of 300 µΩ.cm and an optical transparency of about 75% at 550 nm. However, since the microstructure and conductivity may have a strong influence on the macroscopic properties, motivating us to investigate the local electrical conductivity of the SVO film , by Scanning Spreading Resistance Microscopy (SSRM) [3]. For this Atomic Force Microscopy (AFM) mode, a highly conductive diamond tip is used to map the local resistance of the indium-free transparent conductor (TCO) film. The topography (Figure 1a) reveals clearly the nano-textured structure of the SVO film, three distinct areas can be localized: (1) SVO directly deposited on glass (without NS), (2) SVO on a single layer of NS and (3) SVO on multiple NS layers. The AFM friction map (Figure 1b) shows a granular structure on area (1) and does not show a difference between areas (2) and (3), indicating the same mechanical properties and probably the same chemical composition. In the resistance acquisition (Figure 1c), with V_{DC} bias of +500 mV applied to the sample, the conductivity of the SVO on NS (areas 2 and 3) presents a very low resistance of 20 kΩ, confirming the macroscopically observed metallic conduction. In SSRM spectroscopy mode, a quasi-symmetric I-V spectra is observed in the range of ± 2 V. Interestingly, the area 1 present also some conductivity, which could probably result from polycrystalline state of the SVO due to proximity effect of the nanosheets. In addition, we can note that, the grain boundaries between different NS seem to be largely conducting, an insulating character is only observed if the SVO film is physically interrupted around NS agglomerates.

[1] A. Boileau et al, Adv. Optical Mater. **7**, (2019), 1801516
[2] M. Nijland et al, Adv. Funct. Mater. **25**, *(*2015), 5140
[3] R. Coq Germanicus, et al Applied Surface Science, **510,** (2020), p.145522

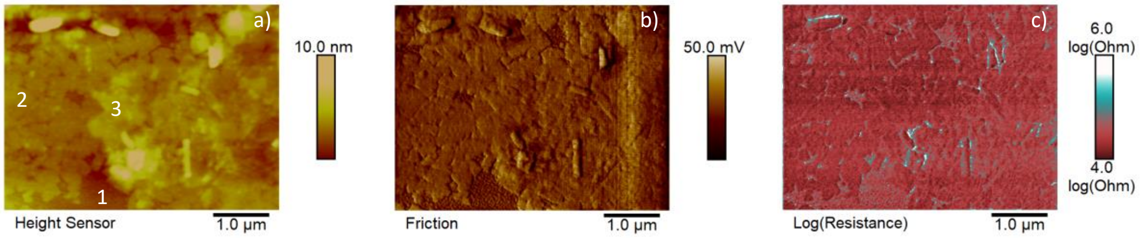


Figure 1. Conductivity of the vanadate TCO on glass covered by nanosheets: a) topography, b) friction acquisition, c) local resistivity SSRM results.

Probing novel functionalities at ferroelastic twin domain using scanning probe microscopy

Kumara Cordero-Edwards[,1]*, Philippe Tückmantel[1], Iaroslav Gaponenko[1], Sahar Saremi[2], Lane Martin[2,3], and Patrycja Paruch[1]

[1]DQMP, University of Geneva, Geneva, Switzerland
[2]Department of Materials Science and Engineering, University of California, Berkeley, USA.
[3]Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, USA.

*Corresponding author: rohini.corderoeduards@unige.ch

In ferroelectrics, domain walls are thin interfaces separating regions with different orientations of electric polarization, either along the same crystalline axis (180° domain walls), or as ferroelastic twins. The domain walls can present physical properties quite different from the surrounding domains, allowing them to be used as active components in future device applications.

Recent studies of domain walls using scanning probe microscopy have focused on mapping their response to different parameters such as temperature, applied pressure and electric field, in order to understand their structure-property relationships. In particular, the role of high strain gradients present at ferroelectric twins has been shown to enhance their electrical conduction [1] and can lead to complex rotational polarization textures [2,3].

Here, I will present our investigation of ferroelastic twin domains (90° domain walls) in epitaxial $PbTiO_3$ thin films grown on $SrTiO_3$, explored with scanning probe microscopy. Our results suggest a complex polarization structure, with unique mechanical response distinct from the surrounding ferroelectric phase, and enhanced electrical conduction.

References
[1] Stolichnov, I., et al. Nano Lett. 15, 8049−8055 (2015)
[2] Catalan, G., et al, Nat. Mat. 10, 963 (2011)
[3] Cao, Y., et al, Appl. Phys. Lett. 110, 202903 (2017)

Fabrication of ferroelectric tunnel nano-junctions in P(VDF-TrFE)-thin layers with conductive-AFM control

Kevin DALLA FRANCESCA[1]*, Anthony FERRI[1], Antonio DA COSTA[1], Luc MAËS[1], Walaa JAHJAH[2], Abir NACHAWATY[3], Liufang CHEN[3], Jean-Christophe LE BRETON[2], Mairbek CHSHIEV[4], Philippe SCHIEFFER[2], Yuan LU[3], Rachel DESFEUX[1]

[1.] Univ. Artois, CNRS, Centrale Lille, Univ. Lille, UMR 8181, Unité de Catalyse et Chimie du Solide (UCCS), F-62300 Lens, France
[2.] Univ. Rennes, CNRS, Institut de Physique de Rennes (IPR), UMR 6251, Beaulieu, 263 avenue du General Leclerc, 35042 Rennes, France
[3.] Univ. Lorraine, CNRS, Institut Jean Lamour (IJL), UMR 7198, Campus ARTEM, 2 Allée André Guinier, 54011 Nancy, France
[4.] Univ. Grenoble Alpes, CEA, CNRS, Grenoble INP, INAC-SPINTEC, 38000 Grenoble, France
* Corresponding author: kevin.dallafrancesca@univ-artois.fr

In the frame of the FEOrgSpin project (*Ferroelectric control of organic/ferromagnetic spinterface*) funded by the National *French* Research Agency (ANR), one of the objectives is to control the hybrid ferroelectric organic/ferromagnetic metal interface, as known as "spinterface", for the development of future advanced data storage devices based on spin electronics. For example, the spin polarization of spinterface at the Fermi level can be different or even of opposite sign to that of the adjacent ferromagnetic electrode, annihilating the spin properties of the device.

Recently, we demonstrated the possibility of modulating the spin polarization at the poly(vinylidene fluoride) (PVDF)/Co interface by changing the direction of the ferroelectric polarization within the PVDF material [1]. We obtained important results regarding the surface morphology, the conduction properties as well as piezo-/ferroelectric behavior of these organic ferroelectric barrier layers by using different techniques/tools of Atomic Force Microscopy (AFM), such as piezoelectric force microscopy, for nanoscale investigation. Now, in order to optimize the parameters of spin transfer, it becomes necessary to develop nanometric-size organic tunnel junctions to improve the effects of both electroresistance (TER) [2] and magnetoresistance (TMR). The solution chosen consists in the development of tunnel nano-junctions indentation by the use of conductive-AFM probes (CT-AFM) [3]. Here the feedback control of the process is operated by the current measurement during indentation and allows for a sub-nanometer control of the thickness of the junction (Fig. 1).

[1] S. H. Liang *et al.*, Adv. Mater. 28, (2016)
[2] B.B. Tian *et al.*, Nat. Commun. 7, (2016).
[3] K. Bouzehouane *et al.*, Nano Lett. 3, (2003).

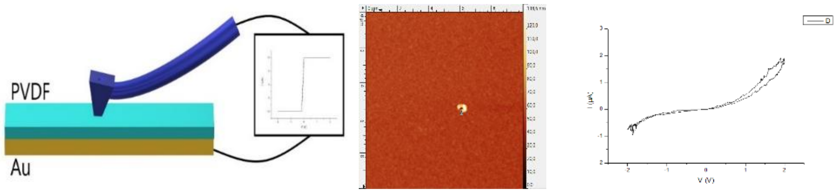


Figure 1. (left) Schematic representation of the nano-indentation step using the CT-AFM technique. (center) AFM image showing the indented area. (right) Local I-V curve showing the evolution of the electrical properties of the nano-junction during indentation.

Functionalization of 2D nanomaterials: a molecular approach

Steven De Feyter[1]

[1.] Department of Chemistry, KU Leuven, Celestijnenlaan 200F, B-3001 Leuven (Belgium)

* Corresponding author: steven.defeyter@kuleuven.be

In this presentation, I will mainly focus on the functionalization of graphite and graphene using molecules, though the concepts can be applied to other 2D materials too. Nanostructuring is at the heart of all functionalization protocols that we develop, because it opens new possibilities of control and functionality. A variety of scanning probe microscopy methods are used for visualization, characterization, and manipulation. A first approach is based on molecular self-assembly at the interface between a liquid or air, and graphite or graphene. I will discuss concepts of nanostructuring emphasizing the effect of solvent, solute concentration and temperature, stimulus-driven self-assembly and self-assembly under nanoconfinement conditions. A second approach is based on grafting molecules on graphite or graphene via covalent chemistry. It will be demonstrated how in addition to bottom-up strategies that in addition to control on the density and layer thickness also provide submicron to nanoscale nanostructuring, also scanning probe microscopy top-down nanolithography can be used to nanostructure such covalently modified surfaces. A third approach does not focus on the functionalization of the surface, but uses the surface as a support for the in-plane covalent stitching of molecules, leading to the formation of on-surface 2D polymers. The (de)polymerization can be controlled by scanning tunneling microscopy.

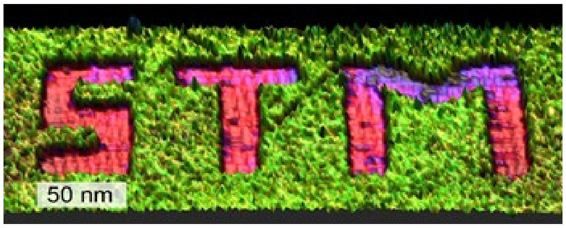


Figure 1. STM image of combined nanolithographic, covalent and non-covalent molecular functionalization of graphite

FluidFM hollow probes in biophysics and life sciences. A versatile extension for AFM.

Paul Monnier[1], Maria Milla[1], Dario Ossola[1], Pablo Dörig[1*]

[1.] Cytosurge AG, Sägereistrasse 25, 8150 Glattbrugg, Switzerland
* Corresponding author: pablo.doerig@cytosurge.com

By introducing a fluidic channel into an AFM probe, fluid force microscopy[1] (FluidFM) greatly extends the application range for AFM. With three distinctive probe designs, the user can pick & place microscopic objects, print on the sub-micron scale, or even inject into adherent cells. Popular applications range from higher throughput single-cell-force-spectroscopy (SCFS)[2], to microbial studies[3], colloidal research, nano-printing as well as single cell injection.

Cytosurge AG, an ETH Zurich spin-off, was founded in 2009 with the aim to make FluidFM conveniently available to interested researchers. Since, more than 100 labs have joined the FluidFM community resulting in a wealth of publications. Here we introduce the underlying technology, outline a typical system, and then present recent application highlights of FluidFM users around the world.

References:

[1] A. Meister, M. Gabi, P. Behr, P. Studer, J. Vörös, P. Niedermann, J. Bitterli, J. Polesel-Maris, M. Liley, H. Heinzelmann & T. Zambelli. (2009) Nano Letters, 9(6), 2501-2507. doi:10.1021/nl901384x
[2] A. Garitano-Trojaola, A. Sancho, R. Goetz, S. Walz, H. Jetani, E. Teufel, N. Rodhes, M. DaVia, L. Haertle, S. Nerreter, C. Vogt, J. Duell, R. Tibes, S. Kraus, A. Rosenwald, L. Rasche, M. Hudecek, M. Sauer, H. Einsele, J. Groll & M. Kortüm. (2019). Blood. doi: 10.1182/blood-2019-129762
[3] M. Mathelié-Guinlet, F. Viela, J. Dehullu, S. Filimova, J.M. Rauceo, P.N. Lipke & Y.F. Dufrêne. (2021) Commun Biol. doi: 10.1038/s42003-020-01498-9

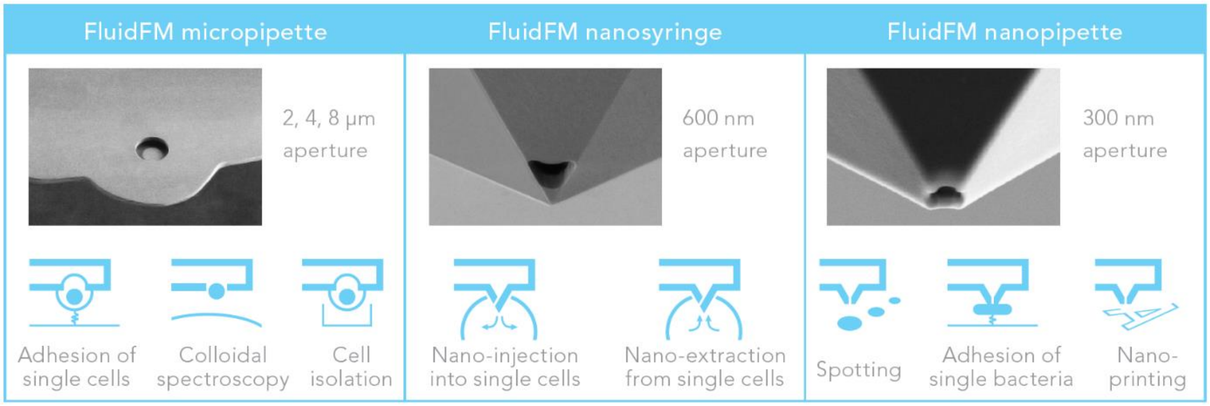


Figure 1. The three FluidFM probe types enable a wide range of applications in mechanobiology, nanotechnology and biotechnology.

Towards quantification of nanoscale electromechanical responses: the contribution of gradient based responses and electrical couplings

Neus Domingo[1]

[1] Institut Català de Nanociencia i Nanotecnologia, CSIC and The Barcelona Institute of Science and Technology, Campus UAB, Bellaterra, Barcelona E-08193, Spain

*Corresponding Author: neus.domingo@icn2.cat

Piezoresponse Force Microscopy (PFM), based on the inverse piezoelectric effect is the basic mode to study surface electromechanics at the nanoscale. Still, quantification of electromechanical responses remains elusive due to the convergence of different physical and electrochemical phenomena that lead to an effective mechanical response. Moreover, but it has been realized that the effect of gradients in electro-mechanical phenomena at the nanoscale can become dominating: the generation of electrical signals after the application of mechanical strain gradients with an AFM tip has been proved, and it has been shown that it is possible to write ferroelectric domains [1] or to move oxygen vacancies and charges.

In this talk, I will review several different phenomena that directly affects quantification of piezoelectric response at the nanoscale, that is, the determination of the d_{33} effective piezoelectric coefficient. First, I will show how gradient-based electromechanical effects couples and interferes with PFM measurements. I will start by demonstrating the asymmetry in mechanical properties induced by the coupling of flexoelectricity to ferroelectricity leading to *ferroelectrics as smart mechanical materials* [2], and opening new opportunities to *mechanically read ferroelectric polarization states* in both, thin films and single crystals, on the base of Contact Resonance Frequency AFM mode. Then, I will put the light in another new aspect: converse flexoelectric effect [3] due to the presence of strong local electric field gradients at the tip end can induce a mechanical strain of the sample in dielectric centrosymmetric materials with magnitudes comparable to piezoelectric d_{33} coefficient. I will compare the output of measurements done using the optical beam detection method with interferometric sensors to isolate artifacts in the response. Finally I will go over the effect of Schottky barriers on the determination of the d_{33} coefficient of piezoelectric semiconductors, and show how the metal-insulator junctions can induce non-linear and multiharmonic electromechanical responses [4].

References

[1] H. Lu, C.-W. Bark, D. Esque de los Ojos, J. Alcala, C. B. Eom, G. Catalan, A. Gruverman, Mechanical Writing of Ferroelectric polarization, Science 336 (2012) 59.

[2] K. Cordero-Edwards, N. Domingo, A. Abdollahi, J. Sort, G. Catalan, Ferroelectrics as Smart Mechanical Materials, Advanced Materials, 29 (2017) 1702210.

[3] Abdollahi, A.; Domingo, N.; Arias, I.; Catalan, G., Converse flexoelectricity yields large piezoresponse force microscopy signals in non-piezoelectric materials. Nature Communications, 10 (2019) 1266

[4] Lozano, E; Catalan, G; Esteve, J; Murillo, G; Domingo, N, Non-linear and Multiharmonic Nanoscale Piezoresponse of Single ZnO nanowires with Schottky Junctions, Nanotechnology 32 (2020) 025202

Sub-10 nm patterning of few-layer MoS2 and MoSe2 nanoelectronic devices by oxidation scanning probe lithography

Y. K. Ryu[1], A. I. Dago[1], Y. He[2,3], F. M. Espinosa[1], C. Munuera[1], E. López- Elvira[1] and R. Garcia[1]

[1] Instituto de Ciencia de Materiales de Madrid, CSIC. Sor Juana Inés de la Cruz 3, 28049 Madrid, Spain
[2] The State Key Laboratory of Robotics and Systems, Robotics Institute, Harbin Institute of Technology, Harbin, Heilongjiang 150080, PR China
[3] Center for Precision Engineering, Harbin Institute of Technology, Harbin, Heilongjiang 150001, PR China
* Corresponding author: francisco.espinosa@csic.es

The properties of 2D materials devices are very sensitive to the physical, chemical and structural interactions that might happen during processing. Low-invasive patterning methods are required to fabricate devices at the nanoscale. Here we developed a process that combines oxidation scanning probe lithography (o-SPL) and oxygen plasma to fabricate nanoribbon field-effect transistors and nano-constrictions on few-layer MoS_2 and $MoSe_2$. The oxygen plasma has a double role in this process. First, it forms a thin, uniform oxide layer on top of the flake surface to enable o-SPL nanopatterning with full control of shape and size. Second, the oxide layer thins down the flake. Both plasma-based and o-SPL oxides are soluble in deionized H_2O, which enabled etching and the definition of electrically isolated nano-constrictions and nanoribbons. The accuracy and robustness of the process was applied to pattern sub-10 nm wide constrictions and nanoribbon transistors.

[1] F. M. Espinosa, Y.K. Ryu, K. Marinov, D. Dumenco, A.Kis, R.Garcia, Appl. Phys. Lett. **106**, 103503 (2015)
[2] A.I. Dago, Y.K. Ryu, R.Garcia, ACS Appl. Mater. Interfaces **10**, 40054 (2018)
[3] Y. K. Ryu and R. Garcia, Nanotechnology **28**, 142003 (2017)
[4] Y. K. Ryu, A. I. Dago, Y. He, F. M. Espinosa, C. Munuera, E. López- Elvira and R. Garcia, Appl. Surface Science **539**, 148231 (2021).

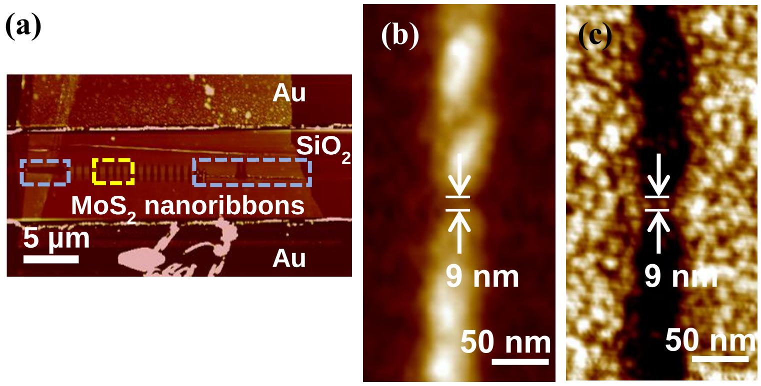


Figure 1. (a) AFM topographic image of MoS_2 nanoribbons FET. The channel consists of an array of 17 nanoribbons of 300 nm half-pitch and 5 nm of thickness. (b) AFM topographic image of a sub-10 nm constriction fabricated by o-SPL on an oxygen plasma treated MoS_2 flake (c) AFM topographic image of the constriction shown in (b) after water etching.

Stereospecific Epitaxial Growth of Bilayered Porous Molecular Networks

Yuan Fang[1,2], Benjamin D. Lindner[3], Iris Destoop[1], Takashi Tsuji[3], Zhenzhe Zhang[2], Rustam Z. Khaliullin[2], Dmitrii F. Perepichka[2], Kazukuni Tahara[3,4], Steven De Feyter[1],* and Yoshito Tobe[3,5]*

[1] Department of Chemistry, Division of Molecular Imaging and Photonics, KU Leuven, Leuven B-3001, Belgium
[2] Department of Chemistry, McGill University, Montreal, Québec, H3A 0B8, Canada
[3] Division of Frontier Materials Science, Graduate School of Engineering Science, Osaka University, Ibaraki, Osaka 567-0047, Japan
[4] Department of Applied Chemistry, School of Science and Technology, Meiji University, Kawasaki 214-8571, Japan
[5] Department of Applied Chemistry, National Chiao Tung University, Hsinchu 30010, Taiwan
* Corresponding author: steven.defeyter@kuleuven.be and tobe@chem.es.osaka-u.ac.jp

Stereocontrolled multilayer growth of supramolecular porous networks at the interface between graphite and a solution was investigated. For this study, we designed a chiral dehydrobenzo[12]annulene (DBA) building block bearing alkoxy chains substituted at the 2 position with hydroxy groups, which enable van der Waals stabilization in a layer and potential hydrogen-bonding interactions between the layers. Bias voltagedependent scanning tunneling microscopy (STM) experiments revealed the diastereospecificity of the bilayer with respect to both the intrinsic chirality of the building blocks and the supramolecular chirality of the self-assembled networks. Top and bottom layers within the same crystalline domain were composed of the same enantiomers but displayed opposite supramolecular chiralities.

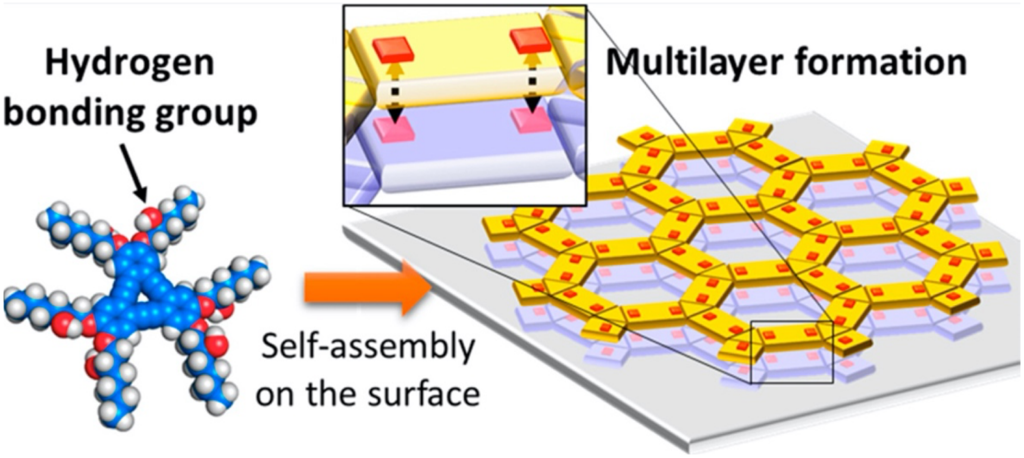


Figure 1. Schematic of a self-assembled porous double-layer structure formed by an alkoxy-substituted dehydrobenzo[12]annulene (DBA) molecules.

Time resolved scanning ion conductance microscopy: taking a gentle peak at cell surface dynamics.

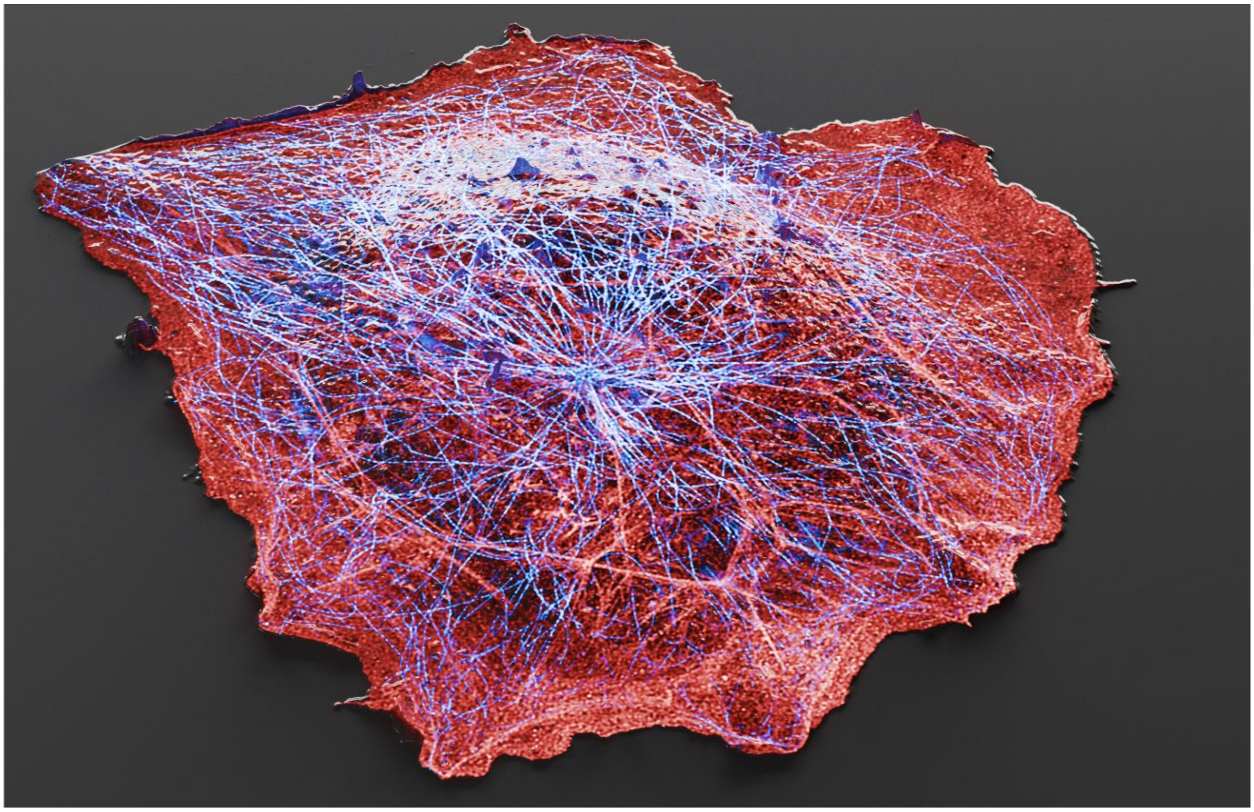
Georg Fantner

Laboratory for Bio- and Nano-Instrumentation, Interfaculty Institute for Bioengineering, École Polytechnique Fédéral de Lausanne, Lausanne, Switzerland.

The evolution of the 3D morphology of cells is at the heart of many biologically relevant process ranging from stem-cell differentiation, to cancer metastasis. Things become even more interesting when looking at the evolution of systems comprising the interaction of multiple cells such as the growth of organoids, the formation of networks by neurons or the infection of cells by pathogens. When studying these systems, the information we are after is not just in their static structure, but in how this structure changes over time. Time resolved imaging has proven to be an invaluable tool in this regard. While many excellent optical microscopy techniques exist for time resolved imaging in the sub-micrometer scale at both 2D and 3D, the options for 3D time lapse characterization at the nanometer scale are very limited. Atomic force microscopy has long since promised a solution but has struggled to deliver except for cases of relatively sturdy cells such as bacteria[1,2] or yeast.

Scanning ion conductance microscopy (SICM) on the other hand has been developed specifically for imaging of fragile surfaces of eukaryotic cells[3]. This true non-contact technique is ideally suited for label-free imaging of cell surfaces and achieves exquisite resolution down to the nanometer regime[4,5]. The challenge to harness this technique for time resolved 3D nanocharacterization of living cells lies in the relatively slow imaging speed of SICM. In this presentation I will show how we apply what we have learned from high-speed AFM to the field of SICM. By reengineering the SICM microscope from the ground up, we were able to reduce the image acquisition time for SICM images to 0.5s while extending the imaging duration to days[6]. I will also discuss the combination of 3D surface data from SICM with 2D and 3D volume data from SOFI imaging for correlative high resolution imaging of the cell interior as well as the cell membrane[7].

1. Eskandarian, H. A. *et al.* Division site selection linked to inherited cell surface wave troughs in mycobacteria. *Nat. Microbiol.* **2**, 17094 (2017).
2. Odermatt, P. D. *et al.* Overlapping and essential roles for molecular and mechanical mechanisms in mycobacterial cell division. *Nature Physics* (2019) doi:10.1038/s41567-019-0679-1.
3. Hansma, P., Drake, B., Marti, O., Gould, S. & Prater, C. The scanning ion-conductance microscope. *Science (80-.).* **243**, 641–643 (1989).
4. Novak, P. *et al.* Nanoscale live-cell imaging using hopping probe ion conductance microscopy. *Nat. Methods* **6**, 279–281 (2009).
5. Korchev, Y. E., Bashford, C. L., Milovanovic, M., Vodyanoy, I. & Lab, M. J. Scanning ion conductance microscopy of living cells. *Biophys. J.* **73**, 653–658 (1997).
6. Leitao, S. M. *et al.* Time-resolved scanning ion conductance microscopy for three-dimensional tracking of nanoscale cell surface dynamics. *bioRxiv* 2021.05.13.444009 (2021) doi:10.1101/2021.05.13.444009.
7. Navikas, V. *et al.* Correlative 3D microscopy of single cells using super-resolution and scanning ion-conductance microscopy. *bioRxiv* (2020) doi:10.1101/2020.11.09.374157.



Correlative imaging using scanning ion conductance microscopy and stochastic optical fluctuation imaging (*bioRxiv* (2020) doi:10.1101/2020.11.09.374157)

Multiparametric analytical quantification of materials at nanoscale in tapping force microscopy

Amir Farokh Payam, Alessio Morelli and Patrick Lemoine

Nanotechnology and Integrated Bioengineering Centre (NIBEC), School of Engineering, Ulster University,
Jordanstown Shore Road, Northern Ireland BT37 0QB, UK.

Atomic force microscopy (AFM) is a powerful technique for accurate, reliable and non-destructive imaging and characterization of materials at the nanoscale [1]. Among the numerous AFM methods, amplitude modulation or tapping mode AFM (AM-AFM) is an established method for imaging and characterization for most commercial AFM systems [3]. Despite its high spatial resolution and sensitivity, quantitative characterization by AM-AFM lag behind other advanced AFM methods as far as quantification of materials properties is concerned [2]. In this work a fully analytical multiparametric approach for AM-AFM is proposed which simultaneously quantifies the Hamaker constant and viscoelastic properties of materials [4]. The main advantage of the proposed method lies in the inclusion of adhesion to calculate viscoelasticity, which makes it superior to the current equations used in the AFM community. The accuracy of the proposed method is validated by several simulations and experiments and comparison with nanoindentation results, which strongly support its candidacy as a method of choice for material properties quantification by dynamic AFM.

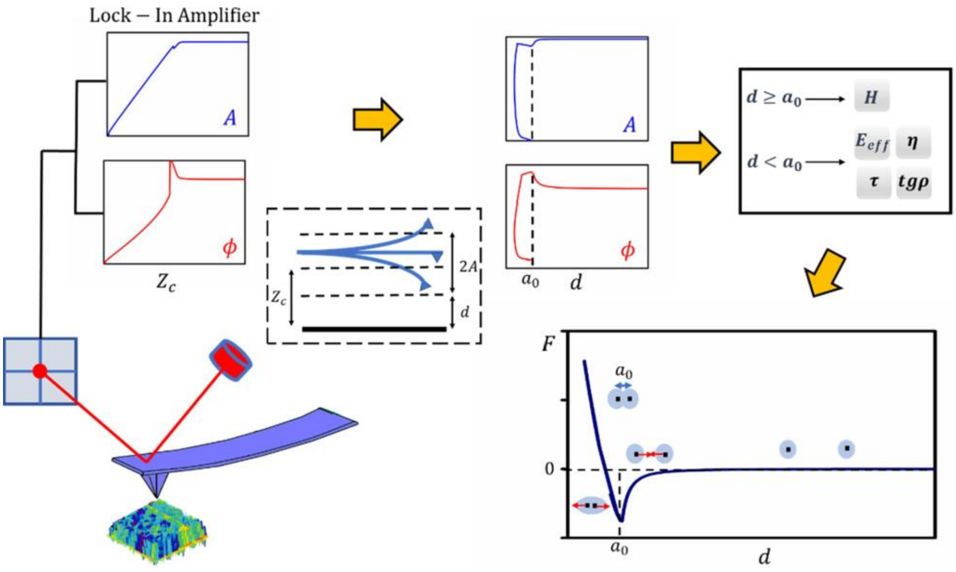


Fig. 1. Schematic of proposed method. The probe interaction with the sample's surface (bottom left) is acquired in the form of amplitude and phase vs average distance between tip and sample (top left). Calculating the closest distance from the amplitude of cantilever oscillation and the average separation distance (middle center), the curves are converted (top center). By applying the proposed equations on the observable data using the known cantilever parameters, the effective Hamaker constant and Young modulus are calculated (top right). Using the definition of energy dissipation and virial, the resonance frequency of cantilever and the obtained Young modulus, the coefficient of viscosity is calculated (up right). Finally, the calculated parameters are used to reconstruct the force based on DMT force model (bottom right).

References

1- R. Garcia, *Chemical Society Reviews,* 2020, 49 (16), 5850-5884.
2- R. García, R. Magerle, R. Perez, *Nature Materials*, 2007, 6, 405-411.
3- A. F. Payam. D. Martin-Jimenez, R. Garcia, *Nanotechnology*, 2015, 26 (18), 185706.
4- A. F. Payam, A. Morelli, P. Lemoine, *Applied Surface Science*, 2021, 536, 147698.

Nanoscale investigation of functional properties of piezoelectric PVDF-based nanocomposites

Anthony Ferri[1*], Antonio Da Costa[1], Juliette Defebvin[2], Marie-Hélène Chambrier[1], Adeline Marin[2], Rudy Bourrez[1], Sébastien Leroy[1], Sophie Barrau[2], Jean-Marc Lefebvre[2], Rachel Desfeux[1]

[1.] Université Artois, CNRS, Centrale Lille, ENSCL, Université Lille, UMR 8181, Unité de Catalyse et Chimie Du Solide (UCCS), F-62300, Lens, France.
[2.] Université Lille, Sciences et Technologies, CNRS, ENSCL, INRA, UMR 8207, Unité Matériaux Et Transformations (UMET), F-59655, Villeneuve D'Ascq, France.
* Corresponding author: anthony.ferri@univ-artois.fr

Polymers with electroactive properties provide numerous opportunities for applications in domains such as biomedicine or energy generation owing to their flexibility, good mechanical acoustic impedance and biocompatibility compared to ceramic materials. Among this class of polymers, poly(vinylidene fluoride) (PVDF) is a semi-crystalline polymer with unique piezo-, pyro- and ferroelectric properties allowing its use for various development in sensors, actuators and energy harvester systems. Furthermore, the addition of nanofillers is an interesting way to strongly enhance properties of such fluoropolymers. In this context, advanced atomic force microscopy tools are fully suitable for probing the response of the nanometric inclusions, which is a crucial step for optimizing macroscopic performance of devices based on such piezocomposites.

In our work, both carbon nanotubes/PVDF and stretched barium titanate ($BaTiO_3$)/PVDF nanocomposites were studied. By means of a combination of several AFM modes, such as Tapping mode AFM for surface morphologies, piezoresponse force microscopy (PFM) for piezo-/ferroelectric properties, conductive-AFM for electrical conductivity and amplitude modulation−frequency modulation (AM-FM) method for nanomechanical behavior, a deep investigation on the microstructure and physical properties of the composites were performed on the nanoscale in addition to macroscopic studies. As significant results, (i) the direct identification of PVDF α- and γ-phases was successfully achieved (figure 1), enabling better understanding of the impact of the carbon nanotubes on the structural and functional properties of the PVDF-based nanocomposites [1], while (ii) the ability of the PFM tool to directly image individual piezoelectric nanometric fillers ($BaTiO_3$) embedded into a polymer matrix and control polarization as well was demonstrated [2], allowing for in-depth study of electroactive composites on the nanoscale.

[1] S. Barrau, A. Ferri, A. Da Costa, J. Defebvin, S. Leroy, R. Desfeux, J.-M. Lefebvre, ACS Applied Materials & Interfaces **10** (2018) 13092-13099.
[2] A. Ferri, S. Barrau, R. Bourez, A. Da Costa, M.-H. Chambrier, A. Marin, J. Defebvin, J.-M. Lefebvre, R. Desfeux, Composites Science and Technology **186** (2020) 107914.

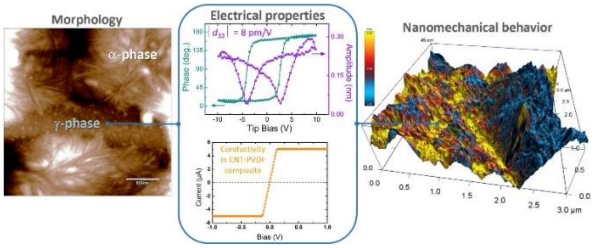


Figure 1. The local behavior of PVDF *γ*-phase induced by carbon nanotube incorporation has been investigated by atomic force microscopy tools [1].

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

Takeshi Fukuma[1, *]

[1.] Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, 920-1192 Kanazawa, Japan.

* Corresponding author: fukuma@staff.kanazawa-u.ac.jp

Recently, three-dimensional atomic force microscopy (3D-AFM) has been proven to be a powerful tool for investigating various structures and phenomena at solid-liquid interfaces [1,2]. In the method, a tip is scanned in the *XY* and *Z* directions in a 3D interfacial space. During the tip scan, the variations in the force applied to the tip is recorded to produce a 3D force image. At a solid-liquid interface, the tip interacts with surrounding solvent molecules during the tip scan. Thus, the obtained 3D image represents the distribution of solvent molecules. So far, the method has been used for visualizing 3D hydration structures on minerals, organic thin films, and biological systems with subnanometer-scale resolution. This emerging technology has attracted attention due to its potential applications in the research on interfacial control technologies for anti-fouling, lubrication, anti-freezing, colloidal dispersion, cosmetics and cleaning.

In the meanwhile, here I would like to draw attention to another important implication of the success of the 3D hydration measurements. In the AFM community, it has been a common sense that we should fix atoms or molecules to a solid surface to visualize them with atomic or molecular resolution. However, 3D-AFM allows us to visualize subnanometer-scale distribution of mobile water molecules that are not fixed on a solid surface. This is a big surprise and may lead to the breakthrough for the aforementioned limitation of AFM. Then, the next question would be what is the requirements to be visualized by 3D-AFM. We believe that the answer is capability of self-organization. For example, in the case of 3D hydration measurements, the hydration structure is significantly disturbed during the vertical tip scan yet it is quickly recovered before starting the next vertical scan. Such a self-organization capability is essential for visualizing inside of 3D structures. One may think this is too severe condition yet we can find large number of important 3D self-organizing systems in both natural and artificial environments. Examples include interfacial phenomena and devices (hydration, lubrication, electric double layer devices and liquid crystal devices) to biological systems (cells, nucleus, chromosomes and proteins). 3D-AFM may allow us to directly visualize inside of these various 3D self-organizing systems.

Based on this idea, we have recently started to explore inside of various 3D self-organizing systems. At polymer-water interfaces, gel-phase polymer chains with a thickness of a few nanometers were visualized. At an ionic liquid - Au electrode interface, ordered ionic liquid distributions with ~5 nm thickness were visualized. Furthermore, a carbon nanotube (CNT) tip was developed and used for visualizing inside of chromosomes with a thickness higher than 500 nm. Finally, a focused ion milled Si needle probe was fabricated and used for visualizing inside of a live cell with a thickness of several microns. With these examples, here I would like to propose to apply 3D-AFM not only for visualizing hydration structures but also for imaging inside of various 3D self-organizing systems.

[1] T. Fukuma, Y. Ueda, S. Yoshioka, H. Asakawa, “Atomic-Scale Distribution of Water Molecules at the Mica-Water Interface Visualized by Three-Dimensional Scanning Force Microscopy”, Phys. Rev. Lett. 104 (2010) 016101.
[2] T. Fukuma, R. Garcia, “Atomic- and Molecular-Resolution Mapping of Solid−Liquid Interfaces by 3D Atomic Force Microscopy”, ACS Nano 12 (2018) 11785−11797.

AFM force-distance curve viscoelastic characterization of single cells

Juan G. Sánchez[1], Francisco M. Espinosa[1], Ruben Miguez[1] and Ricardo Garcia[1,*]

[1.] Instituto de Ciencia de Materiales de Madrid, CSIC, Madrid, Spain

* Corresponding author: r.garcia@csic.es

The atomic force microscope has significantly contributed to advance mechanobiology (1-2). In turns, mechanobiology has stimulated the development of advanced force microscope methods and models to determine the viscoelastic response of a living cell (3-5).

In this contribution, we show that the nanomechanical response of eukaryotic cells depends on the spatial (nucleus versus cytoplasm) and temporal (force rate) properties. Here, we discuss some relevant factors that influence the quantitative accuracy of AFM measurements. Specifically, we study the choice of the linear viscoelastic model used to parameterize the response, the deformation rate or the influence of the stiffness of the solid support on the mechanical property values. We show that the stiffness of the solid support might lead to an overestimation of the Young's modulus by about 15%. However, this effect is corrected by using a bottom-effect theory (3). We show that the use of elastic contact mechanics models leads to overestimate the effective Young's modulus of cells. The agreement obtained between AFM nanomechanical measurements performed on fibroblasts and HeLa cells and a power-law viscoelastic model supports the quantitative accuracy of the experimental data.

References:

[1] Y. F. Dufrêne, et al. Nat. Nanotechnol. **12** (2017), 295–307.
[2] G. Zhou et al. Advances in Physics: X **6** (2021).
[3] P.D. Garcia and R. Garcia. Nanoscale **10** (2018) 19799–19809.
[4] Y.M. Efremov et al. Sci. Rep. **7** (2017) 1–14.
[5] B.R. Bruckner, H. Nöding and A. Janshoff, Biophys. J. **112** (2017) 724-735.

Nonstationary Fourier Mode Decomposition: A New Time-Frequency Analysis Method for Time-Resolved Electrostatic Force Microscopy

Rajiv Giridharagopal,[1*] Daniel E. Shea,[2] Steven L. Brunton,[3] J. Nathan Kutz,[4] David S. Ginger[1]

[1.] Department of Chemistry, University of Washington, Seattle, USA
[2.] Department of Materials Science, University of Washington, Seattle, USA
[3.] Department of Mechanical Engineering, University of Washington, Seattle, USA
[4.] Department of Applied Mathematics, University of Washington, Seattle, USA
* Corresponding author: rgiri@uw.edu

One of the most exciting developments in atomic force microscopy (AFM) over the past decade is the emergence of "big data" approaches to analyzing cantilever motion to extract hidden information. Such methods have enabled new insight into dynamic processes at the nanoscale by applying time-frequency analysis to cantilever motion. One method, time-resolved electrostatic force microscopy (trEFM) can measure dynamic information at sub-microsecond timescales by applying time-frequency analysis to the cantilever's response to a transient stimulus such as photoexcitation of a solar cell [1-2]. In dynamic AFM methods like trEFM, the cantilever motion is a *nonstationary process* where the frequency changes with time. Extracting this instantaneous frequency is a difficult problem in signal processing.

Here, we present a new method that circumvents the limitations of conventional time series methods to extract instantaneous frequency and apply that to trEFM. This method, nonstationary Fourier mode decomposition (NFMD), combines stochastic gradient descent, Fourier mode decomposition [3], and knowledge of the oscillator physics to achieve two critical results. First, we show that NFMD extracts the instantaneous frequency with significantly lower noise (Fig. 1A). Second, NMFD can measure the time constant of the excitation *directly*, providing a significant improvement to conventional trEFM (Fig. 1B) by extracting otherwise hidden information. We demonstrate this method on test cases such as ion motion in doped polymer systems and charge generation dynamics in perovskites. NFMD therefore provides a powerful new approach to explore time-frequency analysis in dynamic AFM measurements.

References:

[1] R. Giridharagopal, et al. *Nano Lett.* **12** (2012), p. 893-898
[2] D. U. Karatay, et al. *Rev. Sci. Instrum.* **87** (2016), p. 053702
[3] H. Lange, et al. *J. Mach. Learn. Res.* **22** (2021), p. 1-38

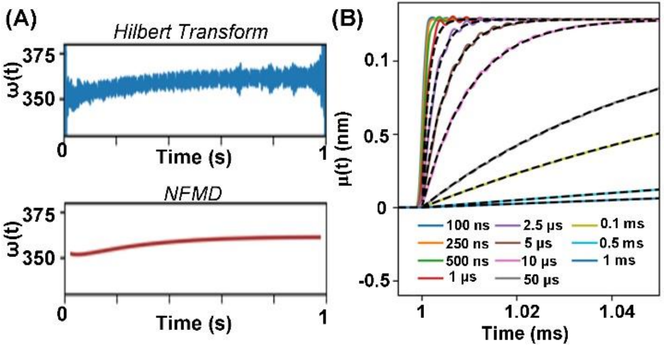


Figure 1. (A) Instantaneous frequency in AFM simulations extracted by Hilbert transform (top) compared to NFMD (bottom) (B) NFMD (solid) can extract the ground truth time constants (dashed) in real data.

Mapping the electric properties at the nanoscale in electrolyte solutions

Gabriel Gomila

Department of Electronic and Biomedical Engineering, University of Barcelona, C/ Marti i Franquès, 1 08028-Barcelona, Spain
Nanoscale Bioelectrical Characterization, Institut de Bioenginyeria de Catalunya (IBEC), The Barcelona Institute of Science and Technology, Baldiri i Reixac 11, 08028, Barcelona, Spain.
E-mail: ggomila@ibecbarcelona.eu

Mapping the electric properties of materials at the nanoscale in electrolyte solutions is of great interest in Life and Materials Sciences. Here, I will review the developments we performed in this field of research in recent years by using in-liquid Scanning Dielectric Microscopy in force detection mode [1]. In-liquid Scanning Dielectric Microscopy is a scanning probe microscopy technique in which a metallic coated atomic force microscopy probe, with an alternating voltage applied, is used to probe the local electric force acting on it when interacting with the sample of interest. From the measured electric force, and by using suitable theoretical models, the local electric properties of the samples (conductivity and/or dielectric constant) can be obtained with nanoscale spatial resolution. The basics of the technique [1], including some relevant theoretical aspects [2], will be presented together with some examples of application. Among the applications I will consider the mapping of the dielectric properties of planar supported lipid bilayers [3], [4], self-assembled monolayers [5], single liposomes [6], fixed eukaryotic cells [7] and electrolyte gated field effect transistors under operation [8]. I will close the presentation with a discussion on the future perspectives of the technique.

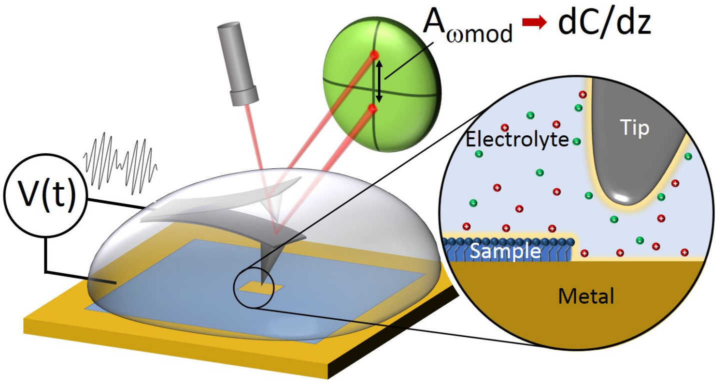


Figure: Schematic representation of in-liquid Scanning Dielectric Microscopy in force detection mode.

[1] G. Gramse, et al., Appl. Phys. Lett. 101, 213108, (2012).
[2] M. Checa, et al., Phys. Rev. E 100, 022604, (2019).
[3] G. Gramse et al., Biophys. J. 104, 1257, (2013).
[4] M. Di Muzio et al. Langmuir 36, 12963 (2020).
[5] R. Millan-Solsona, et al. Nanoscale 12, 20658, (2020).
[6] M. Di Muzio et al. (submitted)
[7] M. Checa et al. (in preparation)
[6] A. Kyndiah, et al. Adv. Func. Mater. 31, 2008032, (2020).

Dynamic force quadratures and the viscoelastic response of soft material surfaces

David B. Haviland[1*], Daniel Forchheimer[2], Riccardo Borgani[1] and Per-Anders Thorén[1].

[1.] KTH Royal Institute of Technology, Department of Applied Physics, Stockholm, Sweden.
[2.] Intermodulation Products AB, Segersta, Sweden.
* Corresponding author: haviland@kth.se.

AFM nano-mechanics typically characterizes soft materials with a local modulus, determined by fitting force-distance curves to an idealized model borrowed from macroscopic contact mechanics. The method breaks down at higher tip velocity were viscous force becomes significant. Furthermore, the very large curvature of an AFM contact enhances capillary force (Laplace pressure) in comparison with macroscopic contacts. The capillary force, derived from *interfacial* energy and surface curvature, is independent of elastic modulus, describing force resulting from *volumetric* deformation. Soft-mater nano-mechanics needs a new paradigm; one based on dynamic analysis of tip-surface force and interaction models which include interfacial energy.

Dynamic force quadratures are part of this new paradigm [1]. Closely related to Dynamical Mechanical Analysis (DMA) used in the study soft materials in bulk, force quadratures result from a cyclic measurement of force. They reveal the elastic and viscous components of the tip-surface force at the cantilever resonance frequency, as functions of the oscillation amplitude A, at fixed probe height. The conservative force quadrature $F_I(A)$ displays the component of force in-phase with the cantilever motion. Similarly, the dissipative force quadrature $F_Q(A)$ displays the component in-phase with the velocity. These 'dynamic force curves' are rapidly acquired (typically 2 ms per curve) and thus well suited to high-density nanomechanical mapping. AFM Force quadrature curves on soft materials reveal viscoelastic surface dynamics [2]. Hysteresis in $F_I(A)$ and $F_Q(A)$ is due to the relaxation time of the surface exceeding the period of cantilever oscillation. We incorporated this relaxation time in to a one-dimensional moving surface model which includes capillary forces in an approximate fashion [3].

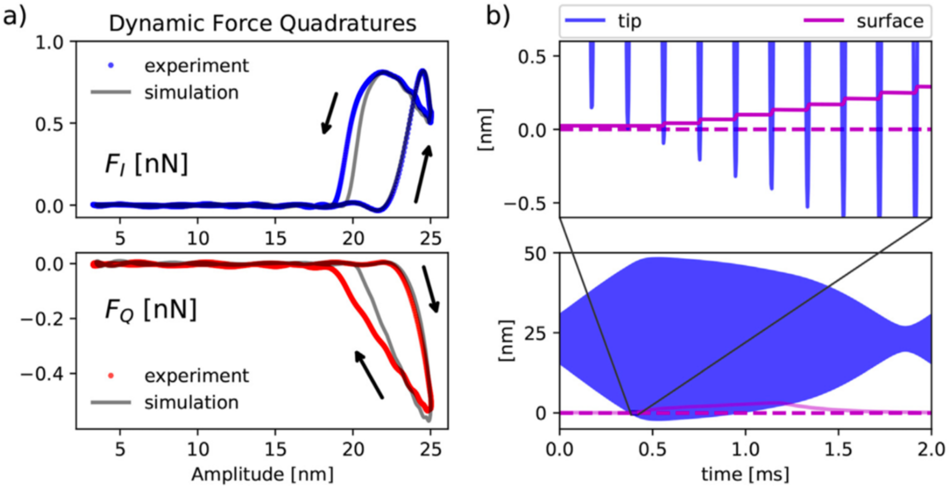


Fig. 1: Taken from [2]. Force quadrature curves measured on a rubber region of a thermoplastic alloy.

[1] D. B. Haviland, Current Opinion in Colloid & Interface Science **27,** 74 (2016).
[2] H. G. Kassa *et al.*, Polymer **135**, 348 (2017).
[3] P. A. Thorén *et al.*, Phys. Rev. Applied **10**, 024017 (2018).

Quantitative Viscoelastic Mapping of Cellulose Nanofibrils Using Low-Total-Force Contact Resonance Force Microscopy

Kristen Hess[1*], Jason Killgore[2] and Wil Srubar[1]

[1.] Department of Civil, Environmental, and Architectural Engineering University of Colorado Boulder, Boulder, Colorado 80309, United States
[2.] Applied Chemicals and Materials Division, National Institute of Standards and Technology, Boulder, Colorado 80305, United States
* Corresponding author: khess@colorado.edu

In this work, a new atomic force microscopy (AFM) characterization method has been developed to quantify the viscoelastic loss tangent (tan δ) of stiff rod-shaped cellulose nanofibrils (CNFs) with thin diameters (5 nm to 15 nm). The method is called low-total-force contact resonance force microscopy (LTF CR-FM) and uses Brownian motion to achieve the thermally limited lowest dynamic force, while approaching adhesive pull-off to achieve the lowest static force. The two-fold advantage of this approach is (1) to minimize substrate and geometry effects by limiting the dynamic stress field to within the CNF cross section and (2) to quantify local instead of bulk tan δ at spot locations along the CNF structure by taking measurements at single points. The LTF CR-FM measurements were shown to generate analyzable data without evidence of nonlinear resonance softening and without damage to the CNF over static forces ranging from 11.6 nN to 84.6 nN. The measured tan δ of CNFs was 0.015 ± 0.0094, which is the first reported tan δ measurement of an isolated CNF. Finally, as shown in **Figure 1**, LTF CR-FM successfully mapped tan δ along the length of CNFs. However, no significant change in tan δ in the transverse direction was observed over kinked defects, which are known to affect nanomechanical and biofuel processing performance, compared to straight sections. This result may be attributed to the length scale of the measurement.

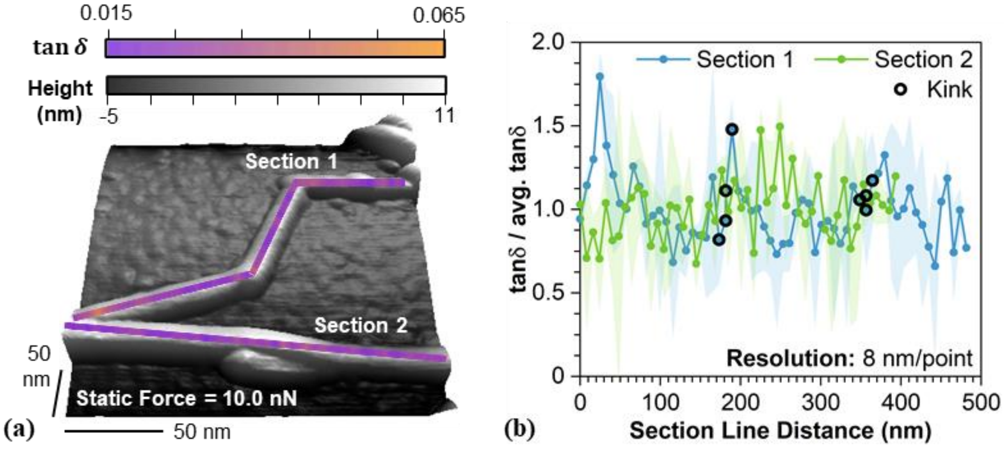


Figure 1. AFM topographical image (a) of 2 selected CNF arrangements for mapping tan δ and a corresponding plot (b) of tan δ at points along the section line normalized by the average tan δ value for the entire section line. To determine tan δ along the top of the CNF, the computed tan δ was correlated to the height measurement taken within the same single point CR-FM measurement. As such, the section lines along the nanofibrils shown in the AFM topographical images (a) correspond to the approximate location of the tan δ result and shown for illustration. The average tan δ values (b) are 0.028 and 0.026 for Sections 1 and 2, respectively. The plotted error (b) represents the coefficient of variation.

IgGs are walking and oligomerize on antigenic surfaces

Jürgen Strasser[1], Rob N. de Jong[2], Frank J. Beurskens[2], Janine Schuurman[2],
Paul W. H. I. Parren[3,4], Johannes Preiner[1] and Peter Hinterdorfer[5*]

[1.] University of Applied Sciences Upper Austria, Linz, Austria.
[2.] Genmab, 3584 CM Utrecht, Netherlands.
[3.] Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Netherlands.
[4.] Lava Therapeutics, ‘s Hertogenbosch, Netherlands.
[5.] Johannes Kepler University Linz, Linz, Austria.
[*] Corresponding author: peter.hinterdorfer@jku.at

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

[1] J Preiner et al., Nature Comm. **5** (2014), p. 4394.
[2] CA Diebolder et al., Science **343** (2014), p. 1260.
[3] RN Jong et al., PLOS Biology **14** (2016), e1002344.
[4] J Strasser et al., Nano Lett. **19, 7** (2019), p.4787.
[5] J Strasser, et al., ACS Nano (2019), doi.org/10.1021/acsnano.9b08347.

Customized MFM probes based on magnetic nanorods

Miriam Jaafar[1,2*], Javier Pablo-Navarro[3], Eider Berganza[2], Pablo Ares[1], César Magén[3,4], Julio Gómez-Herrero[1], Aurélien Masseboeuf [5], Christophe Gatel[5], Etienne Snoeck[5], Jose Maria de Teresa[3,4], Agustina Asenjo[2]

[1]Departamento de Física de la Materia Condensada and Condensed Matter Physics Center (IFIMAC), Universidad Autónoma de Madrid, 28049, Madrid, Spain.
[2]Instituto de Ciencia de Materiales de Madrid, ICMM-CSIC. Cantoblanco, Madrid, Spain,
[3]Instituto de Nanociencia y Materiales de Aragón (INMA), Universidad de Zaragoza-CSIC, 50009 Zaragoza, Spain,
[4]Laboratorio de Microscopías Avanzadas (LMA), Universidad de Zaragoza, 50018 Zaragoza, Spain.
[5]CEMES, CNRS, 29 rue Jeanne Marvig, F-31055 Toulouse, France
* Corresponding author miriam.jaafar@uam.es

Atomic Force Microscopy (AFM) is a powerful technique in biophysics and nanomedicine, since it allows imaging and manipulating nanostructures in physiological conditions on a single molecule level [1]. Magnetic Force Microscopy (MFM) is an AFM-based technique where a nanometric magnetic probe is scanned in close proximity to a surface detecting the local magnetic fields gradients near it. MFM has been applied to the study of a variety of magnetic systems, including magnetic nanoparticles. Despite the importance of studying magnetic nanostructures with biological applications in physiological conditions, the applicability of MFM to these systems was limited up to now because of the difficulty in developing MFM for detecting magnetic interactions in liquids. This is a consequence of the higher damping forces acting on the cantilever when working in liquid environment, as compared to air, which results in a significant loss of sensitivity of the MFM signal. In the work presented here, we start by introducing the necessary development for MFM imaging in liquid media [2] and discussing the influence of the chosen cantilever on the obtained MFM signal-to-noise ratio. Then, we present the development of new magnetic probes fabricated by Focused Electron Beam Induced Deposition (FEBID) using specially designed cantilevers for liquid medium and demonstrating that further improvement on the performance can be gained [3].

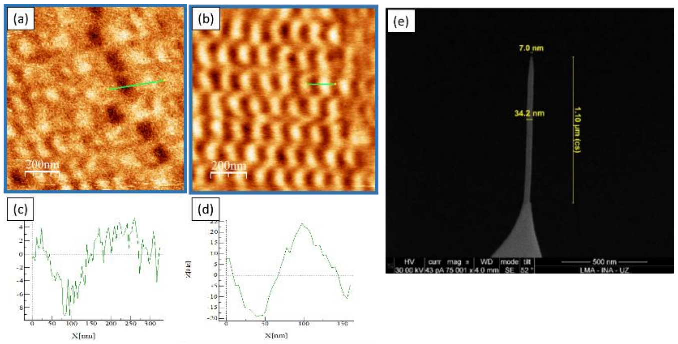


Figure 1: MFM images of a reference sample acquired in water with a commercial MFM tip (a) and with a nanorod tip (b). (c) and (d) are the corresponding profiles (e) SEM image of an Fe nanorod tip grown by FEBID.

[1] Y. F. Dufrêne et al. Nature Nanotechnology **12**, 295–307 (2017)
[2] P. Ares et al. Small **11**, 36, 4731–4736 (2015)
[3] M. Jaafar et al, Nanoscale, **12**, 10090–10097 (2020)

Integrated Simultaneous Chemical, Surface Potential, Mechanical, and Topographic Imaging at < 10 nm Spatial Resolution

Devon S. Jakob[1*] and Xiaoji G. Xu[1]
[1]Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania, United States.
*Corresponding Author: dsj316@lehigh.edu

Multimodal measurements of chemical composition, electrical properties, mechanical properties, and topography by atomic force microscopy (AFM) deliver correlations across properties at the nanoscale and provide clues to the structure-function relationship of materials. In the past, measurements with these modalities are operated separately with different operational modes of AFM. Not only do sequential measurements require additional operation time and are subject to scanner/sample drift, but also, different modalities of SPM have different spatial resolutions, which undermine correlative analysis. For example, the popular frequency-modulated Kelvin probe force microscopy measures the surface potential with 30~50 nm spatial resolution under ambient conditions, whereas the AFM measurements of chemical composition, mechanical properties, and topography can routinely achieve < 10 nm spatial resolution.

At the conference, we will present our recent invention of an integrated AFM technique that can simultaneously provide chemical, surface potential, mechanical, and topographic imaging at < 10 nm spatial resolution in ambient conditions. We name it peak force infrared-Kelvin probe force microscopy (PFIR-KPFM), as we achieved it through an integration of peak force infrared microscopy and pulsed force Kelvin probe force microscopy. In a single scan, the integrated PFIR-KPFM delivers simultaneous multimodal measurement at a comparable and high spatial resolution of < 10 nm. As a demonstration, we measured a naturally-degraded $CH_3NH_3PbBr_3$ perovskite single crystal. AFM topography, mechanical modulus, contact potential difference (CPD), and nano-IR imaging at infrared absorption of perovskite are simultaneously acquired and shown in **Figure 1a-d**, respectively. Correlation analysis among infrared absorption, mechanical modulus, and CPD are performed and displayed in **Figure 1e-f**.

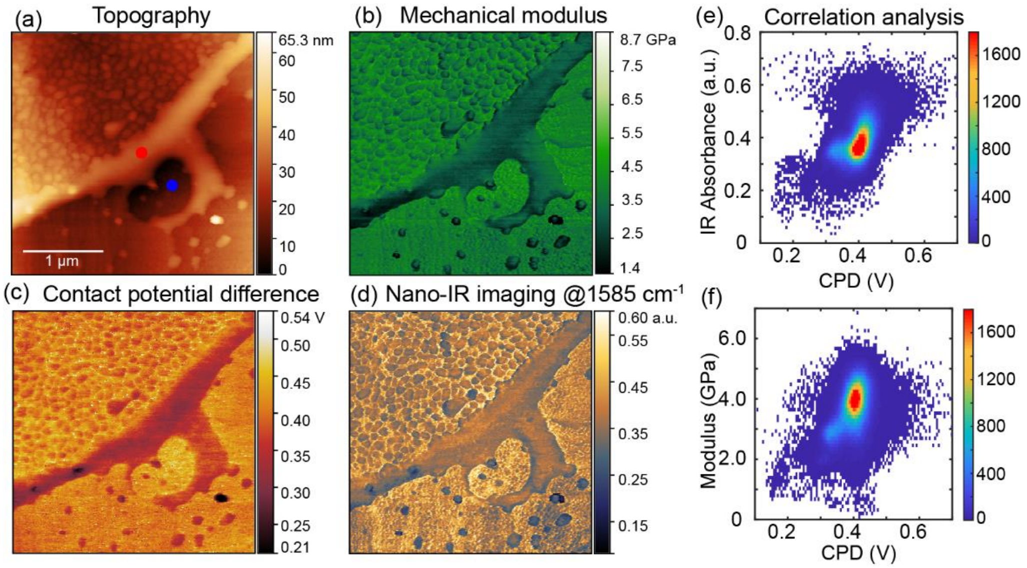


Figure 1. PFIR-KPFM measurement of a naturally degraded $CH_3NH_3PbBr_3$ perovskite crystal. (a) Topography. (b) Modulus. (c) CPD. (d) The nano-IR image at the infrared frequency of 1585 cm^{-1} (e) Correlation between CPD and IR signal. (f) Correlation between CPD and modulus.

Fractional Differential Equation-Based Viscoelastic Material Characterization Using Atomic Force Microscope Contact Resonance Spectroscopy

Md Tasmirul Jalil[1], Rafiul Shihab[1], and Ryan Tung[1*]

[1]Department of Mechanical Engineering, University of Nevada Reno, NV USA
[*]Corresponding author: rtung@unr.edu

In AFM, methods such as Contact Resonance (CR) Spectroscopy [1] have been used to characterize viscoelastic material properties over a wide range of frequencies. CR operates in the net-repulsive contact regime and uses information about the in-contact natural frequencies and quality factors of the system. This information, coupled with a suitable mathematical material model, is used to estimate the sample's material properties. Traditionally, integer-order, differential equation-based viscoelastic material models are used to extract the material properties. However, these models accurately predict properties for a narrow frequency band and exhibit challenges while predicting properties for a larger frequency range, including: ringing phenomena and poor extrapolation properties. These models are also numerically cumbersome, due to the large array of parameters they require. These challenges, however, can be overcome by utilizing fractional differential equation-based viscoelastic material models.

In this work, we begin by analyzing the capabilities of fractional models to capture the behavior of viscoelastic polymers over a wide frequency range. Broad band, bulk complex modulus values of different polymers are taken from the literature and are used as a reference [2]. This reference data, along with a Hertzian contact mechanics model and a traditional CR model, are used to calculate our test data, which consists of the calculated in-contact natural frequencies and quality factors of several "fictitious" numerical test cantilevers, for several eigenmodes for each material. The test data spans several decades of frequency for each material by use of many fictitious test cantilevers. Note that, for each cantilever the calculated spring and damping parameters for the material under test will be different. Next, an analytical Contact Resonance model of an AFM cantilever with a fractional viscoelastic element at its tip is derived. Using the test data and the characteristic equation of the fractional cantilever, the global fractional model parameters of the system are calculated – which are valid for all test cantilevers.

To experimentally validate the idea, CR spectroscopy experiments, along with creep and relaxation experiments, are conducted on the same polymers used in the numerical study. A Time Temperature Superposition (TTS) scheme is used to obtain a broadband, frequency-dependent complex modulus master curve for each material. Finally, a fractional viscoelastic model is applied to these experimental data and corresponding material parameters are extracted. A comparative study regarding the capability of capturing the power-law behavior observed for these materials, in terms of a fractional model and its classical integer-order counterpart model is also presented.

References:

[1] U Rabe, and W Arnold, Applied Physics Letters, Vol. 64(12) (1994), p. 1492-1495.
[2] A Schmidt et al., Proceedings of IMAC XXVI, Vol. 3 (2008), p. 1557-1566.

Reovirus directly engages integrin to recruit clathrin for entry into host cells

Melanie Koehler[1], Simon J.L. Petitjean[1], Jinsung Yang[1], Pavithra Aravamudhan[2], Xayathed Somoulay[2], Cristina Lo Giudice[1], Mégane A. Poncin[1], Andra C. Dumitru[1], Terence S. Dermody[2*] and David Alsteens[1,3*]

[1.] Louvain Institute of Biomolecular Science and Technology, Université catholique de Louvain, Louvain-la-Neuve, Belgium
[2.] Department of Pediatrics, University of Pittsburgh School of Medicine, PA, USA
[3.] Walloon Excellence in Life sciences and Biotechnology (WELBIO), 1300 Wavre, Belgium
*Corresponding author: david.alsteens@uclouvain.be or terence.dermody@chp.edu

As all obligate intracellular microbes, reoviruses must cross the plasma membrane to enter host cells and requires the concerted action of viral and host factors. After interaction of reovirus attachment protein σ1 with cell-surface carbohydrates and proteinaceous receptors [1], additional host factors mediate virus internalization. In particular, β1 integrin is required for endocytosis of reovirus virions following junctional adhesion molecule A (JAM-A) binding. While integrin-binding motifs in the surface-exposed region of reovirus capsid protein λ2 are thought to mediate integrin interaction, evidence for direct β1 integrin-reovirus interactions and knowledge of how integrins function to mediate reovirus entry is lacking. Here, we present an experimental approach using a combination of single-virus force spectroscopy, confocal and fluidic force microscopy, to discover a direct interaction between reovirus and β1 integrins. We proved that λ2 is the viral ligand for integrin and identified a functional role for β1 integrin interaction in promoting clathrin recruitment to cell-bound reovirus, which may in turn facilitate endocytosis. Our study demonstrates a direct interaction between reovirus and β1 integrins and offers insights into the mechanism of reovirus cell entry [2]. These results provide new perspectives for the development of efficacious antiviral therapeutics and the engineering of improved viral gene delivery and oncolytic vectors.

References:

[1] M Koehler et al., Nat. Commun. **10** (2019) 4460
[2] M Koehler et al., Nat. Commun. **in press** (2021)

Remote Bias Induced Scanning Electrostatic Force Microscopy

J. J. Kopanski[1*], L. You[2], and J. Tibbs[3]

[1.]National Institute of Standards and Technology, Nanoscale Device Characterization Division,
Gaithersburg, MD 20889 (USA)
[2.]Micron Semiconductor Inc., Boise, ID 83707 (USA)
[3.]University of Northern Iowa, Cedar Falls, IA 50614 (USA)

The electrostatic force microscope (EFM) produces images proportional to the electrostatic force between the tip and sample. The force between the tip and sample depends on the geometrical capacitance between the tip and sample and the voltage difference (charge) between them. Usually, in EFM, the sample is grounded while an ac voltage is applied to the cantilevered tip to generate an oscillation. Since these forces act over long distances, EFM can image structures beneath the sample surface. We have produced a test chips with a series of subsurface structures with multiple independently biasable electrodes, Figure 1a. Using these structures, we were able to implement a mode of EFM where the AC+DC signal was applied to the buried structures instead of the cantilever, that is, remote bias electrostatic force microscopy (RB-EFM). An external high frequency lock-in amplifier (LIA) monitors the deflection signal of the cantilever, using the AC signal applied to the buried structure as its reference. Small changes in the phase of the cantilever oscillation can then be detected to map subtle electrostatic force variation between the subsurface metal lines and the EFM tip. With a test structure consisting of interdigitated lines, we found that when opposing sets of lines were biased with ac signals 180^{o} out of phase with each other, a substantial enhancement in spatial resolution was achieved. As the tip is scanned across the lines, the phase is forced to go to zero midway between the lines and amplitude is forced to a minimum (Figure 1b). We project that lines biased in this manner can be detected even if covered by 10's of micrometers of dielectric, comparable to the depth that MFM can detected current carrying buried lines. This technique may be useful for imaging buried metal lines in integrated back end of the line metallization. Other structures on the same chip contain of a small center electrode and a much larger surrounding electrode. In this case, if the surrounding electrode is biased with an ac voltage 180^{o} out of phase with the bias on the inner electrode, images formed of material covering the center electrode would only consist of the signal arising from the in-phase excitation of the center electrode. This type of self-shielding or co-axial electrode may result in enhanced spatial resolution in the region of the center electrode.

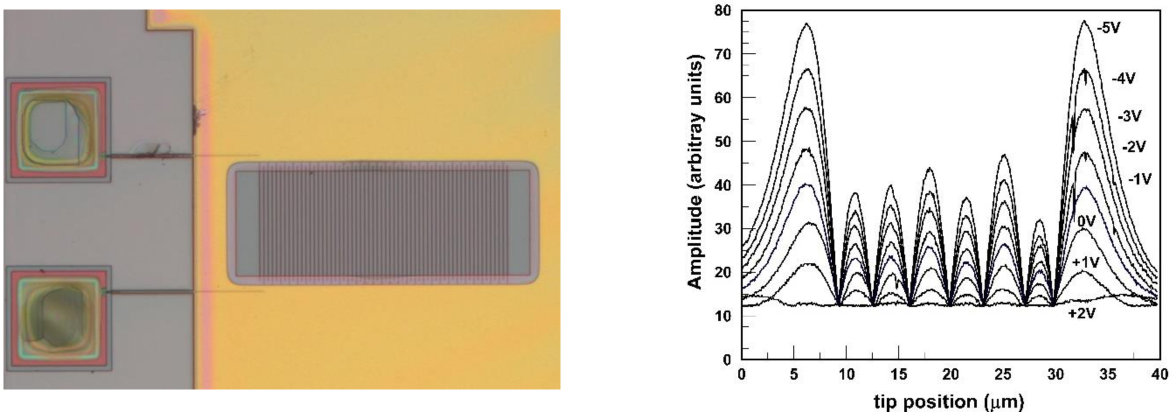


Figure 1 a) Test structure with interdigitated electrodes beneath 1 μm of dielectric. a) RB-EFM amplitude measured for a single set of interdigitated lines at dc bias voltages increasing from -5 V to +2 V in 1 V steps.

A New Approach for Ultra High Resolution Imaging of Polymers: an Outlook and Perspectives

Vladimir Korolkov[1]

[1.] Park Systems UK Ltd, Nottingham, United Kingdom

* Corresponding author: vladimir@parksystems.com

The structural studies of polymers is vital, since polymers are ubiquitous and varied in their use – from micellar drug carriers to bulletproof vests. Their exquisite molecular architecture provides polymers with a wide range of properties. The ability to image this molecular structure in real-space is, therefore, critical. The only technique that can perform such imaging is Atomic Force Microscopy.

In this work we will show how the use of higher eigenmodes imaging [1] provides a routine approach to achieving molecular, and in some instances submolecular resolution, on a wide range of polymers. This approach, unlike others, does not require any special cantilevers or custom modified AFM components. Here, we have implemented this technique on a commercial AFM – Park Systems NX20 – to achieve molecular resolution on a real-world samples of Teflon (Figure 1), polyethylene and i-polypropylene as well as epitaxially grown films of these polymers on 2D-materials.

We will discuss challenges and advantages of applying higher eigenmode imaging technique in structural studies of polymers.

[1] V. Korolkov et al., Nat. Commun. **10**, (2019), 1537.

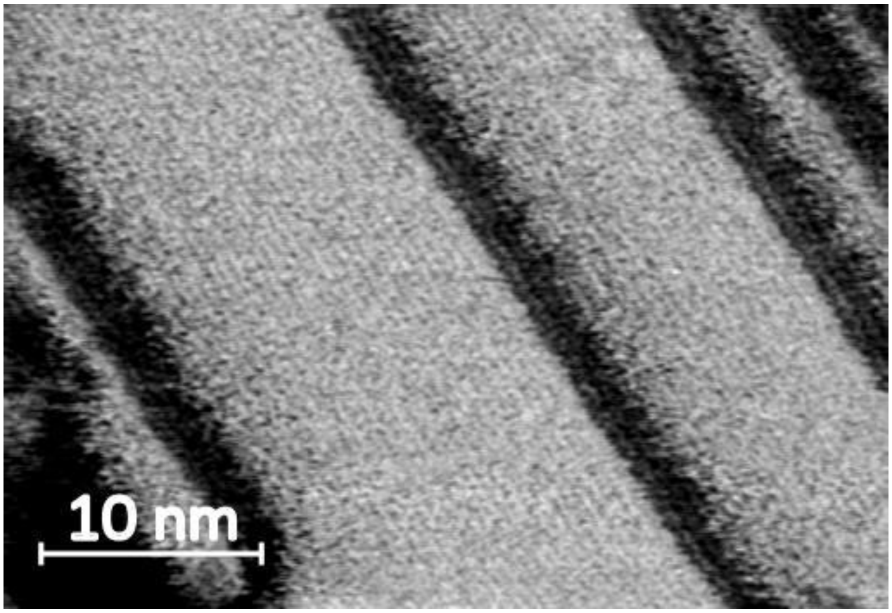


Figure 1. High resolution AFM images of Teflon surface showing single PTFE molecules with a molecule-to-molecule distance of ~5.6Å.

Cell wall stiffness loosening of *Egeria Densa* leaves interacting with functionalized gold nanoparticles

Thomas Le Néel[1*], Maxime Bayle[1] and Patricia Bertoncini[1*]

[1.] Institut des Matériaux Jean Rouxel, University of Nantes, CNRS UMR6502, Nantes, France.
* Corresponding authors: thomas.leneel@cnrs-imn.fr, patricia.bertoncini@cnrs-imn.fr

Gold nanoparticles are frequently used in emerging fields such as biotechnologies due to their remarkable optical properties. Therefore, these particles are brought in contact with various living samples, such as plant cells[1]. However, little information is available about their impact on the mechanical properties of living plants at sub-cytotoxic levels.

In this work, AFM force spectroscopy measurements were performed on cells present on mature leaf epidermis of *Egeria Densa* immersed in an aqueous solution containing functionalized gold nanoparticles. Force maps were recorded on top of cells with applied forces of 0.3 nN and 1.0 nN in order to investigate their wall stiffness at different depths[2]. Our measurements show a decrease of the cell wall stiffness whatever the nanoparticle functionalization, decrease that can be as high as one order of magnitude depending on nanoparticle concentration and time exposure. Moreover, the cell wall stiffness decreases more rapidly with a higher nanoparticle concentration in the surrounding water.

Force measurements were also performed on the same leaves after exchanging the solution containing the nanoparticles by water. An increase of the cell wall stiffness almost to its initial value after 4 hours is observed. This return to the original state suggests that the softening is directly due to the presence of the nanoparticles.

References:

[1] N. Li, P. Zhao, D. Astruc, Anisotropic Gold Nanoparticles: Synthesis, Properties, Applications, and Toxicity, Angew. Chem. Int. Ed. 53 (2014) 1756–1789.
[2] C. Roduit, S. Sekatski, G. Dietler, S. Catsicas, F. Lafont, S. Kasas, Stiffness Tomography by Atomic Force Microscopy, Biophysical Journal. 97 (2009) 674–677.

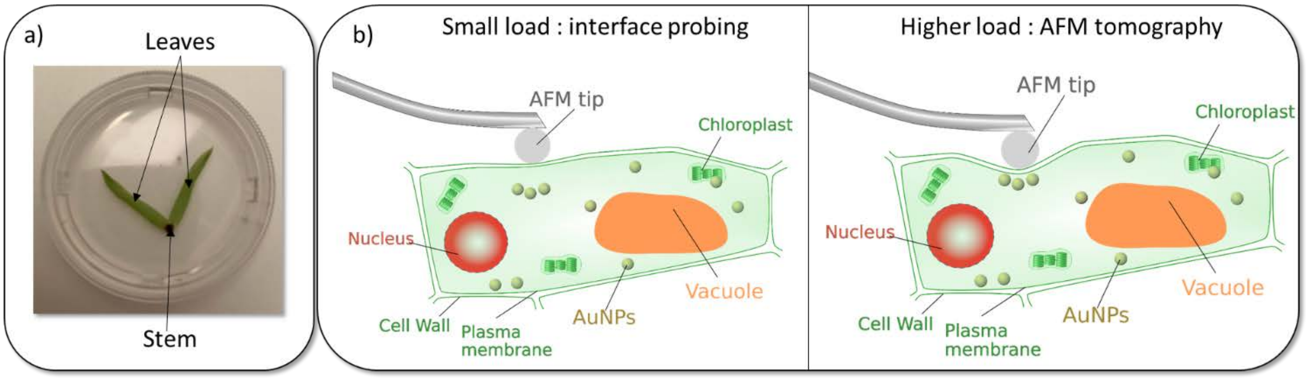


Figure 1. a) Photograph of a piece of Egeria Densa on top of an agarose gel and immersed in solution, and b) schematic illustrations of the AFM experiments: a high-density carbon colloidal tip is put in contact with the epidermis cell wall, and a force is applied in order to monitor the stiffness of the cell.

Modeling AFM in the SMFS mode by Steered Molecular Dynamics simulations: Effects on Molecular Recognition of Protein Adsorption on Silane Monolayers

Solène Lecot[1], Yann Chevolot[1], Christelle Yeromonahos[1] and Magali Phaner-Goutorbe[1*]

[1.] Univ Lyon, ECL, INSA Lyon, CNRS, UCBL, CPE Lyon, INL, UMR5270, 69130 Ecully, France.
* Corresponding author: magali.phaner@ec-lyon.fr

Protein adsorption on surfaces is used in biosensing tools as an immobilization mean to trap the analyte to be detected. However, adsorption can lead to conformational changes in the protein structure, resulting in a loss of bioactivity. Among surfaces, self-assembled monolayers of silane molecules are widely used to functionalize SiO_2, as the surface charge and hydropathy can be tuned by using silane molecules with different the head-group charges and alkyl chain lengths. The objective of this study is to decipher the impact of streptavidin adsorption on silane monolayers on its further interactions with biotin.

Molecular Dynamics (MD) simulations are well-suited to investigate protein-surface interactions and adsorption-induced conformational changes at atomic scale. Furthermore, Steered Molecular Dynamics (SMD) simulations, that mimic Atomic Force Microscopy (AFM) experiments, provide additional information regarding forces and dynamics of individual ligand-receptor interactions.

Firstly, a MD simulation system (GROMACS – OPLS-AA) was developed and several silane monolayers were modeled. Their structural properties were qualitatively validated by FTIR and XPS experimental results. Then, streptavidin adsorption on silane monolayers and its subsequent interactions with biotin were investigated by coupling MD and SMD simulations. It was shown that adsorption-induced conformational changes in streptavidin, which depend on the type of silane molecules, induce a decrease of the streptavidin-biotin rupture force [1]. Individual streptavidin-biotin interactions were further investigated by SMD simulations at various pulling velocities, from 0.002×10^6 to 20×10^6 µm/s, to approach the pulling velocities obtained with AFM experiments. By comparison with previous experimental results and SMD rupture forces obtained without adsorption on silane monolayers [2], it was demonstrated that silane molecules with uncharged head-group and short alkyl chain allow streptavidin immobilization while keeping biotin interactions better than silane molecules with positively charged head-groups. Impact of the nature of silane molecules on the unbinding process and binding energy were also deciphered.

The same methodology was applied to another complex, including the cell receptor ACE2 and the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Contrary to the streptavidin-biotin complex, it was demonstrated that ACE2 adsorption on mixed silane monolayers induces an increase in the rupture force of spike protein RBD, indicating a reinforcement of protein-protein interactions.

References:
[1] S. Lecot, Y. Chevolot, M. Phaner-Goutorbe and C. Yeromonahos, J. Phys. Chem. B, **124** (2020), p.6786-6796.
[2] F. Rico, A. Russek, L. Gonzalez, H. Grubmüller and S. Scheuring, PNAS, **116** (2019), p.6594-6601.

Mechanical and adhesive properties of colon cancer cells upon inhibition of proprotein convertases.

Yarong Shi[1,2,3], Jean Descarpentrie[4], Cecile Feuillie[1], Alexandre Berquand[2], Jean-Marc Millot[2], Abdel-Majid Khatib[4,*] and Michael Molinari[1,*]

[1] Institute of Chemistry & Biology of Membranes & Nano-Objects(CBMN UMR CNRS 5248), University of Bordeaux, IPB; Bordeaux, France.
[2] Laboratory Research Nanoscience (LRN EA4682), University of Reims Champagne-Ardenne, Reims, France.
[3] Brüker Nano, Santa Barbara, CA, United States
[4] Angiogenesis and Cancer Microenvironment Laboratory, INSERM U1029, University of Bordeaux, Bordeaux, France
* Corresponding authors: majid.khatib@inserm.fr, michael.molinari@u-bordeaux.fr

Proprotein Convertases (PCs) can directly or indirectly control the malignant phenotype and metastatic potential of tumor cells, such as their proliferation, survival, invasion, and migration [1,2]. The purpose of the present study was to explore the effect of PCs inhibitors, both endogenous (profurin, the prosegment of furin which is able to inhibit the maturation and function of various substrates of the convertases) and exogenous (Dec-RVKR-CMK, which can block the activity of all the convertases), on the metastatic potential of the colon cancer cell line, CT26. To that end, we investigated the nanomechanical properties of the cells, qualitatively and quantitatively, using Atomic Force Microscopy (BrukerNano, USA) combined with a confocal microscope (Zeiss, Germany) at physiological temperature (37°C). The results showed that both inhibitors have an effect on the apparent Young's modulus of the CT26 cells. The observed difference may be attributed to changes in the organization of sub-membrane actin structures, which directly contribute to cell elasticity [2]. To go further in the understanding of the effects of the inhibitors, FluidFM (Cytosurge) experiments were performed, allowing to probe cellular adhesion properties; The results also show that the inhibitors could change cytoarchitecture and adhesion during malignant transformation.

[1] Scamuffa N, et al., J Clin Invest. 118 (2008) 352-63.
[2] Siegfried G, Descarpentrie J, Evrard S, Khatib AM., Cancer Lett. 31 (2020) 50-61.
[3] Li, Q. S., et al. Biochem. Biophys. Res. Comm. 374 (2008) 609-613.

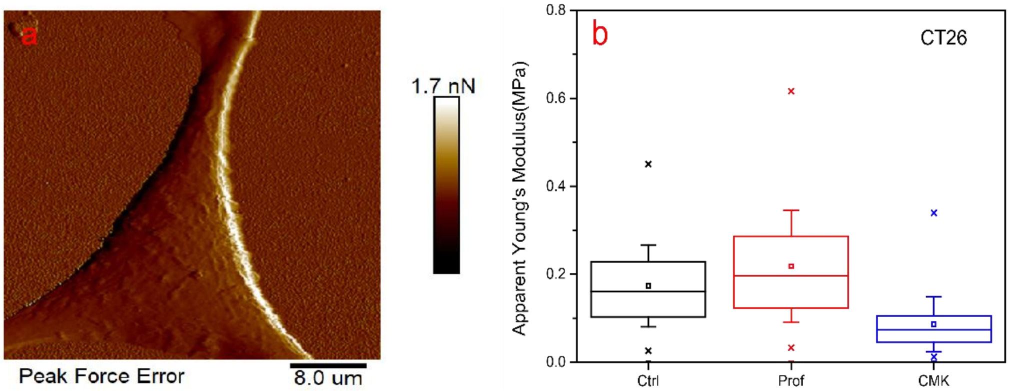


Figure 1. *(a) AFM PeakForce error image of the colon cancer cells CT26 inhibited cell by profurin. (b) Apparent Young's modulus of control and profurin-inhibited CT26 cells.*

Nano-Mechanics and Chemical Reactivity in Atomically Defined Contacts

Damla Yesilpinar[1], Bertram Schulze Lammers[1], Alexander Timmer[1], Zhixin Hu[2], Wei Ji[3], Saeed Amirjalayer[1], Harald Fuchs[1], and Harry Mönig[1*]

[1.] Department of Physics, University of Münster, Münster (Germany)
[2.] Department of Physics, Tianjin University, Tianjin (China)
[3.] Department of Physics, Renmin University of China, Beijing (China)
* Corresponding author: harry.moenig@uni-muenster.de

Controlling the identity of the tip-terminating atom or molecule in low-temperature atomic force microscopy (AFM), is the key for chemical imaging, as well as for quantitative and reproducible force measurements with piconewton precision. However, lacking a direct comparative tip-performance assessment, a profound standardization in such studies is not yet achieved. In this contribution we compare the imaging and force-spectroscopy capabilities of four atomically defined probe tips, namely Cu-, Xe-[1], CO-[1], and O-terminated Cu- (CuOx-) tips [2,3]. Using a nanostructured copper-oxide surface as a benchmark system, we investigated both, their imaging characteristics and capabilities in quantitative force measurements during the lateral manipulation of single adsorbed Xe atoms. Supported by DFT, our results allow a detailed discussion about the interplay between chemical reactivity, tip relaxations, and dynamic processes within the tip-sample junctions. Hereby, the CuOx-tip termination stands out with its ability to obtain a clear chemical-specific contrast on the metal-oxide and showing a superior performance in the manipulation experiments. Its high chemical and mechanical stability qualifies the CuOx-tip as a valuable standard probe in scanning probe microscopy and force measurements with ultrahigh resolution.

References:

[1] F Mohn et al., Appl. Phys. Lett. **102** (2013) p. 073109
[2] H Mönig et al., Nat. Nanotechnol. **13** (2018) p. 371.
[3] L Liu et al., Nat. Chem. accepted (2021).

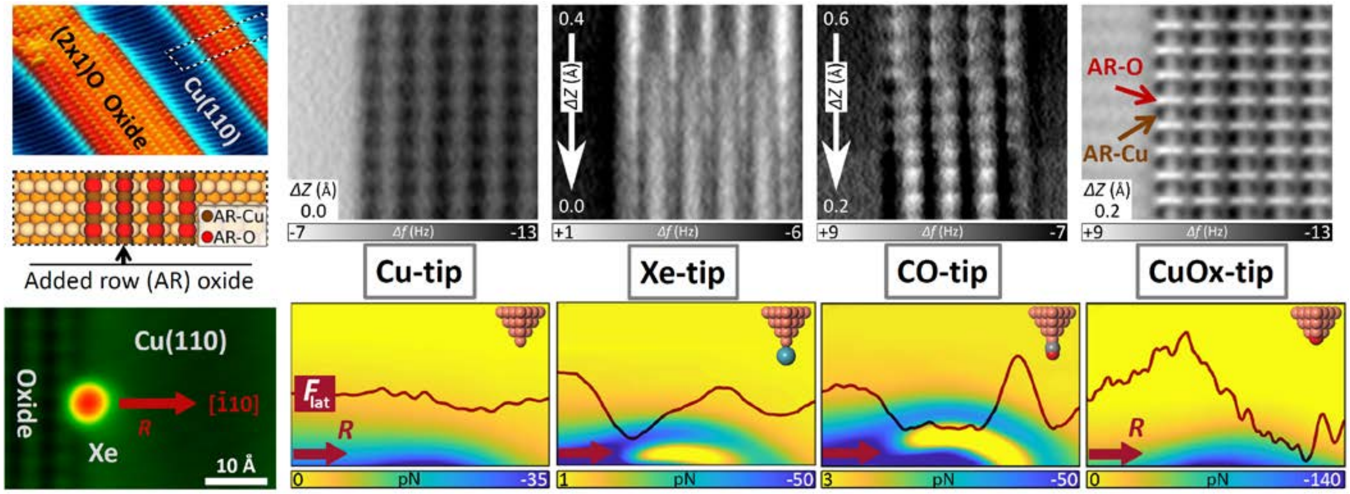


Figure 1. A partially oxidized Cu(110) surface with its typical (2x1)O-reconstructed oxide stripes was used as template for two benchmark experiments with four atomically defined AFM tips. (1) Height-dependent contrast analysis (upper panels). (2) Quantitative force measurements at the threshold for lateral manipulation of single Xe atoms nucleated at the metal/metal-oxide boundary (lower panels).

An ESD-STM comparison of alkyl and glycol side-chains in the assembly of conjugated polymers

Stefania Moro[1]*, Rawad Hallani[2], Oscar Drury[1], Joseph Parker[1], Iain McCulloch[2,3], Giovanni Costantini[1]

[1] Department of Chemistry, University of Warwick, Coventry, UK
[2] King Abdullah University of Science and Technology (KAUST), Physical Science and Engineering Division, Thuwal, Saudi Arabia
[3] Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Oxford, UK.
*stefania.moro@warwick.ac.uk

Conjugated polymers (CPs) are promising materials for modern organic electronics, as they combine structural and electronic adaptability to deliver smart, efficient, low-cost and environmentally friendly devices [1]. Existing applications for these polymers include organic photovoltaics, organic light-emitting diodes, organic electrochemical transistors (OECTs) and biochemical sensors [2]. The molecular-scale characterisation of CPs is, however, still unsatisfactory, as analytical techniques that can reliably provide high-resolution information on their structure and composition are scarce. Here, we utilise an innovative approach to investigate CPs at the (sub-)molecular scale, namely the combination of ultra-high vacuum (UHV) electrospray deposition (ESD) with scanning tunnelling microscopy (STM) [3]. This method allows for intact deposition of thermally fragile macromolecules and their characterisation at the ultimate spatial resolution of UHV-STM, revealing self-assembly, length distribution, sequence and exact chemical structure, including the presence of defects.

Following the recent employment of CPs in OECTs, the typically used alkyl side-chains, which provide stability to the polymeric materials through inter-strand interdigitation, have been substituted with polyethylene glycol (PEG) side-chains for optimal ion transport in aqueous conditions. Although the performance of PEG-functionalised materials rivals that of other established materials [4], the effects of PEG side-chains on the microstructure of polymers have not yet been examined in the literature. However, a precise understanding of the inter- and intra-molecular interactions is of fundamental importance, as structural modifications of CP assembly have been shown to strongly impact the overall order properties and charge-transport mechanisms [5]. Here, we present the results of a comparative study that shows the relationship of the widely employed poly(2,5-bis(3-alkylthiophene-2-yl)thieno[3,2-b]thiophene) (pBTTT) [6] to two PEG derivatives. The polymers have been independently synthetised, deposited in ultra-high vacuum by ESD and studied by STM. Our findings allow us to derive clear parallels and differences between alkyl and PEG side-chains, leading to the formulation of a new hypothesis for PEG side-chain interactions in polymeric films. New insight into the relationship between side-chains behaviour and polymer microstructure is also attained, allowing for predictions of optimised polymer chemical structures to be proposed.

[1] Pankow R. et al, Polymer **207** (2020), 122874; Pankow R. et al, Polym. Chem. **11** (2020, 630.
[2] Leclerc M. et al., Polym. J. **52** (2020), 13; Aldrich T. et al, Chem. Mater. **31** (2019), 4313.
[3] Warr D.A. et al., Sci. Adv. **4** (2018), 9543.
[4] C. B. Nielsen et al., J. Am. Chem. Soc. **138**, (2016) 10252; A. Giovannitti et al., Proc. Natl. Acad. Sci. U. S. A. **113**, (2016) 12017.
[5] Y. Yao et al., **28** (2016), 4513; Y. Zheng et al., Adv. Funct. Mater. **29** (2019), 1; S. Himmelberger and A. Salleo, MRS Commun. **5** (2015), 383.
[6] J. E. Northrup, Phys. Rev. B - Condens. Matter Mater. Phys. **76** (2007), 1; J. E. Northrup et al., J. Appl. Phys. **104** (2008), 083705; D. M. DeLongchamp, et al., Adv. Mater. **19** (2007), 833; I. McCulloch, et al., Nat. Mater. **5** (2006), 328.

Probing the behaviour of surface water and ferroelectric PbTiO3 thin films as a function of relative humidity and temperature

Loïc Musy*[1], Iaroslav Gaponenko[1], Christian Weymann[1], Patrycja Paruch[1]

[1]DQMP, University of Geneva, Switzerland
* Corresponding author: loic.musy@unige.ch

Surface adsorbates play an essential role in the stability of as-grown and written ferroelectric domains, particularly in thin films, since they provide the electrostatic screening necessary to stabilise polarisation bound charge. The study and control of the ferroelectric-adsorbate interaction is, therefore, key to engineering complex ferroelectric patterns which could be used for electromechanical actuation and sensing applications as well as catalytic processes [1,2]. Previous studies have demonstrated that polar adsorbates such as water are strongly influenced by both the orientation of the polarisation and the switching history of the ferroelectric [3], with screening strongly dependent on relative humidity [4].

Here, we report on our nanoscale investigation on the behaviour of water on ferroelectric PbTiO3 thin film surfaces as a function of relative humidity, temperature and electric boundary conditions, using Kelvin probe force microscopy, piezoelectric force microscopy and high resolution non-contact resonant imaging, and a home-built humidity control system [5]. We observe extremely low charge dissipation, and high localisation of both positive and negative screening charge on patterned domain structures in films with varying as-grown polarisation states, which we attribute to the extremely high quality and low surface roughness of the films, as compared with previous studies where much more extensive charge dynamics were observed [6]. In local measurements during temperature cycling at low and high humidities, we also note that both polarisation orientation and the presence of written domains appear to influence the nucleation and growth of water layers: growth appears to be inhibited by written structures. We measure a significant change of growth speed and water island shape between samples with a polydomain polarisation state compared to monodomain polarised ones.

[1] M.A. Khan et al., Surf. Sci. Rep., **71** 1 (2016)
[2] Setter, N et al., J. Appl. Phys, **100** 051606 (2006)
[3] N. Domingo et al., Nanoscale **11** 17920 (2019)
[4] J.J. Segura et al., J. Appl. Phys. **113** 187213 (2013)
[5] I. Gaponenko et al., Eng. Res. Express **1** 025042 (2019)
[6] I. Gaponenko et al., manuscript under review

Nano-scale viscoelastic measurement on heterogeneous polymeric materials

K. Nakajima[1,2]
[1] Department of Chemical Science and Engineering, Tokyo Institute of Technology, Meguro, Tokyo 152-8552, Japan.
[2]Department of Applied Physics, The University of Tokyo, Bunkyo, Tokyo 113-8656, Japan.

Nano-scale viscoelastic measurement based on AFM is reviewed with showing several examples of heterogeneous polymeric materials. Dynamic modulus determination, such as storage (E'), loss (E'') moduli and loss tangent with wide-frequency range is now realized by this technique [1].

The first example uses its capability of wide frequency measurement and its temperature control function to validate time-temperature superposition (TTS) principle. We used styrene-butadiene rubber (SBR) vulcanizate of which glass-rubber (GR) transition temperature, T_g is –27°C [2]. It was confirmed that the viscoelastic values of a pure SBR vulcanizate were quantitatively equivalent to bulk DMA results. TTS holds even at nanoscale if just viewing “average,” while some breakdown occurs due to heterogeneous nature of GR transition.

The miscibility between two polymer species is the second example. Nanorheological AFM was utilized to investigate the nature of a SBR/butadiene rubber (BR) partially miscible blend [3]. The blend seemed to be immiscible according to conventional AFM techniques. However, when both E' and E'' for the blends were compared with those for the homopolymers, values for both the SBR-rich and BR-rich regions did not coincide with those for the pure component. In particular, the BR-rich region, exhibited a dynamic heterogeneity, where the frequency responses were much more complicated than those of the BR homopolymer.

References:
[1] T. Igarashi, S. Fujinami, T. Nishi, N. Asao, K. Nakajima, *Macromolecules*, **46**, 1916 (2013).
[2] M. Arai, E. Ueda, X. Liang, M. Ito, S. Kang, K. Nakajima, *Jpn. J. Appl. Phys.*, **57**, 08NB08 (2018).
[3] E. Ueda, K Nakajima, *J. Soc. Rheol. Jpn.*, **48**, 91 (2020).

Visualizing crystal-solution interfaces: The promises and challenges of 3D fast force mapping

Elias Nakouzi,[1] Andrew G. Stack,[2] Sebastien Kerisit,[1] Benjamin A. Legg,[1,6] Christopher J. Mundy,[1,3] Gregory K. Schenter,[1,4*] Jaehun Chun,[1,5*] and James J. De Yoreo[1,6*]

[1] Physical and Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA 99354, USA.
[2] Chemical Sciences Division, Oak Ridge National Laboratory, 1 Bethel Valley Rd., Oak Ridge, TN 37830, USA.
[3] Department of Chemical Engineering, University of Washington, Seattle, WA 98195, USA.
[4] Department of Chemistry, Washington State University, Pullman, WA 99164, USA.
[5] Levich Institute and Department of Chemical Engineering, CUNY City College of New York, New York, NY 10031, USA.
[6] Department of Materials Science and Engineering, University of Washington, Seattle, WA 98195, USA.

In mineral systems, interfacial hydration structure creates ion distributions, chemical potential gradients, and inter-particles forces that influence surface reactivity, heterogeneous catalysis, mineral nucleation, and growth by particle assembly. Recent advances in atomic force microscopy–specifically 3D fast force mapping (FFM) in amplitude modulated mode–have enabled the direct observation of interfacial solution structure with sub-nanometer resolution. We use this capability to probe multiple mineral-solution systems, including layered silicates (phlogopite and muscovite mica) and aluminum (oxy)hydroxide (boehmite) exposed to salt solutions of different pH, ionic strength, monovalent and divalent cations, as well as using different AFM probes to conduct the measurements. Depending on the system, we typically observe three to five structured layers spaced 0.2–0.5 nm apart with lateral features templated by the underlying crystal lattice. For the case of boehmite, we observe four laterally structured water layers within one nanometer of the surface, with the highest water densities occurring at sites adjacent to the surface hydroxyl groups. The key features beyond the first two layers are not observed in molecular dynamics simulations of the free boehmite-water interface; and can only be predicted using a full-scale simulation that incorporates the AFM tip. These results demonstrate the complex relationship between crystallography, water density, and particle interactions; and present important advances towards quantitative data interpretation in 3D FFM.

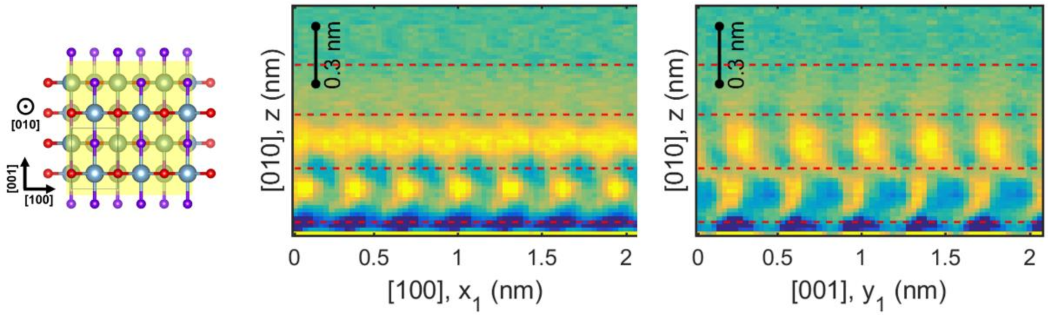


Figure 1. (Left) Schematic of boehmite (010) basal plane and (Center, Right) Slices of force gradient map corresponding to the solution structure at two planes normal to the boehmite-water interface

Insight into Nanoscale Elastic and Adhesive Heterogeneity in Epoxy Network with Bimodal AFM

Hung K. Nguyen[1*], Mika Aoki[2], Xiaobin Liang[1], Satoru Yamamoto[2], Keiji Tanaka[2] and Ken Nakajima[1]

[1.] Department of Chemical Science and Engineering, Tokyo Institute of Technology, Tokyo, Japan.
[2.] Department of Applied Chemistry, Kyushu University, Fukuoka, Japan.
* Corresponding author: nguyen.k.af@m.titech.ac.jp

Epoxy thermosets are important in a wide range of applications covering adhesives, structured composites, coatings, and so forth, thank to their advanced mechanical and thermal properties, and solvent stability. Epoxy thermosets are based on a three-dimensional network formed via the curing reaction of functional groups such as epoxy and amino. The formation of network structures under curing therefore plays key roles in determining the final performance of epoxy materials. Epoxy thermosets are generally believed to exhibit a nanoscale heterogeneous network structure as a result of an uneven growth of cross-linked fragments under curing [1]. However, many associated properties, such as heterogeneous behaviour of the mechanical and adhesive responses of epoxy thermosets at nanoscale remains little known.

In this study, we employ a bimodal AFM approach, namely amplitude-modulation (AM) and frequency-modulation (FM) [2], to simultaneously characterize nanoscale elastic and adhesive responses of an epoxy-amine system: hydrogenated bisphenol A diglycidyl ether (HBADGE) and 1,4-cyclohexanebis(methylamine) (CBMA) [1]. The surface of epoxy samples of different cured conditions was smoothed using a microtome (Leica Microsystems UC6). AM-FM measurements were performed using a Cypher S AFM (Asylum Research Inc.) in air condition. Panels (a), (b), and (c) of Figure 1 show the topographic height, elastic modulus (E_{elas}), and energy dissipation (E_{dis}) images of an epoxy sample cured at 298 K for ~24 h corresponding to a conversion degree of ~80%. Heterogeneous features were observed in both E_{elas} and E_{dis} images, suggesting a direct effect of heterogeneous network structure on the elastic and adhesive properties of epoxy materials at nanoscale. The hard and soft domains in the E_{elas} image might represent the high and low cross-linked regions, which appeared during the network formation [1]. The correlation lengths of these heterogeneous domains were ~27 and ~21 nm for E_{elas} and E_{dis} images, respectively. We also found that these length scales decreased to ~12 nm upon full cure.

References:

[1] M. Aoki, A. Shundo, R. Kuwahara, S. Yamamoto and K. Tanaka, Macromolecules **52** (2019), 2075.
[2] M. Kocun, A. Labuda, W. Meinhold, I. Revenko and R. Proksch, ACS Nano **11** (2017), 10097.

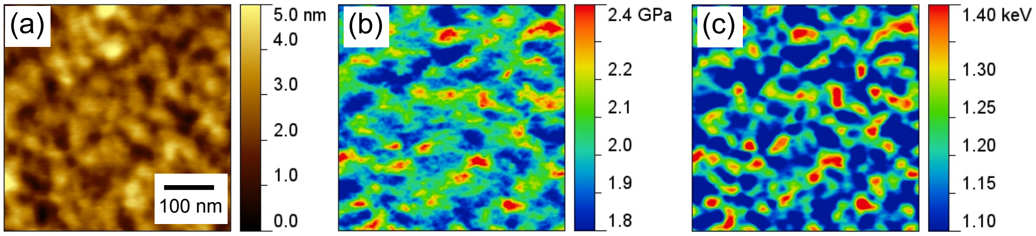


Figure 1. (a) Height, (b) elastic modulus, and (c) energy dissipation images of HBADGE/CBMA epoxy cured at 298 K for ~24 h simultaneously obtained using AM-FM mode.

Study of the mechanical, viscoelastic, and chemical properties of polymer blend-based thin films by dynamic atomic force microscopy

P. Nickmilder[1], Th. De Muijlder[1], J. Mathurin[2], A. Dazzi[2] and Ph. Leclère[1*]

[1] University of Mons (UMONS), Laboratory for Chemistry of Novel Materials, B 7000 Mons, Belgium.
[2.]Institut de Chimie Physique, UMR8000, Université Paris-Saclay, CNRS, 91405 Orsay, France

*Corresponding author: philippe.leclere@umons.ac.be

During the last decades, the knowledge about nanoscale properties has known a leap forward thanks to the development of Atomic Force Microscopy, particularly concerning the mapping of their mechanical and viscoelastic properties. These progresses are of the utmost importance since the nanoscale has a direct influence on the macroscale behavior. Among the different dynamic modes available nowadays, Peak Force Tapping, Intermodulation AFM, and nano Dynamic Mechanical Analysis are able to estimate these properties from the force curves using the approach-retract movement of the AFM tip and provide quantitative mapping.

In this study, we aim to systematically compare these AFM modes in term of accuracy as well as their applications and limitations using polymer blend thin films as model systems. In this study, we are focusing on thin films made of poly(styrene) (PS) and poly(methyl methacrylate) (PMMA) or PS and poly (ε-caproplactone) blends prepared by drop-casting. These polymers are chosen because they are exhibiting different elastic modulus and different viscoelastic behaviors linked to their chemical characteristics.

For the data analysis, we also used Machine Learning (ML) algorithms as a promising tool for the detection of the different domains in the materials (by partitioning the recorded data (i.e. the observables) into clusters according to their similarities. In this study, we will discuss ML algorithms dealing with data clustering (such as K-Means or Automatic Gaussian Mixture Model).

In fine, the observed morphologies have been confirmed by analyzing their local chemical properties (Figure 1) by AFM-IR which is a combination of the contact or tapping AFM imaging mode with a tunable infrared laser to make infrared spectroscopy far below the diffraction limit. This allows us to obtain a chemical identification mapping at the nanoscale as well as an IR spectrum from a region of the sample and try to estimate the actual composition of the different phases from the spectra and the IR images.

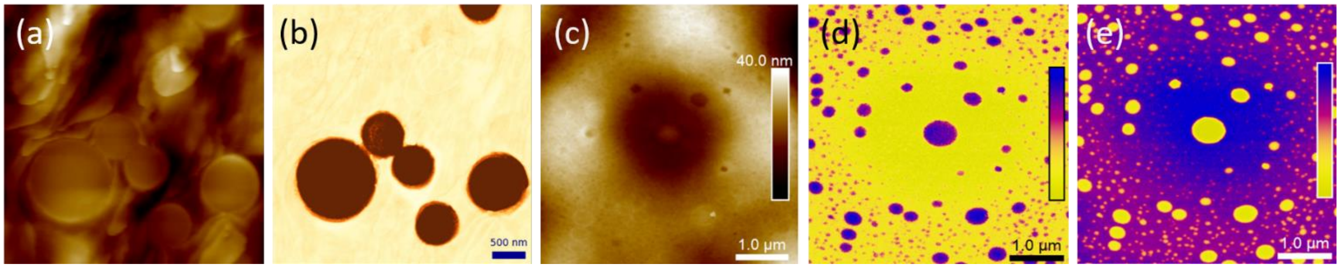


Figure 1. PS-PCL blend thin film: (a) Topography; (b) Mapping of the rigidity modulus; **PS-PMMA blend thin film:** (c) Topography; Mapping of the IR laser absorption at the fixed wavelength (d) 1600 cm^{-1}, and (e) 1735 cm^{-1}.

Frontiers of force microscopy at solid-liquid interfaces: From single ions to single cell nanomechanics

Ricardo Garcia[1]

[1.] Instituto de Ciencia de Materiales de Madrid, CSIC, Madrid (Spain)
* Corresponding author: r.garcia@csic.es.

This contribution introduces some of the main challenges faced by force microscopy to image at subnanometer-scale spatial resolution solid-liquid interfaces. The presentation is divided in three sections. The first section is devoted to introduce the capabilities of 3D-AFM to image with atomic-scale resolution the 3D interfacial structure of surfaces immersed in aqueous solutions (1-3). The second section shows the applications of high-speed bimodal AFM to map the nanomechanical properties of several biological processes (4). The third section discusses some fundamental issues involving the imaging and nanomechanical characterization of live cells with the AFM (5-6).

References:

[1] D. Martin-Jimenez, E. Chacon, P. Tarazona, R. Garcia, Nat. Commun. **7 (**2016**)**, p. 12164.
[2] M.R. Uhlig, D. Martin-Jimenez and R. Garcia, Nat. Commun. **10** (2019) p.2606.
[3] M.R. Uhlig, S. benaglia, R. Thakkar, J. Comer and R. Garcia, Nanoscale 13 (2021), p. 5275.
[4] V. G. Gisbert, S. Benaglia, M. R. Uhlig, R. Proksch, R. Garcia, ACS Nano **15** (2021), p. 1850.
[5] C.R. Guerrero, P.D. Garcia, R. Garcia, ACS Nano **13** (2019), p. 9629.
[6] P.D. Garcia, C. R. Guerrero, R. Garcia, Nanoscale **12** (2020), p.9133.

Correlative imaging of ferroelectric domain walls

Iaroslav Gaponenko
University of Geneva

In the last decades, ferroelectric materials have shown promise in photovoltaics applications, high efficiency actuation and sensing, as well as information storage. These materials possess an intrinsic polarization switchable under the application of an electric field, as well as a wealth of associated functional properties including nonlinear optical activity, very high piezo- and pyro-electric responses, and in some cases magneto-electric coupling. Recently, ferroelectric domain walls - regions separating domains of differing polarization orientation - have been the focus of numerous studies due to their intrinsically nanoscale nature and unique functional properties, which make them potentially useful for device applications. Whilst most of investigations of domain wall functionalities are conducted via a combination of high resolution scanning probe microscopy (SPM) based techniques probing their electromechanical and electrochemical responses, optical approaches - in particular second harmonic generation (SHG) nonlinear optical microscopy and polarimetry analysis - are increasingly being used as a way to non-perturbatively probe the internal structure, chirality and polarization of domain walls. Combining the two felicitously complementary techniques - with sequential SPM and SHG measurements of the same intrinsic or engineered domain structure - provides an opportunity to paint a complete structural and functional portrait of domain walls. However, despite their complementarity, the very different nature of the two techniques has so far made a fully quantitative and correlative analysis of their observables extremely challenging. Here, we leverage a combination of machine learning based techniques with data stacking approaches in order to enable a fast, correlative and objective analysis of SHG and SPM data sets. First, the correlative approach is introduced and discussed from the workflow point of view. Then, a comparative study of the different data analysis approaches is presented. Based on a data set acquired on a lead zirconate titanate thin film, the analysis aims to disentangle the contributors to the SHG polarimetry response of different origins in ferroelectric domain walls delimiting triangular shape c-domains. We specifically discuss the merits of K-means clustering on the stacked SHG dataset, showing not only similarity but significant improvements over the manual analysis published before. The correlative workflow enables us to find additional behaviors revealed by machine learning that might have been discarded as noise or experimental error through manual analysis. Finally, we extend the analysis to include and correlate the SHG signal to the observed PFM response through spatially correlative machine learning - enabling a better discrimination of domain and domain wall functional response.

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

Victor G. Gisbert[1*], Simone Benaglia[1], Manuel Uhlig[1], Roger Proksch[2] and Ricardo Garcia[1]

[1.] Instituto de Ciencia de Materiales de Madrid, 28049 Madrid, Spain
[2.] Asylum Research an Oxford Instruments Company, Santa Barbara, California 93117, United States
* Corresponding author: victor.gisbert@csic.es

Collagen is the most abundant structural protein of the extracellular matrix. The assembly of collagen fibrils play relevant roles in a variety biological processes. The formation fibrils during the self-assembly process of collagen I have been studied by AFM [1,2]. Those studies lacked the time and mechanical properties resolution to clarify the mechanism of the earlier stages of collagen assembly and fibril structure formation. We have developed a high-speed bimodal AFM [3] that combines the *ms* time resolution of high-speed AFM [4] with the nanomechanical force sensitivity of bimodal AFM [5,6]. High-speed bimodal AFM characterizes the earliest stages of the self-assembly of the collagen fibrils by proving time-resolved and high-spatial resolution maps of the evolution of the elasticity of the fibrils during the growth.

[1] F. Jiang, H. Hörber, J. Howard, D.J. Müller., J. Struct. Biol. **148** (2004), 268–278.
[2] D.R. Stamov, E. Stock, C.M. Franz, T. Jähnke, H. Haschke, Ultramicroscopy **149** (2015), 86–94.
[3] V.G. Gisbert, S. Benaglia, M. Uhlig, R. Proksch and R. Garcia, ACS Nano **15,** 1 (2021), 1850-1857.
[4] T. Ando. Nanotechnology **23** (2012), 062001.
[5] S. Benaglia, V.G. Gisbert, A.P. Perrino, C.A. Amo, R. Garcia, Nat. Protoc. **13** (2018), 2890.
[6] E.T. Herruzo, A.P. Perrino, R. Garcia, Nat. Commun. **5**, (2014), 3126.

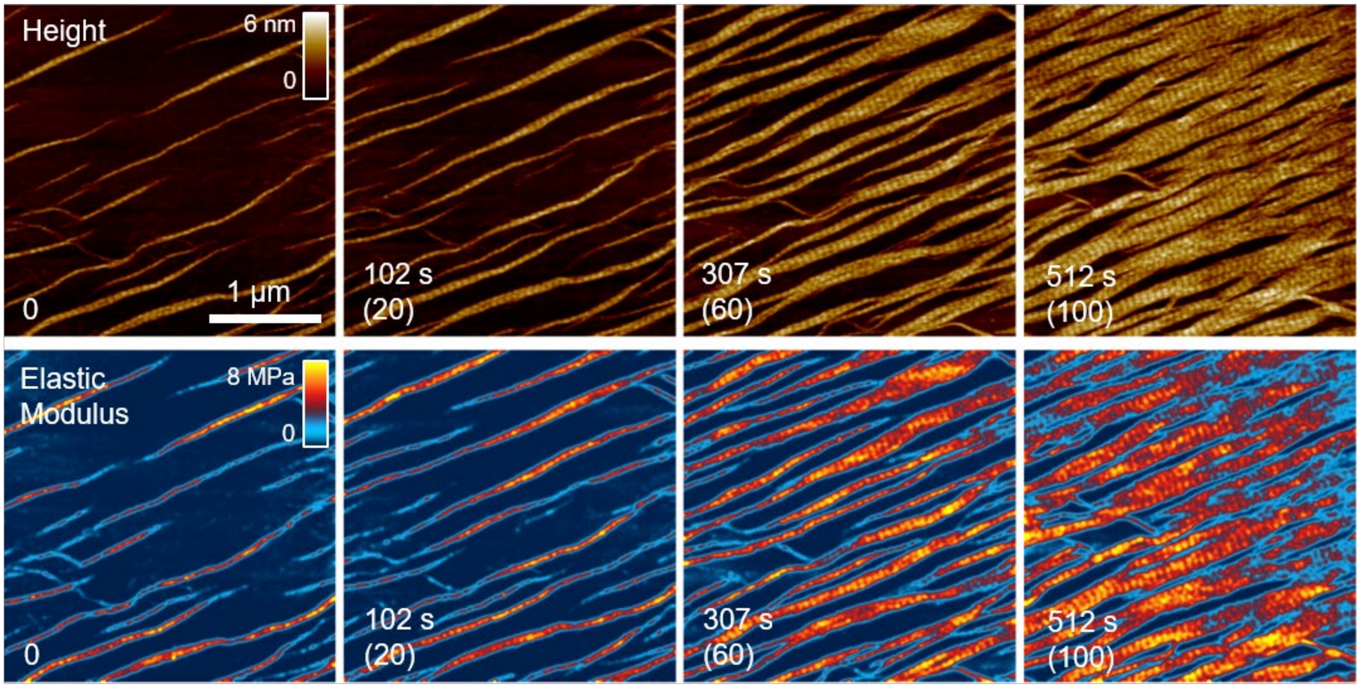


Figure 1. Sequence of High-speed bimodal AFM maps of topography and elastic modulus showing collagen microribbon growth on mica and evolution of physical properties.

AFM characterization of Teflon fiber filters exposed to airborne pollutants

Angelina E. Beck[1], David E. Beck[2], Gan-Jyun Siang[3], Yan-Pin Shi[3], Chia C. Wang[3,4], and Shuchen Hsieh[3,4*]

[1] Gushan Elementary School, Kaohsiung Municipal Gushan District, Kaohsiung 80443, TAIWAN
[2] Asylum Research (an Oxford Instruments Company), Santa Barbara, CA 93117 USA
[3] Aerosol Science Research Center, National Sun Yat-sen University, Kaohsiung 80424, Taiwan
[4] Department of Chemistry, National Sun Yat-sen University, Kaohsiung 80424, Taiwan

Airborne pollutants are a serious and growing human health crisis. Annual international expenditures in 2013 were estimated at between 240 billion and 630 billion US dollars, depending on the determinist model applied, or roughly 3-9% of the world's total expenditure on health care.[1] Cancer, chronic respiratory disease, and cardiovascular illness make up the largest percentage of health care conditions linked to airborne pollution. Teflon fiber filters can be used to trap airborne particles in a variety of high pollutant environments. In this report, we show AFM topographical images of Teflon filters that have been exposed to pollutants and show that the particles are trapped at levels consistent with the pollution type and exposure time.

[1] A. Preker, O. Odeyi, M. Lapetra, D. Simon and E. Keuffel, Annals of Global Health **82** (2016), p. 711.

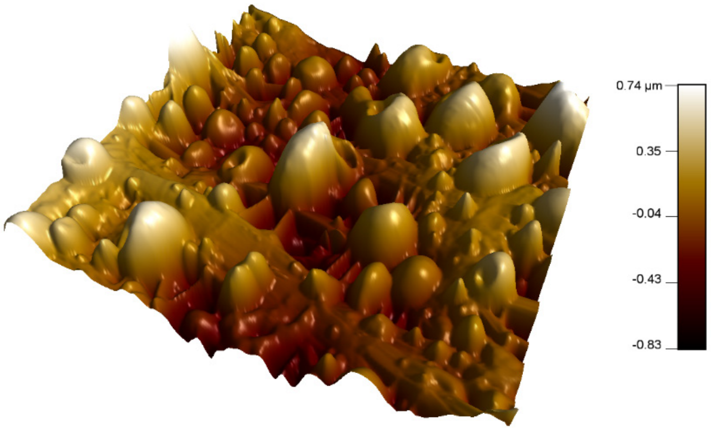


Figure 1. AFM image of a PTFE filter after 12 hour exposure to ambient air. Protrusions show a high density of particles trapped in the filter matrix. 10µm X 10µm.

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

Simone Benaglia[1*], Carlos A. Amo[1], Ricardo Garcia[1]
[1.] Instituto de Ciencia de Materiales de Madrid, CSIC, c/ Sor Juana Ines de la Cruz 3, 28049 Madrid, Spain
* Corresponding author: simone.benaglia@csic.es

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

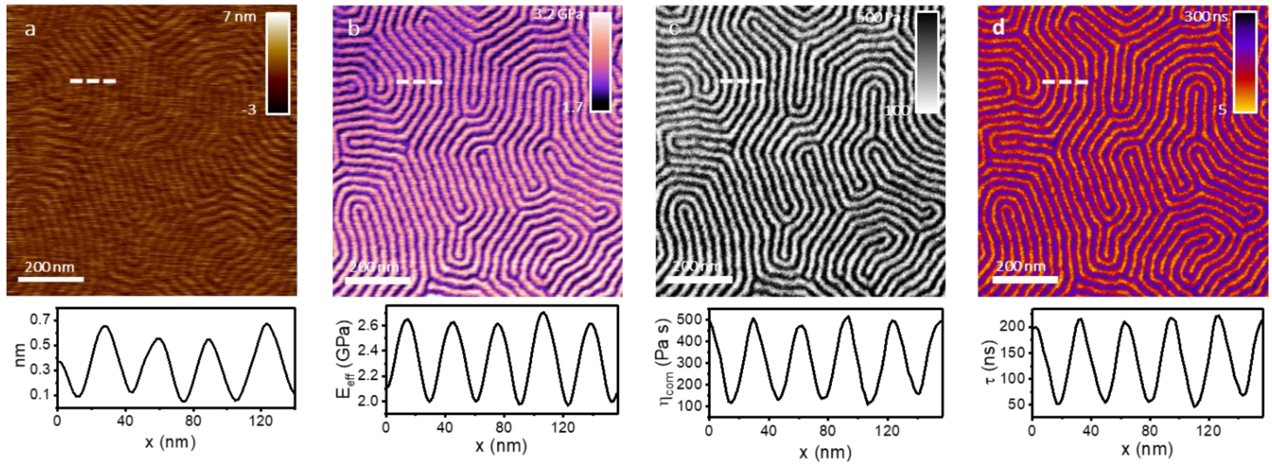


Figure 1. Apparent, deformation and true topography and bimodal nanomechanical maps of a PS-b-PMMA block co-polymer. (a) True topography. (b) Young's modulus. (c) Viscosity coefficient. (d) Retardation time.

References:

[1] R. Garcia et al., Phys. Rev. Lett. 97, 1 (2006).
[3] S. Benaglia, V. G. Gisbert, A.P. Perrino, C. A. Amo R. Garcia, Nat. Protoc. 13, 2890 (2018).
[4] S. Benaglia, C. A. Amo, R. Garcia, Nanoscale 11, 15289 (2019).

On the Nanoscale Characterization of the Mechanical and Electrical Properties of Nanodielectrics: The Case Study of Photoablated Silver Nanoparticles dispersed in a Poly(styrene) Matrix

Thomas De Muijlder[1], Daniel Forchheimer[2], Michel Voué[3], Philippe Leclère[1*]

[1.] University of Mons (UMONS), Laboratory for Chemistry of Novel Materials, B 7000 Mons, Belgium.
[2.] Intermodulation Products AB, 82393 Segersta, Sweden.
[3.] University of Mons (UMONS), Laboratory for Materials Physics and Optics, B 7000 Mons, Belgium.

* Corresponding author: philippe.leclere@umons.ac.be

During the last decades, nanodielectrics (i.e. nanocomposite materials based on a polymer matrix mixed with an inorganic filler) have shown a great potential for electronic applications. In particular, they show clear improvement in electrical properties (capacitance, electric field concentration, dielectric breakdown) as well as mechanical properties (tensile strength, flexibility) for a wide range of temperature. These improvements are promising for the design of energy harvesting devices or electrical insulation systems.

More and more studies highlighted the major role of the interphase between the matrix and the filler. Because of its nanoscopic nature, the study of the interphase properties requires cutting edge tools as scanning probe microscopy (SPM).

As model polymer matrix, we choose poly(styrene) (PS), a well-known polymer, easy to handle and one of the most used in this context with epoxy resin. As inorganic filler, we selected silver nanoparticles since silver is known to show the highest electrical and thermal conductivity. They are produced by laser photoablation in Tetrahydrofuran (THF) or toluene as organic solvent. The laser photoablation is known to produce nanoparticles with good colloidal stability while the use of THF aims to add an extra carbonic shell surrounding the silver nanoparticles.

For the analysis of our samples, we used different experimental techniques. we used optical measurements at different steps of the thin film fabrication process: reflectometry to determine the thickness of the polymer matrix, UV-VIS spectroscopy to monitor the production of the silver particles, and finally ellipsometry to characterize the optical properties of the composite films and more particularly the complex permittivity with lateral resolution of 1-2 micrometers.

For the measurements at the nanoscale, on one hand, we used PeakForce Tapping (PFT) and Intermodulation AFM (ImAFM) methods, to systematically analyze and quantitatively map the mechanical properties (rigidity modulus, adhesion, deformation), and viscoelastic properties (storage, loss moduli, and tan δ) of the thin films. On the other hand, for their electrical properties (contact potential difference, capacitance and its derivatives) we used Electrostatic Force Microscopy (EFM), Kelvin Probe Force Microscopy (KPFM), and Intermodulation EFM (ImEFM).

In fine, we compared the different existing interphase models with the data to explain the dielectric properties of our model samples.

Nanomechanical behavior of Human IgG mAbs binding to bacterial surface polysaccharide Poly N-Acetylglucosamine

Begüm Dikecoglu[1*], Gerald B. Pier[2], Peter Hinterdorfer[1]

[1.] Institute of Biophysics, Johannes Kepler University, Gruberstrasse 40, 4020 Linz, Austria
[2.] Division of Infectious Diseases, Department of Medicine, Brigham and Women`s Hospital/Harvard Medical School, Boston, Massachusetts 02115

*Corresponding author: beguem.dikecoglu@jku.at

Poly-N-acetylglucosamine (PNAG) has been identified as surface polysaccharide antigen expressed by many microbial pathogens during human and animal infection. The group of Gerald Pier developed a human IgG monoclonal antibody (MAbs) with quantitative difference in effector functions due to differences in epitope binding and affinity. Human IgG MAbs that bound to acetylated PNAG show different activities with respect to their complement-depositing activity.[1,2] Here, nanomechanical properties of antigen-antibody binding was characterized using single molecule force spectroscopy technique (SMFS). Two subclasses of human IgG mAbs (F598 IgG1 and F628 IgG2) was studied with purified PNAG and surface PNAG expressing Staphylococcus aureus bacterial cells. As control bacteria lacking PNAG expression was used. Cells from overnight culture were fixed with formaldehyde, placed onto a glass slide. MAbs were coupled to AFM tips via a flexible polyethylene glycol (PEG) linker and the purified PNAG was tethered to a silicon nitride substrate using the same chemistry. The antibody binding behavior on the bacterial surface and purified polysaccharide was analyzed using a home-built force detection and analysis software.

References

[1] Maira-Litran, T., A. Kropec, C. Abeygunawardana, J. Joyce, G. Mark III, D. A. Goldmann, and G. B. Pier. 2002. Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. Infect. Immun. 70:4433–4440

[2] Kelly-Quintos C, Cavacini LA, Posner MR, Goldmann D, Pier GB. 2006. Characterization of the opsonic and protective activity against Staphylococcus aureus of fully human monoclonal antibodies specific for the bacterial surface polysaccharide poly-N-acetylglucosamine. Infect Immun. 74:2742-2750.

Surface Conduction of Silicon Surfaces Measured by Two-probe Scanning Tunneling Microscopy with Ohmic Contact

Jo Onoda[1*], Ali Khademi[2], Robert Wolkow[1, 2, 3] and Jason Pitters[2].

[1.] Department of Physics, University of Alberta, Edmonton, Alberta T6G 2J1, Canada
[2.] Nanotechnology Research Centre, National Research Council Canada, Edmonton, Alberta T6G 2M9, Canada
[3.] Quantum Silicon, Inc., Edmonton Alberta T6G 2M9, Canada
* Corresponding author: jonoda@ualberta.ca

Relentless downscaling of conventional electronic devices demands continuous improvement in characterization of microscopic surface electronic properties. Moreover, in the pursuit of more exploratory device concepts there is great need for characterization of extremely small one- and two-dimensional structures. We describe here a refinement of a two-probe technique. We first establish the reliability of this approach by reproducing measurement of some established silicon surface properties. We then exemplify opportunities this approach offers through a study of minute islands and strips of silicon that are cut away from surrounding surface contributions.

Scanning tunneling microscope (STM) with one-probe is typically used to image and fabricate nano- and atomic-scale structures and to measure highly local electronic properties. To assess the electrical conduction properties lateral to the surface two-probe (2P-) and four-probe (4P-) STM has been developed. The measurement of conductance with 4P-STM is generally preferred over 2P-STM as it can eliminate probe-to-surface contact uncertainties by employing two tips to inject current and two additional probes to make a high impedance voltage measurement. The typical drawback of the contact resistance appearing in two-probe I-V measurement is shown in Fig. 1 (non-Ohmic contact). In order to achieve Ohmic probe-to-surface contact, we prepared tip by means of field evaporation. We found that we are able to obtain a linear (Ohmic) curve on the Si(111)-(7×7) surface with this method (Fig. 1), and that the measured surface conductivity was comparable with those obtained by 4P-STM. We also utilized STM lithography to create electronically isolated regions from the otherwise surface area, finding that we can measure their conduction properties correctly. Since the smallest tip-to-tip distances of 25 nm to 50 nm is achievable in 2P-STM [1], the present method will be useful in a wide range of fields of material sciences.

References:

[1] J. Onoda, D. Vick, M. Salomons, R. Wolkow and J. Pitters, AIP Adv. **10** (2020) 105213.

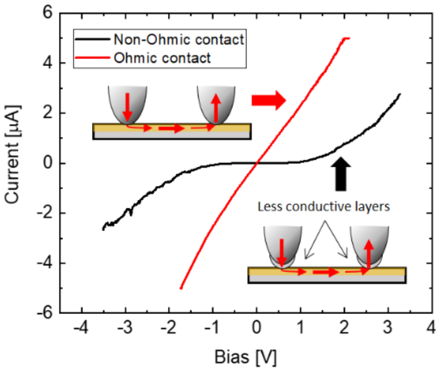


Figure 1. I-V curves obtained by 2P-STM with non-Ohmic and Ohmic contacts. Bias voltage is swept at one of the probes with another probe grounded and the sample floated. Tip-to-tip distance is 3 μm.

Electrodeposited silver nanowire network for flexible transparent electrodes

Sy Hieu Pham[1,2], Anthony Ferri[2], Antonio Da Costa[2], Van Dang Tran[1,3], Rachel Desfeux[2] and Philippe Leclere[1*]

[1.] Laboratory for Chemistry of Novel Materials (CMN), Center for Innovation and Research in Materials and Polymers (CIRMAP), University of Mons (UMONS), B-7000 Mons, Belgium.
[2.] University Artois, CNRS, Centrale Lille, University Lille, UMR 8181, Unit of Catalysis and Solid Chemistry (UCCS), F-62300 Lens, France.
[3.] International Training Institute for Materials Science (ITIMS), Hanoi University of Science and Technology, No.1 Dai Co Viet, Hai Ba Trung, Hanoi, Viet Nam.

* Corresponding author: Philippe.LECLERE@umons.ac.be

Silver nanowire network (AgNW) has demonstrated high optical transparency, low sheet resistance, and promises to be a next-generation transparent conductive electrode (TCE). The electrical properties of the AgNW network strongly depend on the contact resistance and the number of junctions. In this work, we used an electroplating method of Ag on AgNW to improve the contact resistance of the AgNW network. In addition, electrodeposition of Ag on AgNW TCEs can provide higher conductivity than spincoating AgNW TCEs at the same transparency due to increased diameter of the nanowire by epitaxial. The optimized experimental conditions have revealed a sheet resistance as low as 12 Ohm/sq associated to a high transmittance larger than 88% measured at λ = 550 nm on PET. In this study, electrical behavior of the AgNWs network was thoroughly investigated on the nanoscale by means of the conductive mode of the Atomic Force Microscopy (c-AFM) demonstrated a high percolation of the AgNW network. The obtained results allow us to propose a methodology to elaborate electrodes suitable for flexible displays, organic light-emitting diodes, and thin-film solar cells.

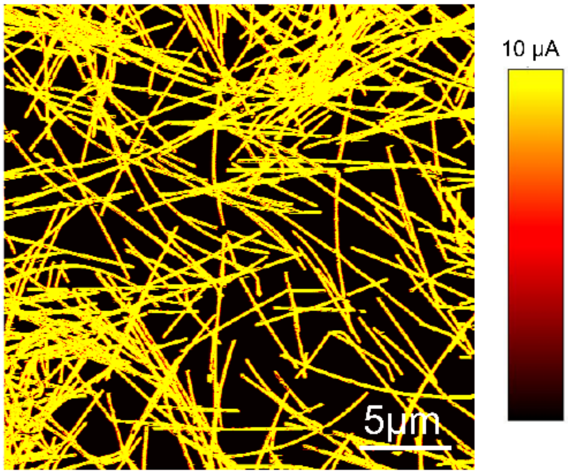


Figure 1. c-AFM current of transparent electrode based on electrodeposited Ag/AgNWs network

Revisiting phase contrast in tapping mode AFM: exploiting fluctuations to deconvolute dissipative pathways with application in contact resonance enhanced AFM-IR

Arindam Phani[1*] and Seonghwan Kim[1*]

[1]Department of Mechanical and Manufacturing Engineering, University of Calgary, Calgary, AB, Canada

[*]Corresponding Author email: arindam.phani@ucalgary.ca, sskim@ucalgary.ca

Abstract:

Phase-contrast in tapping-mode atomic force microscopy (TM-AFM) results from dynamic tip-surface interaction losses which allow soft and hard nanoscale features to be distinguished. So far, phase-contrast in TM-AFM has been interpreted using homogeneous Boltzmann-like loss distributions that ignore fluctuations. Here, we revisit the origin of phase-contrast in TM-AFM by considering the role of fluctuation-driven transitions and heterogeneous loss. At ultra-light tapping amplitudes < 3 nm, a unique amplitude dependent two-stage distribution response is revealed, alluding to metastable viscous relaxations that originate from tapping-induced surface perturbations. The elastic and viscous coefficients are also quantitatively estimated from the resulting strain rate at the fixed tapping frequency. The transitional heterogeneous losses emerge as the dominant loss mechanism outweighing homogeneous losses at smaller amplitudes for a soft-material. Analogous fluctuation mediated phase-contrast also becomes apparent in contact resonance enhanced AFM-IR (infrared), showing promise in decoupling competing thermal loss mechanisms via radiative and non-radiative pathways. Understanding the loss pathways can provide insights on the bio-physical origins of heterogeneities in soft-bio-matter e.g., single cancer cell, tumors, and soft-tissues.

We introduce a method to experimentally decouple energy loss pathways at the tip-surface junction of a soft heterogenous surface in the nanoscale, without necessarily indenting the surface. We render fluctuations relevant to dynamics at the regime between contact and non-contact conditions we are calling transitional tapping. We exploit it to quantitatively determine elastic and time dependent viscoelastic coefficients from estimations of surface deformations on tip-approach. We explain the origin of phase contrast in the light of delay bandwidth product appearing from transient excursions to non-equilibrium states. We show that the losses off equilibrium get encoded in unique phase contrast distributions which characterize nanoscale heterogeneities. Apparently, fluctuations at the tip-surface junction capture more details of the dissipative pathways than conventional steady state dynamics by separating the timescale interactions. We have derived a generalized model to decouple the transients from the steady state losses. Our results and discussed method of exploiting fluctuations at an AFM-probe tip will encourage scientists and biologists across domains. Studying phase contrast distributions can shed new light on energy loss pathways via non-equilibrium routes at scales only limited by tip radius and can provide new impetus for widespread applications in all fields of engineering soft-mater, physical sciences, and experimental biophysics.

Photothermal Plasmonic Actuation of Micromechanical Cantilever Beams

M.D. Pichois[1], M.A. Hurier[1] and M.V. Rastei[1*]

[1.] Institut de Physique et Chimie des Matériaux de Strasbourg, CNRS, Université de Strasbourg, 23 rue du Loess, Strasbourg F-67034, France
* mircea.rastei@ipcms.unistra.fr

Oscillations of charge carriers in plasmonic metal nanoparticles activated by resonant absorption of light are accompanied by local temperature increase due to nonradiative damping. The control of this photothermal effect is considered essential for many applications ranging from photochemistry or nanomedicine to chemical and physical sensing. Here, we present a study on the conversion of visible light into mechanical energy via photothermal plasmonic and nonplasmonic effects. Using atomic force microscopy, cantilevered sensors coated with various materials and excited in vacuum by a wavelength tunable laser show that light generates a resonant oscillation of the cantilever. The photoinduced oscillation amplitude depends on the wavelength of the incident light, allowing for an optimization of energy conversion based on absorption spectroscopy of the coating material. The effect of nonphotonic forces acting on the cantilever is probed in the context of photoinduced force microscopy by placing the cantilever in interaction with a substrate surface at various distances. The findings are relevant for any technique utilizing an optical actuation of a mechanical system, and for photoinduced force detection methods in particular.

References:

[1] M.D. Pichois, M.A.Hurier and M.V.Rastei, Phys. Rev. Applied **15** (2021), p. 3.

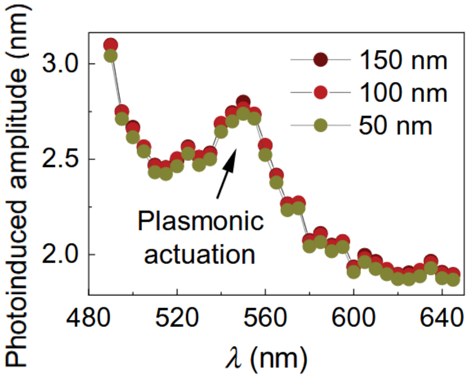


Figure 1. Spectral evolution of the amplitude of a cantilever coated with gold nanoparticles and excited at resonant frequency by a modulated laser.

Investigating Time-Temperature Superposition at the Nanoscale in Polymer Composites

Bede Pittenger[1*], Sergey Osechinskiy[1], Sophie Loire[1], Thomas Mueller[1]

[1] Bruker Nano Surfaces, AFM Unit, Santa Barbara, CA, USA.
[*] Corresponding author: bede.pittenger@bruker.com

The mechanical performance of polymer composites is controlled by the properties of the microstructure of the material. As confinement effects and interphase formation can alter properties of the microphases, only measurements performed directly on the composite can provide the needed local property distribution. Mechanical properties of polymers are generally time dependent, so a full understanding requires measurements over a range of frequencies and temperatures.

With its proven ability to map mechanical properties at the nanometer level, AFM has the resolution and mechanical sensitivity needed to investigate these domains. Unfortunately, established AFM measurement modes do not yield results that are directly comparable to established rheological techniques like Dynamic Mechanical Analysis (DMA). Contact resonance [1] provides mechanical property maps at known frequencies, but the cantilever resonances are many orders of magnitude higher than DMA, making comparisons indirect at best. Intermittent contact methods like TappingMode [2], force volume, and PeakForce Tapping [3] face challenges in calculating intrinsic mechanical properties like storage and loss modulus (or tan delta) at well-defined frequencies due to the non-linear process of making and breaking contact.

This presentation will examine the capabilities of a new mode called AFM-nDMA that provides viscoelastic results which can be directly compared with bulk DMA. It provides spectra of storage and loss modulus across frequency and temperature allowing construction of master curves through Time Temperature Superposition. These measurements are localized by the probe-sample contact, allowing high resolution observations of the microscopic structures within heterogeneous samples.

[1] U. Rabe, S. Amelio, E. Kester, V. Scherer, S. Hirsekorn, and W. Arnold, Ultrasonics, 2000, 38, 430.
[2] O. Sahin, C. Quate, O. Solgaard, and A. Atalar, Phys. Rev. B, 2004, 69, 1.
[3] B. Pittenger and D. G. Yablon, Bruker App. Note 149, 2017, doi: 10.13140/RG.2.2.15272.67844

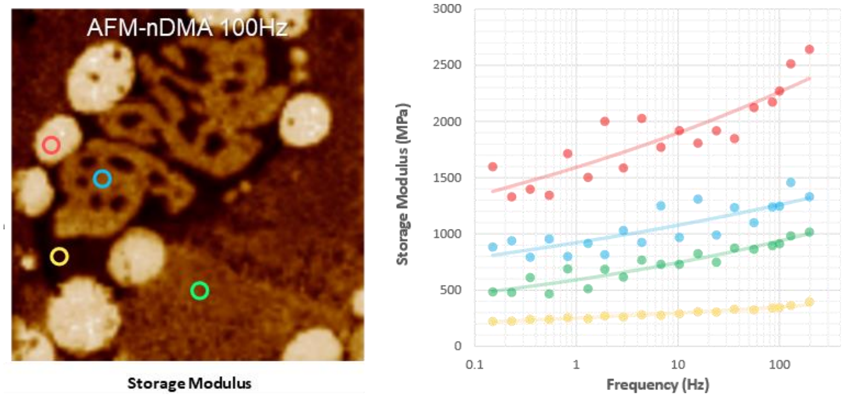


Figure 1. AFM-nDMA map of storage modulus at 100Hz and spectra at selected points. Sample is a polymer blend composed of COC, PP, LLDPE and an elastomer.

Base-pair resolution analysis of the effect of supercoiling on DNA structure and recognition

Alice L. B. Pyne[1]*, Agnes Noy[2], Kavit H. S. Main[3], Victor Velasco-Berrelleza[2], & Sarah A. Harris[4]

[1] Department of Materials Science and Engineering, University of Sheffield, Sheffield, UK.
[2] Department of Physics, Biological Physical Sciences Institute, University of York, York, UK.
[3] UCL Cancer Institute, University College London, London, UK.
[4] School of Physics and Astronomy, University of Leeds, Leeds, UK.
* Corresponding author: a.l.pyne@sheffield.ac.uk

In the cell, DNA is arranged into highly-organised and topologically-constrained (supercoiled) structures. It remains unclear how this supercoiling affects the detailed double-helical structure of DNA, largely because of limitations in spatial resolution of the available biophysical tools. We overcome these limitations, by a combination of high-resolution atomic force microscopy (AFM) and atomistic molecular dynamics (MD) simulations, to resolve structures of negatively-supercoiled DNA minicircles to the base-pair level (Figure 1)[1]. We observe that negative superhelical stress induces local variation in the canonical B-form DNA structure by introducing kinks and defects that affect global minicircle structure and flexibility[2]. We probe how these local and global conformational changes affect DNA interactions through the binding of triplex-forming oligonucleotides to DNA minicircles. Our results provide mechanistic insight into how DNA supercoiling can affect molecular recognition, that may have broader implications for DNA interactions with other molecular species.

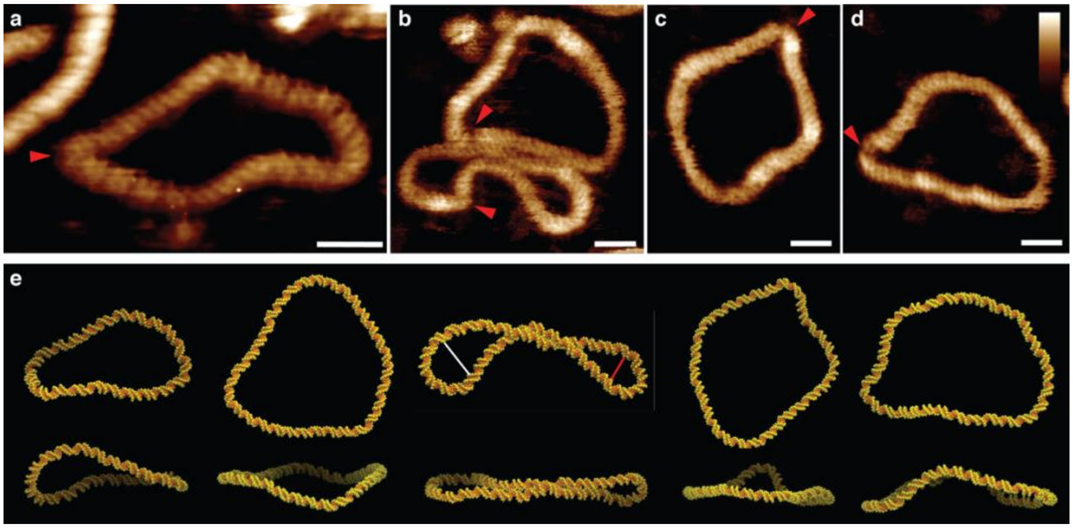


Figure 1. Structural diversity in supercoiled DNA minicircles. Synergistic high-resolution AFM images (a-d) and MD snapshots (e) of natively supercoiled DNA minicircles show striking structural diversity in natively supercoiled DNA minicircles. Scale bars: 10 nm. Height scale (inset): 2.5 nm for all images

References:

[1] Pyne, A. L. B. *et al.* Base-pair resolution analysis of the effect of supercoiling on DNA flexibility and major groove recognition by triplex-forming oligonucleotides. *Nat. Commun.* **12**, 1053 (2021).

[2] Beton, J. G. *et al.* TopoStats – a program for automated tracing of biomolecules from AFM images. *Methods* (2021) doi:10.1016/j.ymeth.2021.01.008.

Quantification of Nano-scale Viscoelasticity using Dynamic Atomic Force Microscopy in Liquid Environment

Shatruhan Singh Rajput[1$], Surya Pratap S. Deopa[1], Jyoti Yadav[1], Vikhyaat Ahlawat, Saurabh Talele, and Shivprasad Patil[1*]

1. Department of Physics, Indian Institute of Science Education and Research, Pune, India.

* s.patil@iiserpune.ac.in, $ shatruhansingh.rajput@students.iiserpune.ac.in

We have developed a methodology to quantify the dissipation and stiffness (viscoelasticity) of nano-scale interactions in liquid environment. We use small-amplitude and off-resonance amplitude-modulation Atomic Force Microscopy technique for measurements. Experiments were performed using two cantilever-deflection detection schemes, optical-beam-deflection commonly used in commercial AFMs and displacement-detection using a fibre interferometer-based home-built setup. Cantilever is excited using two different excitation mechanisms, excited from the base using dither piezo [2] and directly the tip using magnetic fields [3]. A single protein molecule (Titin-I27), tethered between cantilever-tip and substrate, is pulled with constant speed (~ 20 nm/s). Cantilever-tip is driven with very low frequency (~ 100 Hz), amplitude (~ 1Å) while pulling the molecule, and phase and amplitude (or X & Y components of oscillation) are recorded. We analyzed the data using existing theoretical models [4] and also a model proposed by us. Pairing different excitation and detection schemes; and analyzing the data with different models, a robust study of mechanical properties of I27 molecule was made. Our observed results show inconsistencies with previous studies. We observed that the offset in the initial phase, which may appear from various sources and likely to be present in dynamic AFM experiments, introduces artefacts in the measurement. Apart from this, artefacts may appear due to inappropriate selection of experimental parameters and theoretical models. We propose the appropriate methods of performing measurements and suggest appropriate theoretical model for data analysis. We performed careful measurement and data analysis and found that the dissipation in the single unfolded protein molecule is immeasurably low and it is below the detection limit of AFM. The upper bound of dissipation in single unfolded molecule is $5\text{x}10^{-7}$ kg/s. We believe it is due to the inappropriate selection of experimental parameters, initial conditions, and theoretical models which leads to many artefacts in the results [1]. We also discuss the validity of widely used point-mass model. Our proposed methodology is useful in quantifying the viscoelasticity (with better accuracy) of all the nano-scale systems which can be probed using dynamic AFM technique.

References:
[1] S.S. Rajput et al., Nanotechnology, 32(2021), p. 085103.
[2] F. Benedetti et al., Biophys. Journal **111**(2016), p. 1163-1172.
[3] M. Kawakami et al., Biophysical Journal **91**(2006), p. 16-18.
[4] Recardo Garcia, Amplitude Modulation Atomic Force Microscopy, WILEY-VCH (2010), ISBN: 978-3-527-40834-4.

Supramolecular Organization of Model Polycyclic Aromatic Molecules: Comparison of 2D and 3D Assemblies

Gijo Raj[1†], Yoshihiro Kikkawa[2], Luca Catalano[1], Renu Pasricha[1], Yasuo Norikane[2], Panče Naumov[1]

[1]New York University Abu Dhabi P.O. Box 129188, Abu Dhabi, UAE, [2]National Institute of Advanced Industrial Science and Technology (AIST) Tsukuba Central 5, 1-1-1Higashi,Tsukuba, Ibaraki 305-8565, Japan, [†]Present address: Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum 695012, Kerala, India.

Email: pance.naumov@nyu.edu

Abstract:

The spatial organization of polycyclic aromatic molecules during adhesion on a solid/liquid interface is the key event of a plethora of natural and industrial processes. Herein, we report the supramolecular organization of violanthrone-79—a model of asphaltenes, an intractable mixture of polycyclic aromatics from crude oil—at the solid-liquid interface between highly oriented pyrolytic graphite (HOPG) and a hydrophobic medium (1-phenyloctane). High-resolution scanning tunneling microscopy revealed that molecules of violanthrone-79 readily self-assemble on the surface in supramolecular "nanoring" structures. The lattice parameters of the 2D unit cell, observed with STM, are on the same order as those determined from a bulk single crystal X-ray diffraction. Transmission electron microscopy showed long-range ordered patterns, and the spacing between the fringes is in agreement with the inter-planar distances between aromatic cores of molecules that are helically arranged around the [001] axis in the 3D crystal. STM data complemented with TEM and XRD analysis confirm that, upon adsorption on solid/liquid interfaces, polycyclic aromatic molecules such as violanthrone-79 form supramolecular assemblies by interaction with the substrate and self-association, and this process could be the initial step of deposition of asphaltenes on carbonaceous oil reservoir walls and production tubing.

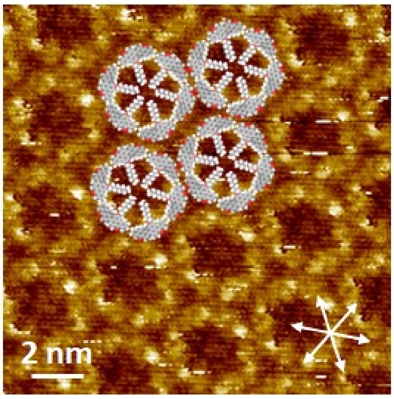


Figure 1: violanthrone-79 molecules assembled at HOPG/1-phenyloctane interface.

PREDICTING HYDRATION LAYERS OVER SURFACES

Yashasvi S. Ranawat[1], Ygor M. Jaques[1], and Adam S. Foster[1,2]

[1]*Department of Applied Physics, Aalto University, Finland*

[2]*WPINano Life Science Institute (WPI-NanoLSI), Kanazawa University,Kakuma-machi, Kanazawa 920-1192, Japan*

email: yashasvi.ranawat@aalto.fi

Several technological and natural processes are dominated by the mineral-water interactions, for example biominerelisation, corrosion etc, and hence, characterization of such interfaces is vital. Atomic Force Microscopy (AFM) has gained prominence in characterizing such surfaces. However, the complex interplay of the tip with the hydration layers over the surface govern the AFM tip's interaction with the hydration layers, and impede high resolution requirements needed for characterization. The theoretical molecular dynamics (MD) approaches have helped with the surface characterization. Given a hydration layer image, the search space is wide and the MD approach becomes prohibitively expensive. Here we introduce deep learning methods to reliably predict the hydration layer over a given surface. These methods are tested on the polymorphs of calcium carbonate.

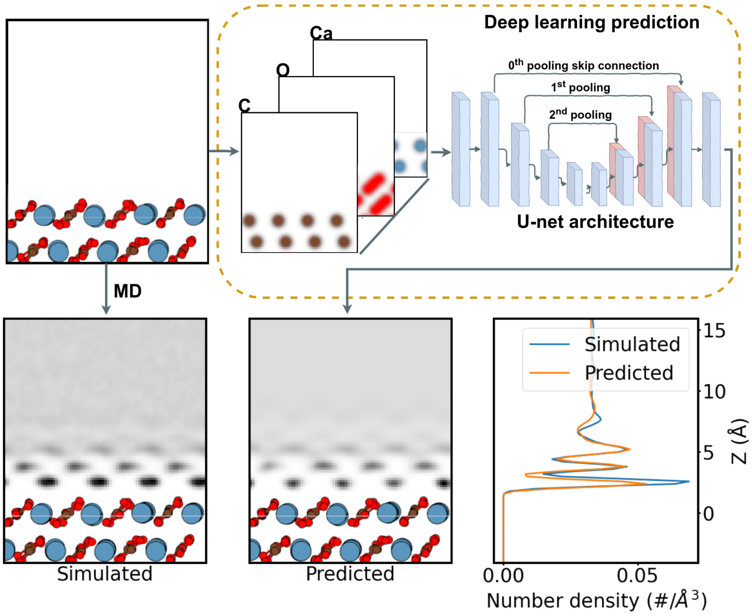


Figure 1: Schematic of the prediction process of hydration layers over a calcite surface. The three-channel density of the surface carbon, oxygen, and calcium atoms is the input of the U-net based neural network. A single-channel density of water over the surface is predicted by the network. The prediction matches the water density derived through the MD-simulations.

Hydrodynamic Damping of Oscillating Microcantilevers in Cross-flow

Bruno Reyes[1] and Ryan Tung[1*]

[1.] University of Nevada, Reno, Department of Mechanical Engineering, Reno, NV USA
* Corresponding author: rtung@unr.edu

High-Speed AFM has seen recent developments [1] and is a useful tool in life science [2]. However, the effect that a high scanning speed has on the hydrodynamic forces acting on the microcantilever has not been explored. In general, flow over an oscillating microcantilever and the effects on the added mass, the forces in phase with the acceleration of the microcantilever, and viscous damping, forces in phase with the velocity, have not been fully investigated. In this work, 2D numerical simulations are performed to investigate the effect that high-speed flow has on the hydrodynamic forces of a microcantilever oscillating in water and far from any surface. The forces in the direction of oscillations are extracted for cases with differing frequencies of oscillations (1000 kHz – 2MHz) and flow velocities (0 m/s – 0.5 m/s) with a cantilever width of 30 μm and thickness of 2 μm.

The hydrodynamic function is used to model the steady-state fluid forces experienced by the microcantilever and is dependent on the frequency of oscillations in the case where the fluid is initially quiescent. The numerical simulation results for the hydrodynamic function are validated for the quiescent fluid case [3] and the introduction of flow in the direction of the width of the cantilever is simulated and changes in the hydrodynamic function are observed. From the current results, a trend is seen where the added mass decreases and viscous damping increases with increasing flow rates and the degree that these are impacted is dependent on a non-dimensionalized velocity value that is the ratio of the maximum velocity of the cross-section of the microcantilever to the flow velocity of the fluid.

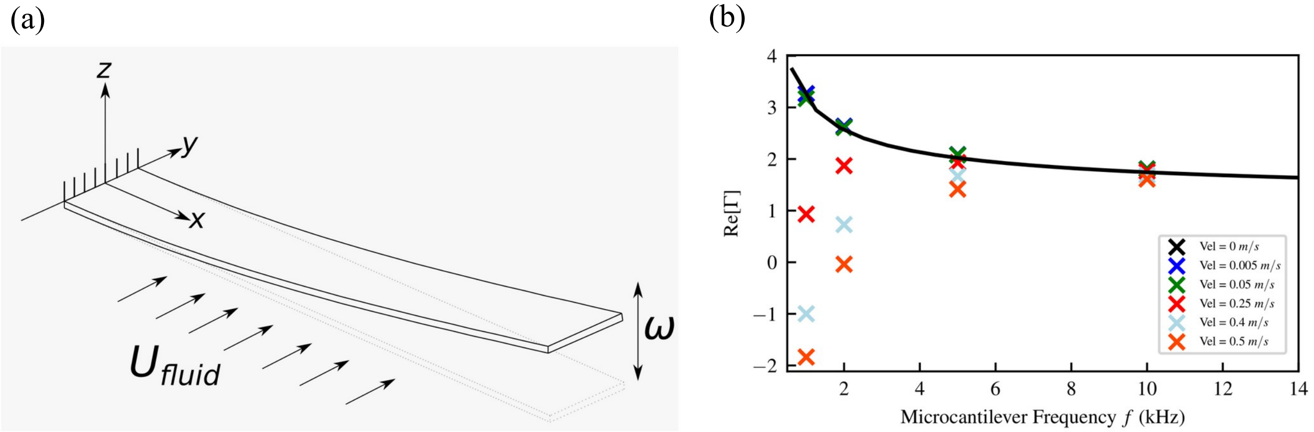


Figure 1. (a) Diagram of the simulated scenario of flow over an oscillating microcantilever; the displacement shown is heavily exaggerated and meant to show the direction of oscillation. (b) Values of the real part of the hydrodynamic function taken from simulated force data; the black line represents the expected results of the hydrodynamic function in an initially quiescent fluid [3].

References:

[1] Fukuda, S. et al. (2021) *Review of Scientific Instruments* 92, 033705
[2] Ando T. (2018) *Biophys Rev*.10(2), 285-292
[3] Sader, J. (1998) *J. Appl. Phys.* 84, 64

Isomorphic contact resonance: demonstration of a new contact resonance force microscopy technique for more direct material properties measurements

Lawrence H. Robins[1*], Matt D. Brubaker[2], Ryan C. Tung[3] and Jason P. Killgore[4]

[1.] Applied Chemicals and Materials Division, NIST, Boulder, Colorado, USA
[2.] Applied Physics Division, NIST, Boulder, Colorado, USA
[3.] Department of Mechanical Engineering, University of Nevada – Reno, Reno, Nevada, USA
* Applied Chemicals and Materials Division, NIST, Boulder, Colorado, USA

We introduce isomorphic contact resonance force microscopy (iso-CRFM), a new CR imaging technique that acquires data at constant frequency and contact stiffness throughout the scan area. Constant frequency is obtained by acquiring a force versus distance curve at each pixel, such that a preselected target frequency is reached somewhere in the force curve. As a consequence, the cantilever maintains an invariant vibrational shape and a constant environmental damping, thus simplifying interpretation of amplitude and quality factor image contrast. Advantages of iso-CR are demonstrated by presenting iso-CRFM (mechanically driven) and iso-CR piezoresponse force microscopy (iso-CR-PFM, electrically driven) images of a piezoelectric AlN thin film containing nanoscale Al-polar and N-polar domains. The domain structure is revealed by iso-CR-PFM phase imaging, which shows nearly 180° contrast between domains of opposite polarity. The PFM amplitude and Q-factor images also show domain contrast, which decreases with increasing CR frequency. Further, the difference between the iso-CR-PFM and iso-CRFM results decreases with increasing frequency. These frequency-dependent effects, summarized in Figure 1, are ascribed to frequency-dependent electrostatic artifacts in the measured PFM signals. Additional experiments were performed to examine the effect of varying the DC tip voltage (and thus varying the strength of the electrostatic force) on the iso-CR-PFM amplitude, Q factor, and other measurable parameters. We conclude that the iso-CR capability to control the CR frequency across multiple excitation schemes helps elucidate the origin of the amplitude and Q-factor image contrast.

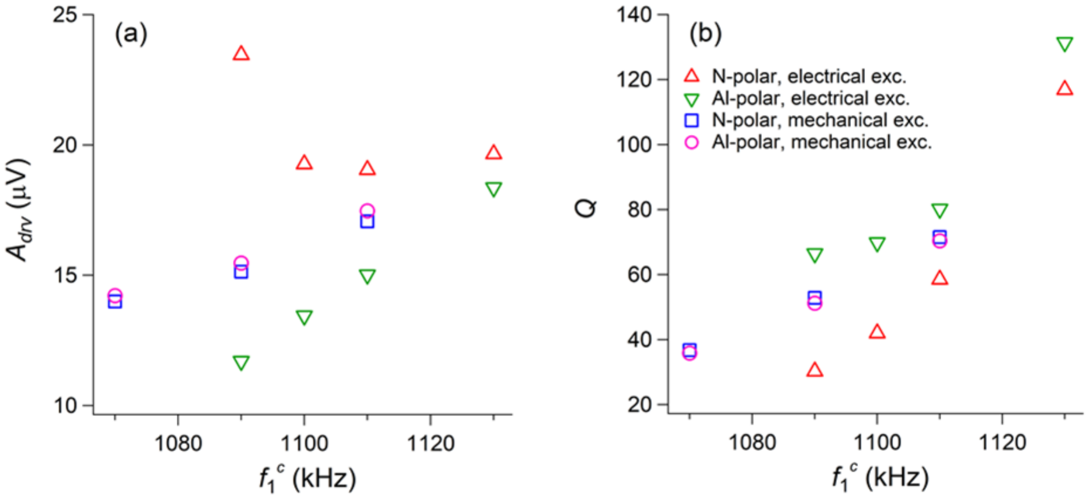


Figure 1. (a) Dependence of median amplitude (A_{drv}) on CR frequency (f_1^c) for electrical excitation in N-polar domains (triangles), electrical excitation in Al-polar domains (inverted triangles), mechanical excitation in N-polar domains (squares), and mechanical excitation in Al-polar domains (circles) of an AlN thin film. (b) Dependence of median Q-factor (Q) on f_1^c, with same labeling of data points as in (a).

Resonant mechanical force sensor based on cavity optomechanics

August Roos[1*], Ermes Scarano[1], Erik Holmgren[1], Gabriele Baglioni[1] and David Haviland[1]

[1.] KTH Royal Institute of Technology, Department of Applied Physics, Stockholm, Sweden
* Corresponding author: augroo@kth.se

The field of cavity optomechanics has recently demonstrated the detection of mechanical displacement at the standard quantum limit. A key feature is the use of a high-Q superconducting microwave resonator coupled to a mechanical mode of vibration. A measurement of the phase of the microwave resonance gives the mechanical deflection, with sensitivity limited by photon shot noise. If the width of the microwave resonance is smaller than the mechanical resonance frequency, so-called side-band cooling of the mechanical mode to its quantum ground state can be realized[1]. Using these ideas, we are developing a practical force sensor for scanning probe microscopy applications.

Our design and fabrication combines concepts from MEMS and circuit QED. We use nanowire meanders of NbTiN to realize 'super inductors' or superconducting structures with very large kinetic inductance and relatively small capacitance. Thus, we form a very compact, high-Q microwave resonance in the range of 500 MHz to 5 GHz. We investigate two separate designs to achieve coupling to the fundamental bending mode of a Si-N cantilever. The first design investigates a strain-induced change in the meander's kinetic inductance, caused by flexure of the supporting cantilever, as shown in Figure 1. The second design aims to modulate the capacitance of the microwave resonator as an inner cantilever flexes in opposition to the bending of an outer supporting plate. This design employs a mechanical lever to enhance the change of capacitance for small motion of the tip, which is fixed to the supporting plate.

[1] Teufel, J., Donner, T., Li, D. *et al.* Sideband cooling of micromechanical motion to the quantum ground state. *Nature* **475,** 359–363 (2011).

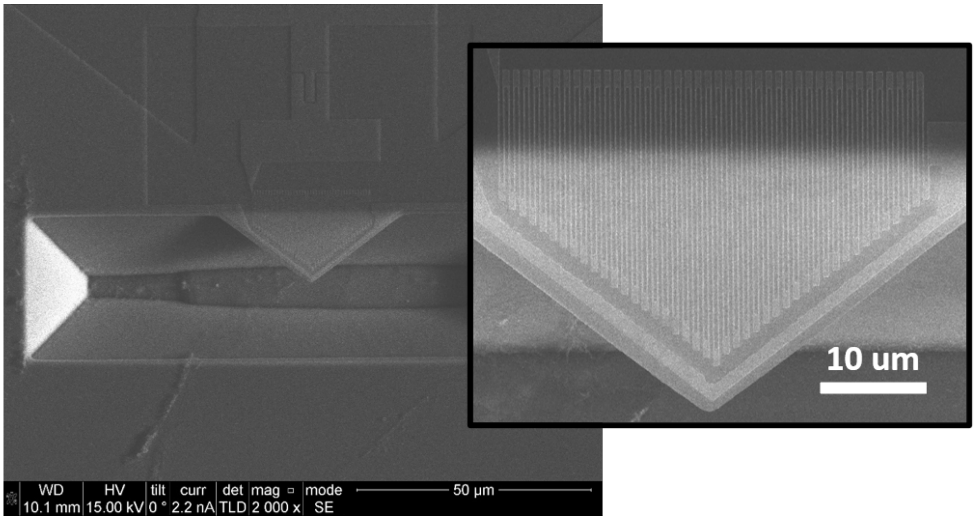
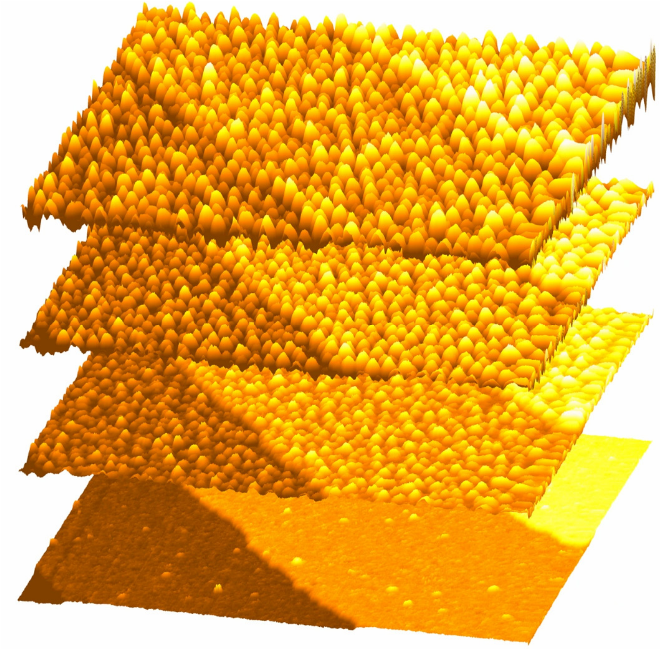
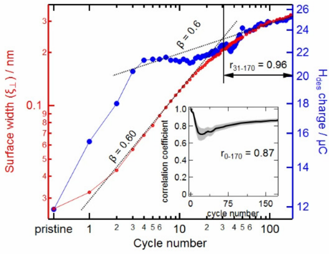


Figure 1. Top view SEM image of a prototype AFM probe based on inductive modulation. The released triangular membrane of silicon nitride forms the cantilever. The inductor is defined by the meandering nanowire and placed on the point of maximum strain near the base of the cantilever.

Correlation of Surface Site Formation to Nano-Island Growth in the Electrochemical Roughening of Pt(111)

Marcel J. Rost
Huygens-Kamerlingh Onnes Laboratory, Leiden University, Leiden, The Netherlands
rost@physics.leidenuniv.nl





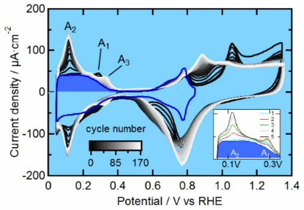


Image size: 230 x 230 nm^2
Measured in the double layer region:
U_s = 0.4 V and U_t = 0.45 V (vs. RHE)
after one or more CVs to 1.35 V

Platinum plays a central role in a wide variety of electrochemical devices. Electrode degradation, especially under oxidizing conditions, forms an important barrier for the widespread of applications. Although it is known that repeated oxidation and reduction of platinum electrodes results in irreversible surface structure changes, over thirty years of research did not yet yield to a conclusive description of this process on the atomic level; even not for well-defined single crystal surfaces.

Using a special EC-STM [1, 2], which is capable of measuring the electrochemical signals simultaneously during imaging in operando, we directly correlate, for the first time, the evolution of the hydrogen adsorption peaks on Pt(111) to the observed roughening of the surface [3-5]. In the later stages, we find a strong correlation between the evolution of the roughness and the absorption peaks clearly indicating that each created step contributes equally strong to the adsorption signal as well as to the roughness. However, surprisingly, in the early stage step edges are created that seem to be chemically "*inactive*". Analyzing this observation further, it turns out that vacancies play a crucial role: the well-established homoepitaxial adatom-growth modes are realized also in their counterpart as vacancy-growth modes [6, 7]. These results present not only an important step forward in understanding the atomic-scale process of the electrochemical roughening of Pt(111), but also provide valuable insight in the degradation of industrially relevant platinum nanoparticles and thus electrodes in fuel cells and electrolyzers, as a large part of their surface is naturally composed out of {111} planes.

[1] Y.I. Yanson, F. Schenkel and M.J. Rost; Rev. Sci. Instr., 84, 023702 (2013)
[2] M.J. Rost "High-Speed Electrochemical STM" in "Encyclopedia of Interfacial Chemistry: Surface Science and Electrochemistry" K. Wandelt, Elsevier, pp. 180-198, (2018), ISBN: 9780128097397
[3] L. Jacobse, Y.-F. Huang, M.T.M. Koper, and M.J. Rost, Nature Materials, 17 (3), 277, (2018).
[4] Movie: https://youtu.be/xSpRcsgxq3Q
[5] L. Jacobse, M.J. Rost, and M.T.M. Koper, ACS Central Science, 5 (12), 1920, (2019)
[6] M.J. Rost, L. Jacobse, and M.T.M. Koper, Nature Communications, 10, 5233, (2019)
[7] Movie: https://www.youtube.com/watch?v=xpN_mswrar8

Integrated superconductive lumped-element resonator on membrane for parametric force transduction in AFM

Ermes Scarano[1*], Elisabeth Arvidsson[1], August Roos[1], Erik Holmgren[1], Daniel Forchheimer[2] and David B. Haviland[1]

[1.] Nanostructure Physics, KTH Royal Institute of Technology, Stockholm, Sweden
[2.] Intermodulation Products AB, Sweden
* ermes@kth.se

In our quest towards quantum limited AFM, we propose a sensor concept based on parametric transduction via coupled oscillators. Parametric transduction is appealing since it is inherently noiseless, with fundamental limitations arising only from noise in the input field and quantum effects such as zero-point fluctuation of the mechanical motion, and back-action[1].

We integrate a superconductive lumped element resonator realized with a high kinetic inductance NbTiN meandering nanowire deposited on a Si_3N_4 membrane. With the relatively small capacitance of coplanar structures, we obtain high characteristic impedance and high Q factor microwave resonance, even when strongly over-coupled to the readout transmission line.

Resonances are probed via microwave reflectometry using a cryogenic circulator and a low noise amplifier. Fig 1b) shows the non-linear bending of the resonance curve with increasing input amplitude due to current-dependent kinetic inductance. We investigate electro-mechanical coupling arising from strain induced modulation of the kinetic inductance. By inertial excitation of the mechanical modes under microwave resonant drive, we detect the instantaneous phase shift of the reflected field or, in the frequency domain, resolve the sidebands in the upconverted mechanical oscillation via multifrequency lock-in.

References:
[1] Aspelmeyer, M., Kippenberg, T. J., & Marquardt, F. (2014). Cavity optomechanics. Reviews of Modern Physics, 86(4), 1391.

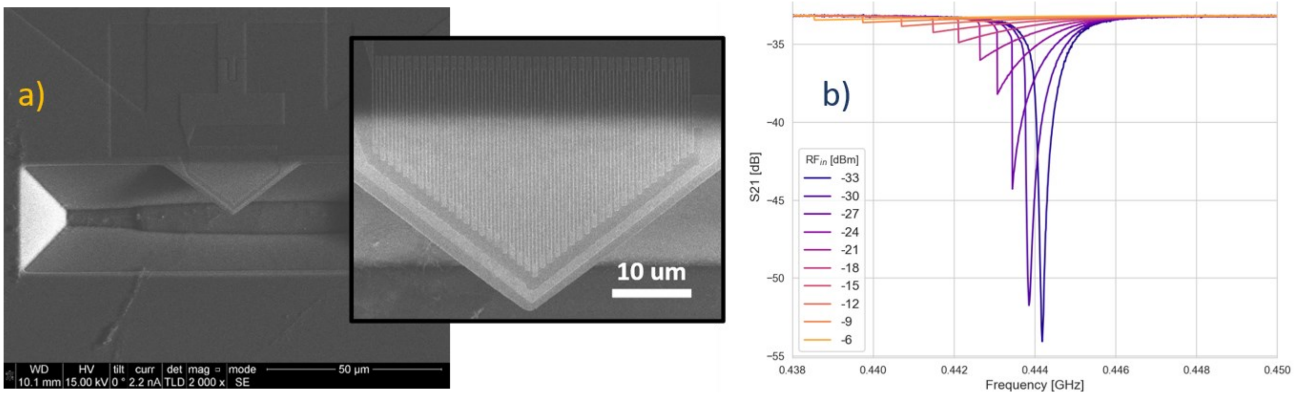


Figure 1. a) SEM image top view of a resonator-on-membrane AFM sensor prototype based on inductance modulation. The meandering nanowire sits on a triangular silicon nitride membrane. b) Measurement of the reflection coefficient of a series LC circuit near resonance. The plot highlights the effect of kinetic inductance non-linearity.

Friction and Adhesion between single DNA molecules and Nanoporous Membranes

Kordula Schellnhuber[1,2], Hanna Hübner[2], Johanna Blass[1] Markus Gallei[2], and Roland Bennewitz[1,2]

[1.]INM - Leibniz for New Materials, Campus D22, 66123 Saarbrücken
[2.]Saarland University, Faculty of Natural Sciences and Technology, 66123 Saarbrücken
* Kordula.Schellnhuber@leibniz-inm.de

Understanding and controlling the dynamics of polymer-surface interactions are key to a functional design of nanoscale objects and to reveal mechanisms underlying biological processes. We study friction and adhesion of single polymers at the solid-liquid interface by means of atomic force microscopy (AFM) with focus on entanglement dynamics. As a model system, a single M13mp18 DNA-molecule with a length of 2.5 µm is attached to an AFM probe. Friction measurements are performed by moving the cantilever in parallel to the surface at a height of a few hundred nanometers. Deflection of the cantilever reveals adhesive interactions between the DNA polymer and the membrane. Entanglement of the DNA in the membrane pores is probed by adhesion measurements after varying waiting time at a constant height of few hundred nanometers above the surface.

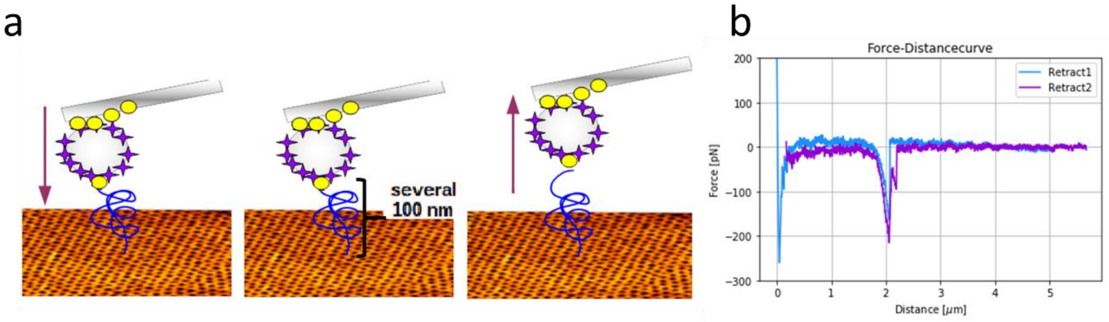


Figure 1. (a) Sketch of AFM force-spectroscopy measurements between a single DNA molecule and a nanoporous membrane. (b) Typical force-distance curve between a single DNA and the porous membrane with a characteristic rupture event at 2 µm.

Giant thermal expansion of 2D supramolecular networks tuned by alkyl chain functionalization

Sebastian Scherb[1*], Antoine Hinaut[1], Rémy Pawlak[1], J.G. Vilhena[1], Yi Liu[2], Xinliang Feng[3], Klaus Müllen[3], Thilo Glatzel[1], Akimitsu Narita[2] and Ernst Meyer[1]

[1.] Department of Physics, University of Basel, Basel, Switzerland
[2.] Max Plank Institute for Polymer Research, Mainz, Germany
[3.] Faculty of Chemistry and Food Chemistry, TU Dresden, Dresden, Germany
* Corresponding author: sebastian.scherb@unibas.ch

Thermal expansion is a fundamental property of solid systems that governs many of their mechanical applications. Due to various mechanisms involving intrinsic molecules flexibility, their propensity to conformational changes or their intermolecular interactions, molecular architecture allows large or negative expansion coefficients.

Here, we investigate the thermal response of 2D supramolecular networks on metal surfaces via molecular scale scanning probe microscopy, at 5 and 300K, of molecules equipped peripheral alkyl side chains [1,2]. By comparing two hexabenzocoronene (HBC) derivates and a shape persistent polyphenylene spoked wheel (SW) molecule, we determine giant thermal expansion coefficients up to 980 ± 10-6 1/K, twice larger than other molecular systems hitherto reported in literature. By exchanging the core or the surface and modifying the side chains length, the expansion can be fine-tuned while maintaining its order of magnitude.

Molecular dynamics simulations of the SW molecule assemblies support the giant expansion coefficient and reveal a mechanism of expansion based on large thermal fluctuations of the alkyl chains as a result of entropic effects and large anharmonic vibrations. This results in temperature-dependent intermolecular interactions that promote the giant expansion of the supramolecular network with temperature [1].

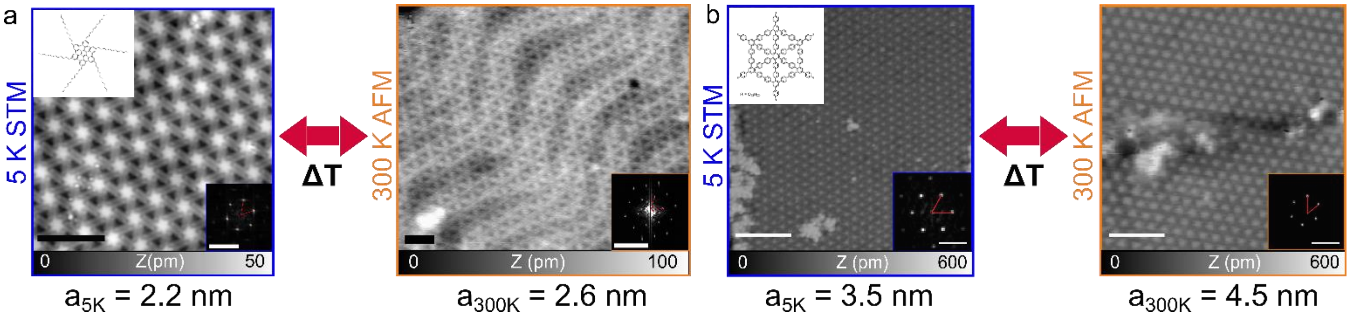


Figure 1. Thermal expansion of molecular assemblies. 5 K STM and 300 K nc-AFM topographies (a) and SW networks (b) showing giant thermal expansion. Scales: a: 5nm b: 20 nm

References:

[1] S. Scherb et al., Commun Mater 1, 8 (2020).
[2] A. Hinaut et al., Nanoscale **10** (2018), p. 1337.

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

Simon Scheuring[1,2]

[1.] Weill Cornell Medicine, Department of Anesthesiology, 1300 York Avenue, New York, NY-10065, USA.
[2.] Weill Cornell Medicine, Department of Physiology and Biophysics, 1300 York Avenue, New York, NY-10065, USA.
* Corresponding author: sis2019@med.cornell.edu.

High-speed atomic force microscopy (HS-AFM) is a powerful technique that provides dynamic movies of biomolecules at work. HS-AFM has the notable advantage that it permits to modulate the applied force during imaging, thus representing an excellent tool to study mechanosssensitive molecules. Piezo proteins are mechanosensitive ion channels that mediate force-detection in eukaryotic cells through translating a mechanical stimulus into an electrical signal. Recent cryo-EM studies have revealed the structure of most parts of the channel, and gating mechanisms have been suggested. However, it is intrinsically difficult to acquire a structural view of the channel exposed to force. Using HS-AFM imaging during force-sweep cycles, we show that Piezo1 undergoes significant conformational changes under force: the channel reversibly flattens into the membrane plane. In the framework of a model where channel flattening relates to in-plane area expansion, we also derive from the HS-AFM experiments the sensitivity of Piezo1 [1].
To break current temporal limitations to characterize molecular dynamics using HS-AFM, we developed HS-AFM height spectroscopy (HS-AFM-HS), a technique whereby we oscillate the HS-AFM tip at a fixed position and detect the motions of the molecules under the tip. This gives sub-nanometer spatial resolution combined with microseconds temporal resolution of molecular fluctuations. HS-AFM-HS can be used in conjunction with HS-AFM imaging modes, thus giving access to a wide dynamic range [2].
To break current resolution limitations, we developed Localization AFM (LAFM). By applying localization image reconstruction algorithms to peak positions in high-speed AFM and conventional AFM data, we increase the resolution beyond the limits set by the tip radius and resolve single amino acid residues on soft protein surfaces in native and dynamic conditions. The LAFM method allows the calculation of high-resolution maps from either images of many molecules or many images of a single molecule acquired over time, opening new avenues for single molecule structural analysis [3].

[1] Lin et al. Nature, 2019, 573(7773):230–234, Force-induced conformational changes in Piezo1
[2] Heath et al. Nature Communications, 2018, 9(1):4983, High-Speed AFM Height Spectroscopy (HS-AFM-HS): Microsecond dynamics of unlabeled biomolecules
[3] Heath et al. Nature, accepted, Localization Atomic Force Microscopy

The effect of hinge region flexibility on the binding behaviour of monoclonal hIgG antibodies to CD40

Hannah Seferovic[1*], Patricia Sticht[1], Javier Chaparro-Riggers[2], Wei Chen[2], Christian Wechselberger[3], David Bernhard[3] and Peter Hinterdorfer[1]

[1.] Institute for Biophysics, Johannes Kepler University, Linz, Austria
[2.] Biotherapeutic Design & Discovery, Pfizer Inc., South San Francisco, USA
[3.] Division of Pathophysiology, Medical Faculty, Johannes Kepler University, Linz, Austria
* Presenting author: hannah.seferovic@jku.at

The recognition of antigens via T-cell receptors is not sufficient for an effective immune response. Additional coupling of T-cell surface receptors to certain ligands on the surface of antigen-presenting cells (APCs) is required, triggering costimulatory signals in both cells and stabilizing their connection. One of these ligands on APCs is the transmembrane protein CD40. It plays an important role in activation of APCs, such as dendritic cells and B-cells, and is often found on the surfaces of different types of cancer cells. In immunological pathways the stimulation of CD40 on APCs is triggered by helper T-cells via the CD40 ligand (CD154) [1].

Cancer cells can block certain host immune mechanisms, leading to immune tolerance. By utilizing special therapeutic antibodies this immune tolerance can be disrupted either by blocking certain checkpoints or stimulating receptors by mimicking their ligands [1]. For this purpose, we investigated the binding behaviour of a set of human IgG monoclonal antibodies to CD40 using single molecule force spectroscopy (SMFS). IgG subtypes, such as IgG1, IgG4 and two isoforms of the human IgG2 (IgG2A and IgG2B) differ in the disulphide-connectivity of their hinge region, which determines the antibody's flexibility. Studies showed that IgG2B is less flexible and has the most compact structure compared to the other IgG subtypes [2]. Furthermore, all antibody subtypes trigger B-cell activation when coupled to CD40, if stabilized additionally via the Fcγ receptor on the APC's surface. Here the exception is IgG2B, which does not need the extra support of the Fcγ receptor, most probably due to its rigid and compact form [2],[3].

With our SMFS experiments we were able to detect ruptures between single bonds of the IgG-CD40 and CD154-CD40 complexes. Interestingly, the bond lifetime differs for the IgG subtypes. The rigid hIgG2B shows a higher bond lifetime to CD40 than the more flexible hIgG1. This leads to the idea that the hinge region flexibility might influence the stability of the IgG-CD40 interaction. For further investigations, SMFS experiments with different surface interaction times will be performed to gain an insight of the antibodies' ability of forming double bonds with CD40 and to compare this behavior between the IgG subtypes.

[1] Lee CS *et al.*, British journal of clinical pharmacology **vol. 76,2** (2013)
[2] Liu X *et al*, Nature Communication **vol. 10,4206** (2019)
[3] White AL *et al.*, Cancer cell **vol. 27,1** (2015)

Sensor Egregium – An Atomic Force Microscope Sensor for Continuously Variable Resonance Amplification

Rafiul Shihab[1], Tasmirul Jalil[1], Burak Gulsacan[1], Matteo Aureli[1], and Ryan C. Tung[1*]

[1.] University of Nevada, Reno, Department of Mechanical Engineering, Reno, NV USA.
* Corresponding author: rtung@unr.edu

Several AFM contact imaging modes measure small surface displacements at specific excitation frequencies, such as scanning joule expansion microscopy, piezoresponse force microscopy (PFM), and electrochemical strain microscopy. Combining these methods with contact resonance (CR) spectroscopy, which utilizes the measurement of the natural frequencies of vibration of the permanently coupled, tip-sample system to obtain quantitative information about the sample, has shown drastic measurement enhancement due to resonance amplification, for instance in PFM methods [1]. However, one primary issue with current CR methods is that the amplification bandwidth of the measurement is constrained to the bandwidth of the discrete in-contact natural frequencies of the system. That is, one can only measure frequency dependent phenomena, with resonance amplification, in very small frequency windows. Additionally, changes in sample topography and material properties can alter the locations of these natural frequencies.

One way to achieve resonance amplification at arbitrary frequencies is to adjust the stiffness of the sensor in-situ. We posit that a continuous plate geometry utilizing curvature-induced in-plane straining will allow in-situ static and dynamic stiffness adjustment across a wide range of frequencies while simultaneously offering increased sensor bandwidth [1]. In this work, we propose a method to arbitrarily tune the stiffness and natural frequencies of a microplate sensor for atomic force microscope applications, thereby allowing resonance amplification at a broad range of frequencies [2]. This method is predicated on the principle of curvature-based stiffening. A macroscale experiment is conducted to verify the feasibility of the method. Next, a microscale finite element analysis is conducted on a proof-of-concept device. We show that both the stiffness and various natural frequencies of the device can be highly controlled through applied transverse curvature. Dynamic phenomena encountered in the method, such as eigenvalue curve veering, are discussed and techniques are presented to accommodate these phenomena. We believe this work is a step towards facilitating significant advances in the field of nanometrology.

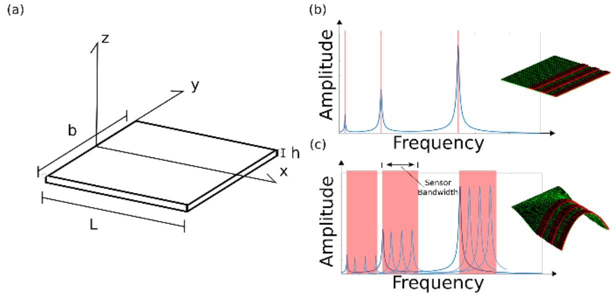


Figure 1. (a) Proposed sensor in its unperturbed state. (b) The natural frequencies of the unperturbed device are shown on the amplitude vs. frequency plot. (c) As transverse curvature is applied, the natural frequencies of the device increase. The location of the natural frequencies of vibration of the sensor can be controlled through applied transverse curvature.

References:

[1] Zhu, Q., et al. (2019). *J. Mech. Phys. Solids*, 126, 76-86.
[1] Aureli, M., Ahsan, S. N., Shihab, R. H., & Tung, R. C. (2018). *J. App. Phys.*, 124(1), 014503.
[2] Shihab, R., Jalil, T., Gulsacan, B., Aureli, M., & Tung, R. (2021). *J. Vib. Acous.*, 143(4), 041013.

Microscale Tissue Stiffening After Spinal Cord Injury

Delphine Sicard[1*], John G. Cooper[2] Sripadh Sharma[2], Stephanie Van Gulden[2], Tammy L. McGuire[2], Miguel Pareja Cajiao[3], John A. Kessler[2] and Daniel J. Tschumperlin[1]

[1.] Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA.
[2.] Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA.
[3.] Department of Anesthesiology, Mayo Clinic, Rochester, MN, USA.
* Corresponding author: sicard.delphine@mayo.edu

Spinal cord injury (SCI) often induces a permanent loss of motor and sensory function due to the formation of chronic scar at the injury lesion site. This scar tissue was long assumed to be mechanically stiffer than normal tissue, representing a physical barrier composed of a meshwork of reactive glia and crosslinked extracellular matrix that contributes to the failure of neuronal regeneration. However, Moeendarbary et al. reported that spinal cord crush injury on rats leads to tissue softening at early time points after SCI [1]. In the literature, there is little information regarding chronic SCI stiffening. This represents a critical gap in knowledge in a field which searches for potential targets and clinical interventions to restore function after SCI.

In our study, we used atomic force microscopy microindentation to evaluate the micromechanical properties of murine spinal cord. We first analyzed important aspects of sample preparation (comparison between fresh and frozen sample), the force applied on sample to perform force curves, and the analysis of the data, yielding an enhanced understanding of the critical parameters for assessing spinal cord micromechanics.

We then applied our approach to demonstrate that at 12 weeks post injury the chronically injured mouse spinal cord is significantly stiffer than healthy tissue (1217 Pa and 405 Pa respectively). Recently, Jeong et al. have reported that acute intravenous treatment after SCI with immune modifying nanoparticles (IMP) selectively reduces chronic fibrotic scarring and improves motor function in mice at 12 weeks post injury [2]. We determined that after treatment with these IMP the spinal cord stiffening (517 Pa) is significantly lower than for the injured tissue with no treatment, and also close to the elastic modulus results for uninjured tissue.

Taken together, these results validate the important influence of spinal cord stiffening after injury on neuronal regeneration.

References:

[1] E Moeendarbary et al, Nature Communications **8** (2017), p. 14787.
[2] S J Jeong et al, Neurobiology of Disease **108** (2017), p. 73.

New Multi Dimensional AFM modes to Identify Cells Using Machine Learning

I. Sokolov[1,2,3 *]

[1.] Department of Mechanical Engineering, Tufts University, Medford, MA 02155, USA
[2] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA
[3] Department of Physics, Tufts University, Medford, MA 02155, USA

* Email: Igor.Sokolov@Tufts.edu

New multi dimensional AFM modalities, sub- resonance tapping, Ringing mode, FT-NanoDMA allow collecting multiple images of a sample surface simultaneously. These images represent different physical/chemical information on the sample surface. As a result, it becomes possible to combine these images to identify different materials by their surface features. For example, Ringing mode, in combination with PeakForce QNM sub-resonance tapping presently allows for simultaneous obtaining 14 maps/images of different physical and chemical properties of the sample surface. FT-NanoDMA allows simultaneous recording of more than 20 dynamical mechanical parameters. This wealth of information requires special methods of data processing. Machine learning seems to be a natural method of classification of sets of complex images. “Classical” deep learning methods, however, are not directly applicable to AFM imaging because they require an unrealistically large number of images for training. This is technically impossible because AFM is a relatively slow technique to obtain the required number of images.

In this talk, I will describe our solution to adapt AFM imaging for use together with machine learning. The key is in a substantial reduction of the dimension of the data space by using so-called surface parameters. An example of this approach will be described. The Ringing mode images of adhesion and height are used to detect the cells coming from urine of bladder cancer patients [1,2]. The reduction of the imaging data space to the space of the surface parameter will be demonstrated. The method of further decrease of the data space dimension by the appropriate choice of the relevant surface parameters will be described. The steps to produce sufficient statistics using bootstrap methods will be overviewed. An important problem of overtraining will be discussed. The described method can be applied to the classification of virtually any surface, not necessarily the medical one.

References:

[1] I. Sokolov, M. E. Dokukin,V. Kalaparthi, M. Miljkovic, A. Wang, J. D. Seigne, P. Grivas, E. Demidenko, Proceedings of the National Academy of Sciences (PNAS), **115**(2018), p. 12920.
[2] Siona Prasad, Alex Rankine, Tarun Prasad, Patrick Song, Maxim E. Dokukin, Nadezda Makarova, Vadim Backman, I. Sokolov* Atomic force microscopy detects the difference in cancer cells of different neoplastic aggressiveness via machine learning, AdvancedNanoBiomed Research, in press.

Enabling Autonomous AFM Imaging of Single Molecules with Deep Learning

Javier Sotres[1,2,*], Hannah Boyd[1,2], Juan F. Gonzalez-Martinez[1,2]

[1] Department of Biomedical Science, Faculty of Health and Society, Malmö University, 20506 Malmö, Sweden

[2] Biofilms-Research Center for Biointerfaces, Malmö University, 20506 Malmö, Sweden

[*] Corresponding author. E-mail: javier.sotres@mau.se

Scanning Probe Microscopies allow investigating surfaces at the nanoscale, in the real space and with unparalleled signal-to-noise ratio. However, these microscopies are not used as much as it would be expected considering their potential. The main limitations preventing a broader use are the need of experienced users, the difficulty in data analysis and the time-consuming nature of experiments that require continuous user supervision. In this contribution, we present a deep learning based strategy that addresses the latter. Specifically, we present an algorithm for controlling the operation of an Atomic Force Microscope (AFM) that, without the need of user intervention, allows acquiring multiple high-resolution images of different molecules. We used DNA on mica as a model sample to test our control algorithm, which makes use of two state-of-the-art deep learning techniques. One is an object detector, YOLOv3, which provides the location of molecules in the captured images. The second is a Siamese network that can identify the same molecule in different images. This allows both performing a series of images on selected molecules while incrementing the resolution, as well as keeping track of molecules already imaged at high resolution, avoiding loops where the same molecule would be imaged an unlimited number of times. Overall, this work brings SPM a step closer to full autonomous operation.

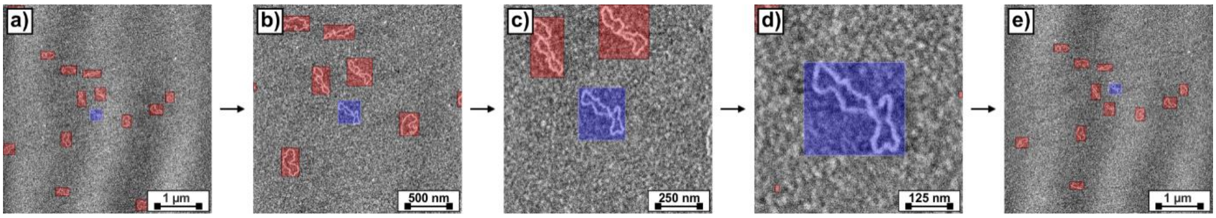


Figure 1. Consecutively acquired AFM images representative of the workflow of the autonomous imaging algorithm. The algorithm detects a suitable single DNA molecule (a), and performs successive zooms (b-c) on the selected molecule until a user-set threshold for enough resolution is achieved. Then, the algorithm zooms out, a different molecules is selected (e), continuously repeating a similar workflow.

Open-loop sideband frequency-modulation Kelvin probe force microscopy

Gheorghe Stan[1*]

[1.] Material Measurement Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Stop 8520, Gaithersburg, MD 20899, USA
* Corresponding author: gheorghe.stan@nist.gov

In the last years new open-loop (OL) Kelvin probe force microscopy (KPFM) modes [1] were introduced to facilitate a faster and more accurate surface potential characterization at the nanoscale. Based on high-speed data capture, the OL KPFM modes observe the tip-sample electrostatic interaction in the time domain, which potentially can increase the temporal resolution by few orders of magnitude in comparison to common closed-loop (CL) KPFM implementations that rely on frequency domain detection [1, 2].
In this work, the high-speed digitization for open-loop sideband frequency-modulation (FM) KPFM is presented in terms of amplitude and frequency responses of the cantilever. In both cases, the tip-sample contact potential difference (CPD) is extracted from the time series analysis of the signals. This interpretation is demonstrated to be superior to the more common used analysis of the parabolic bias dependence of the OL KPFM signals. The OL sideband FM-KPFM amplitude and frequency responses were compared side-by-side to their CL AM-KPFM and CL FM-KPFM counterparts on materials of different surface potentials and subjected to various bias voltages. Notably, the OL sideband FM-KPFM frequency retains the tip-confinement sensitivity that is characteristic to the FM-KPFM based modes while the OL sideband FM-KPFM amplitude suffers from cantilever stray capacitive couplings much in the same way as the common CL AM-KPFM mode.

[1] L Collins, A Belianinov, S Somnath, N Balke, SV Kalinin and S Jesse, Sci. Reports **6** (2016) 30557.
[2] L Collins, M Ahmadi, T Wu, B Hu, SV Kalinin and S Jesse, ACS Nano **11** (2017) 8717.

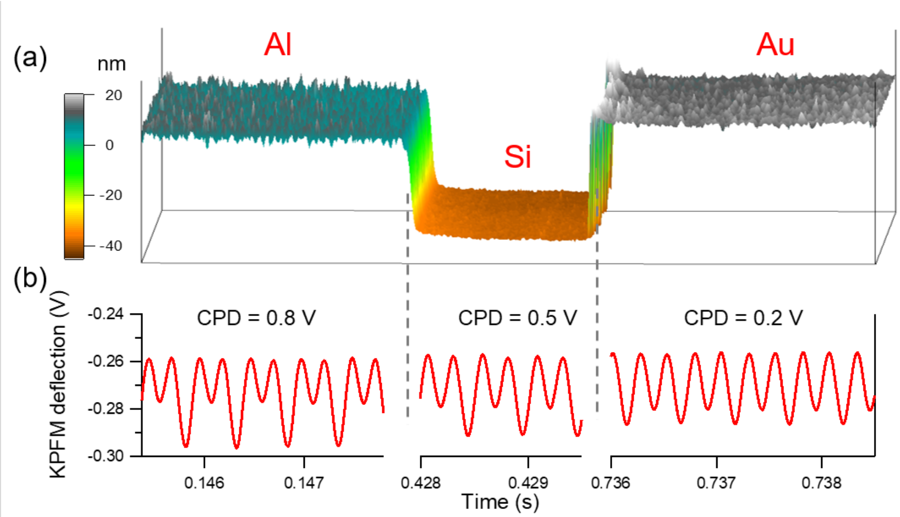


Figure 1. a) Topography of a 15.0 µm x 2.5 µm Al/Si/Au trench. b) KPFM deflection of the cantilever in OL sideband FM-KPFM showing the variability imposed by CPD over each region of the trench.

Polarization-dependent Stiffness of Ferroelectric $BaTiO_3$ Single Crystals at the Nanoscale

Christina Stefani[1], Martí Checa[1], Gustau Catalan[1,2] Liam Collins[3], Stephen Jesse[3], Neus Domingo[1]
[1] Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Campus UAB, Bellaterra, 08193 Barcelona, Spain
[2] ICREA - Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain.
[3] Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA.
* Christina.stefani@icn2.cat

Strain gradients induced under an AFM tip in contact mode on a dielectric surface are huge enough to induce flexoelectricity, that is, the appearance of an effective electric field that has been observed to be high enough to switch the ferroelectric polarization in thin films. [1]

The mechanical response of homogeneous materials does not change when they are turned upside down, even for piezoelectrics and ferroelectrics. This paradigm can be altered, however, in the presence of flexoelectricity. Strain gradients induced under an AFM tip in contact mode are huge enough to induce flexoelectricity. These flexoelectric fields, coupled to ferroelectric properties at the nanoscale, can promote changes in the mechanical properties of ferroelectric materials as a function of the direction of the ferroelectric polarization [2].

Here we will show the change of the mechanical stiffness between oppositely poled ferroelectric domains as measured under an AFM tip and the asymmetry on the effective Young's modulus on different areas of $BaTiO_3$ single crystals as a function of polarization domains and domain walls. We use both Piezoresponse Force Microscopy (PFM) and Contact Resonance AFM (CR-AFM) in Band Excitation mode to identify the different ferroelectric domains, and build up their mean electromechanical and mechanical response. We present a detailed quantification model correlating changes in resonance frequency, as obtained by AFM measurements, with difference in stiffness of the material [3].

Contrast of both sets of data (PFM and CR-AFM) allows us to decouple gradient based mechanical and electromechanical responses from classical electromechanical signals, paving the way towards true nanoscale quantification of piezoelectricity.

References

[1] H. Lu, C.-W. Bark, D. Esque de los Ojos, J. Alcala, C. B. Eom, G. Catalan,*, A. Gruverman,* Science, 336 (2012) 59
[2] K. Cordero-Edwards, N. Domingo, A. Abdollahi, J. Sort, G. Catalan, Ferroelectrics as Smart Mechanical Materials, Advanced Materials,29 (2017) 1702210.
[3] Ch. Stefani, M. Checa, G. Catalan, L.Collins, S. Jesse, N. Domingo, Manuscript under preparation.

Molecular recognition & selective binding of cargo DNA within DNA nanopores

Saanfor Hubert Suh[1], Xing Yongzheng[2], Rong Zhu[1], Stefan Howorka[1,2],and Peter Hinterdorfer[1]

[1]Institute of Biophysics, Johannes Kepler University Linz, Gruberstr. 40, 4020 Linz, Austria

[2]University College London, Department of Chemistry, 20 Gordon Street, London WC1H0AJ

Protein channels in natural lipid membranes are capable of fulfilling a variety of functions in living cells from recognition of substrates to selective transport of ions or large biomolecules between cellular compartments. Several biological and synthetic nanopores can be built from different biological building blocks. Here, we examine transport under controlled nanoscale conditions using large DNA nanopores that offer defined dimensions. Amongst others, our DNA pores particularly allow for precise positioning of multiple recognition sites along the channel lumen.

Our first model pore is composed of several interlinked DNA duplexes that enclose a 7.5 x 7.5 nm^2 central lumen. Such a wide lumen permits electrophoretic transport of folded proteins like trypsin across lipid membranes. The pore's rational design, assembly, structural characterization and transport assays were published in Nature Communications[1]. This pore study is an important step but does not yet demonstrate facilitated transport via molecular recognition.

To enable molecular recognition studies in this work, a pore variant of the aforementioned pore model was studied with AFM based single molecule force spectroscopy and simultaneous topography & recognition microscopy (TREC). This pore variant has a slightly modified design to facilitate binding onto a flat substrate (in this case, streptavidin) as opposed to lipid bilayer insertion.

Generated pores were verified for successful nanopore assembly by agarose gel electrophoresis and structurally characterised by AFM in tapping mode to confirm the overall geometry and dimensions. DNA-functionalised cantilevers were used to probe the interaction between the cargo DNA and the receptors positioned in the lumen. In the microscopy mode, unbinding events (dark spots in recognition images) between cargo and receptors are mapped to the topography of the pore. In the force spectroscopy mode, unbinding events were seen as clear rupture signals in the force distance curves. These unbinding forces were determined to infer the kinetic rate constants and the energy landscape.

Reference

[1] T. Diederichs et *al.*, Nature Communications **2019**, 10, 5018

Effect of Substrate Interaction on Thermodynamics of Prefreezing

Muhammad Tariq[1†], Oleksandr Dolynchuk[1], and Thomas Thurn-Albrecht[1*]

[1.] Institute of physics, Martin Luther University Halle-Wittenberg, Germany.
* Corresponding author: thomas.thurn-albrecht@physik.uni-halle.de
† Presenter: muhammad.tariq@physik.uni-halle.de

It is well-known that crystallization of liquids typically starts at the interface to a foreign solid surface. In general, interface-induced crystallization can take place either via heterogeneous nucleation or via prefreezing. The latter refers to the abrupt formation of a thermodynamically stable crystalline layer at the melt-solid interface above the melting temperature, and is an equilibrium phenomenon.[1] The recently developed phenomenological theory[2] of prefreezing predicts that the transition temperature T_{max} depends primarily on the difference of the interfacial free energies $\Delta\gamma = \gamma_{sm} - (\gamma_{sc} + \gamma_{cm})$, whereas the minimum thickness of the prefrozen layer l_{min} at T_{max} is controlled by the ratio $\frac{\gamma_{sc}+\gamma_{cm}}{\gamma_{sm}}$. To test these predictions, we investigated prefreezing of various polymer-substrate systems by direct in situ AFM experiments performed in net-attractive regime[3] of tapping mode. Because of the reduced tip-sample interactions in this special regime of AFM operation, scanning over the liquid material at elevated temperatures is possible without much contamination of the tip of the AFM cantilever. The results for polyethylene (PE) on a molybdenum disulfide (MoS_2) substrate evidence a much higher T_{max} than on graphite, caused by a larger value of $\Delta\gamma$ of PE-MoS_2. In case of poly(ε-caprolactone) (PCL), where direct measurements of the prefrozen layer thickness are possible, T_{max} of the prefrozen PCL on MoS_2 remains nearly the same as on graphite, whereas l_{min} decreases to a smaller value. With these findings, we confirm that, firstly, $\Delta\gamma$ plays a primary role in determining T_{max} and, secondly, T_{max} and l_{min} can vary independently. It is worthy to mention that our findings are not limited to polymer crystallization only but also valid for crystallization of other liquids on solid surfaces.

[1] M. Tariq, O. Dolynchuk, and T. Thurn-Albrecht, J. Phys. Chem. C 124 (2020), p. 26184.
[2] O. Dolynchuk, M. Tariq, and T. Thurn-Albrecht, J. Phys. Chem. Lett. 10 (2019), p. 1942.
[3] T. Henze, K. Schröter, and T. Thurn-Albrecht, Nanotechnology, 23 (2012), p. 245702.

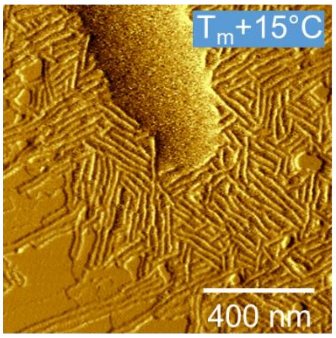


Figure 1. In situ observation of prefreezing. Net-attractive AFM amplitude image of a 10 nm thin PCL film on MoS_2 at 73 °C (well above T_m) shows the stable interfacial crystalline layer around the featureless molten droplet.

On the Quantitative Nanomechanical Properties of Hydrogel Substrates for the Study of Cell Mechanics

Thi Quynh TRAN[1,2], Abir KAZAN[2], Sylvain GABRIELE[2], and Philippe LECLERE[1]

[1] Laboratory for Chemistry of Novel Materials, Center for Innovation and Research in Materials and Polymers (CIRMAP), University of Mons (UMONS), B-7000 Mons, Belgium.
[2] The Mechanobiology & Soft Matter group, Interface and Complex Fluids Laboratory, University of Mons (UMONS), B-7000 Mons, Belgium.

* Corresponding author: philippe.leclere@umons.ac.be

In the field of cell mechanics, hydrogels are popularly used as extracellular matrices (ECMs) due to their tuneable tissue-like properties, biodegradability, and biocompatibility in all different forms of biomaterials. Interestingly, physicochemical properties of the ECM can couple with numerous cell processes, such as differentiation [1], replication, migration [2],[3], and apoptosis [4]. In this work, Polyacrylamide hydrogels (PA) fabricated and commercial hydrogels (from Matrigen) with well-defined stiffness are remarked to study the impact of hydrogel stiffness on the mechanical properties of cells. Firstly, the viscoelastic properties were estimated by nano Dynamic Mechanical Analysis, based on every single force curve while the probe is indenting the surface with nano-Newton forces in physiologic medium. According to the obtained results, a linear stiffness gradient is determined while bis-acrylamide/acrylamide ratio increases. In addition, the adhesion from PeakForce Quantitative NanoMechanics (PF QNM) is higher significantly than the value obtained in Force Volume (~0.2nN). A second step, we managed to quantitatively map the nanomechanical properties of human umbilical vein endothelial cells (HUVECs), the cellular response is investigated at 500 pN of force. We found that the moduli of cell body are in the range of 12-18 kPa higher slightly than cell edge (5-10 kPa), and Tan δ is ~0.53 for cell body.

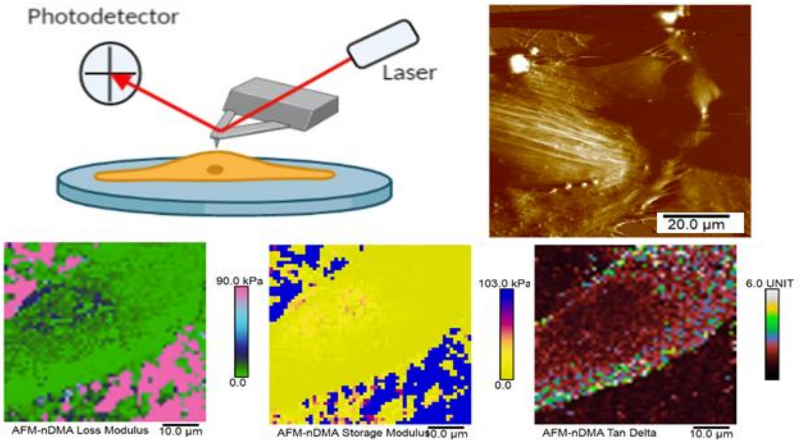


Figure 1: Schematic for tip–cell surface interaction; AFM images (loss modulus and storage moduli, and Tan δ) of live human umbilical vein endothelial cells in liquid.

[1] A. P. Kourouklis, K. B. Kaylan, and G. H. Underhill, *Biomaterials*, 2016.
[2] Q. Luo, D. Kuang, B. Zhang, and G. Song, *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1860, no. 9, pp. 1953–1960, 2016.
[3] W. J. Hadden *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, vol. 114, no. 22, pp. 5647–5652, 2017.
[4] Y. H. Zhang, C. Q. Zhao, L. S. Jiang, and L. Y. Dai, *Matrix Biol.*, vol. 30, no. 2, pp. 135–144, 2011.

SPM and fluorescence microscopy on human osteoarthritic cartilage: Correlation of chondrocyte organization, nanomechanics and collagen structure

Mathaeus Tschaikowsky[1,2], Tanja Neumann[3], Sofia Brander[1], Julien Dengler[1], Heiko Haschke[3], Bernd Rolauffs[2], Bizan N. Balzer[1,4] and Thorsten Hugel[1,4]

[1.] Institute of Physical Chemistry, University of Freiburg, Freiburg, Germany.
[2.] G.E.R.N. Center for Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Medical Center - University of Freiburg; Faculty of Medicine, University of Freiburg, Freiburg, Germany.
[3.] Bruker JPK BioAFM, Berlin, Germany.
[4.] Cluster of Excellence livMatS @ FIT – Freiburg Center for Interactive Materials and Bioinspired Technologies, Freiburg, Germany.

Osteoarthritis is the leading cause of chronic pain and disability. Its underlying mechanisms remain elusive, and the available treatment is symptomatic. Spatially resolved monitoring of articular cartilage will enhance our ability to specifically detect the early stages of osteoarthritis for improving our understanding of the disease and fostering effective therapies[1]. Here we combine a highly stable AFM with fluorescence microscopy and precisely motorized movement to correlate micro- and nanoscopic properties of articular cartilage on a millimeter sized sample under native conditions[2]. This serves us to unravel the relationship between the spatial organization of chondrocytes, micrometer scale changes in articular cartilage properties and nanoscale organization of collagen fibers. We demonstrate that major loss of micro- and nanoscale articular surface stiffness is detectable in regions that display earliest identifiable osteoarthritis. We show that local changes of the chondrocyte organization typical for early osteoarthritis closely co-localize with a severe impairment of the collagen fiber organization. These findings suggest that articular surface degeneration across length scales occurs much earlier than anticipated and that changes in the spatial organization of chondrocytes might be a main cause for cartilage degeneration in early osteoarthritis. Finally, we will discuss how local frequency dependent elastic moduli from SPM experiments can further guide our understanding of hierarchical biomaterials like cartilage.

References:

[1] Tschaikowsky et al., Osteoarthritis and Cartilage **29** (2) (2021), p. 269–279.
[2] Tschaikowsky et al., Acta Biomaterialia (2021) (in press), DOI: 10.1016/j.actbio.2021.03.034.

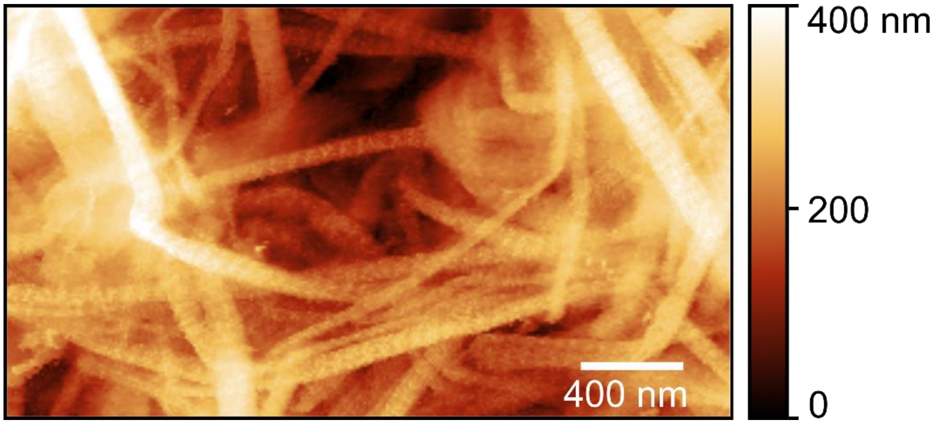


Figure. AFM image recorded under native conditions: Articular surface of a human femoral condyle[2].

Atomically resolved interfacial water structures on crystalline hydrophilic and hydrophobic surfaces

Manuel Ralph Uhlig[1*], Simone Benaglia[1], Ravindra Thakkar[2], Jeffrey Comer[2] and Ricardo Garcia[1]

[1.] Instituto de Ciencia de Materiales de Madrid (CSIC), 28049 Madrid, Spain
[2.] Nanotechnology Innovation Center of Kansas State, Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas, 66506, USA

* Corresponding author: manuel.uhlig@csic.es

Hydration layers are formed on hydrophilic crystalline surfaces immersed in water [1]. Their existence has also been predicted for hydrophobic surfaces, yet the experimental evidence is controversial. Using 3D-AFM imaging, we probed the interfacial water structure of hydrophobic and hydrophilic surfaces with atomic-scale resolution [2,3]. We demonstrate that the nanoscale structure of interfacial water on crystalline surfaces presents two antagonistic arrangements. On mica, a common hydrophilic crystalline surface, the interface is characterized by the formation of hydration layers separated by ≈0.3 nm. On a variety of hydrophobic surfaces such as graphite, h-BN and transition metal dichalcogenides, the interface is characterized by solvation layers separated by ≈0.5 nm. Studying the time-evolution of both solid-liquid interfaces indicated that airborne species are the constituents of the interfacial layers observed on hydrophobic surfaces [3]. By including them in molecular dynamics simulations we reproduced the experimental data and identified the involved species [3]. Experiments and simulations reveal that water molecules are expelled from the vicinity of the surface and replaced by airborne hydrocarbons. This creates a new 1.5–2 nm thick interface between the hydrophobic surface and the bulk water. Our results provide a comprehensive understanding of interfacial water on crystalline surfaces and have direct implications on the interfacial dielectrical properties of hydrophobic surfaces in water [4,5].

[1] T. Fukuma, R. Garcia, ACS Nano **12** (2018) 11785
[2] M. R. Uhlig, D. Martin-Jimenez, R. Garcia, Nat. Comm. **10** (2019) 2606
[3] M. R. Uhlig, S. Benaglia, R. Thakkar, J. Comer and R. Garcia, Nanoscale **13** (2021) 5275
[4] L. Fumagalli et al., Science **360** (2018) 1339
[5] T. T. Duignan and X. S. Zhao, J. Phys. Chem. C **123** (2019) 4085

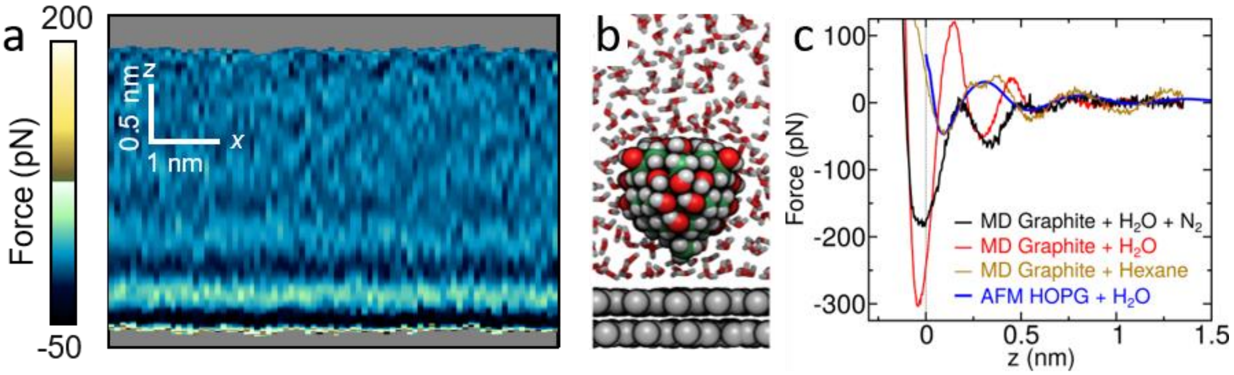


Figure 1. (a) 3D-AFM image of the graphite-water interface. (b) MD simulation snapshot of a tip-water-graphite interface. (c) Comparison between force curves obtained by AFM and MD. Adapted from [3]

Force spectroscopy demonstrates that *Staphylococcus aureus* vWF-binding protein triggers an ultra-strong interaction between the ClfA adhesin and host vWF

Albertus Viljoen[1*], Pietro Speziale[2*] and Yves F. Dufrêne[1*]

[1.] Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, Belgium
[2.] Department of Molecular Medicine, Unit of Biochemistry, University of Pavia, Pavia, Italy
* Corresponding authors: albertus.viljoen@uclouvain.be, pspeziale@unipv.it,
yves.dufrêne@uclouvain.be

The *Staphylococcus aureus* cell surface adhesin ClfA binds to the very large blood circulating protein von Willebrand Factor (vWF) via vWF-binding protein (vWbp), a secreted protein that does not bind the cell wall covalently. We have performed AFM force spectroscopy studies on living bacteria to unravel the molecular mechanism of this interaction (Figure 1).[1] We discovered that the presence of all three binding partners leads to very high binding forces (2,000 pN), largely outperforming other known ternary complexes involving adhesins. Strikingly, our experiments indicated that a direct interaction involving features of the dock, lock and latch mechanism must occur between ClfA and vWF to sustain the extreme tensile strength of the ternary complex. Our results support a previously undescribed mechanism whereby vWbp activates a direct, ultra-strong interaction between ClfA and vWF. This intriguing interaction represents a potential target for therapeutic interventions, including synthetic peptides inhibiting the interactions between ClfA and its ligands.

Reference

[1] A., Viljoen et al., *Staphylococcus aureus* vWF-binding protein triggers a strong interaction between Clumping factor A and host vWF. Communications Biology (2021), **In press**

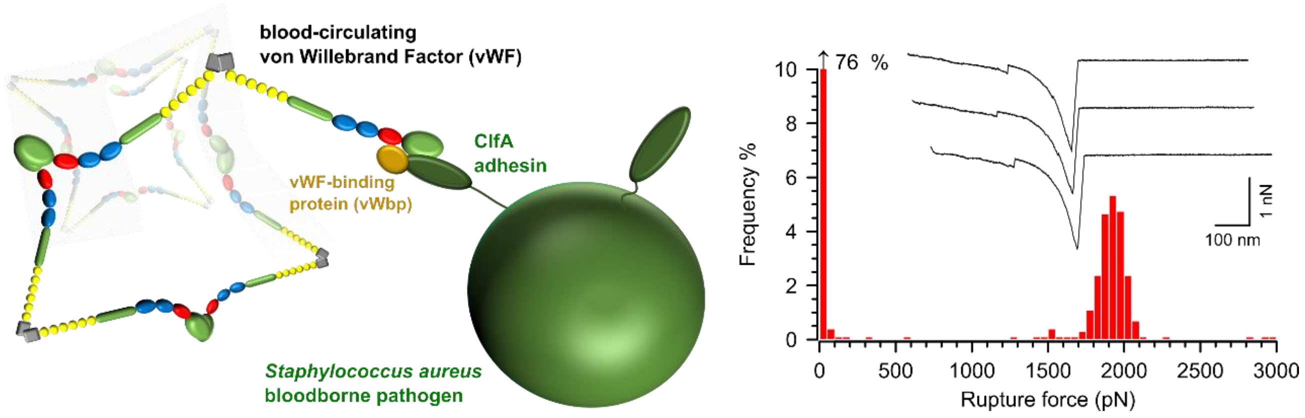


Figure 1. The secreted staphylococcal protein vWbp triggers an ultra-strong interaction between the cell surface adhesin ClfA and human blood circulating vWF.

Alternating Current Scanning Electrochemical Microscopy for Dielectric and Multifrequency Mapping of Mice Colon Sections

Varun Vyas[1]*, Niranjan Kotla[1], Yury Rochev[1] , Abhay Pandit[1], Manus J. Biggs[1]

[1] CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland Galway, Ireland

* Corresponding author: varun.vyas@nuigalway.ie

Alternating current scanning probe microscopy (AC-SECM) has become an instrument of choice for characterizing electrochemical heterogeneities over biological surfaces[1]. This study translates the application of classic electrochemical impedance spectroscopy (EIS)[2] for dielectric loss (tan δ) measurement in the bulk of tissue into tan δ mapping with high spatial resolution. Two dimensional (2D) tan δ mapping was performed over mice colon cross sections (CCS) at a frequency of 10 kHz (Figure 1). This dielectric loss map is an immediate measure of physiological conditions in a living system. At the right frequency, any minute change in protein or lipid chemistry can be measured as dielectric loss. This investigation gave a visual perspective into degree of dielectric loss over biological surfaces. And multifrequency 2D impedance maps give us an alternate route to select an optimal frequency for imaging in a cell culture media. These multifrequency scans were performed at 100 Hz, 10 kHz, 300 kHz, and 900 kHz. Impedance histograms showed that the best contrast that can segregate electrochemical signature for DMEM media as an electrolyte was at 300 kHz. This experimental approach takes into account the complex nature of cell culture media as the electrolyte of AC-SECM imaging. One is able to observe distinct electrochemical signals within the tissue and surrounding glass (amorphous SiO_2) surface. Further conversion of multifrequency scans into tan δ maps, one can observe minute changes in dielectric loss over tissue surface at different frequencies. Significant differences were observed at frequency of 10 kHz and 300 kHz. This approach can be used to measure changes in the lipid composition in disease like colorectal cancer[3]. Any alterations in the composition and distribution of the fatty acids can be used as a potential biomarker to study progression of cancer. With further investigations, one might be able to isolate dielectric signatures of various types fatty acids that are expressed in different pathological conditions.

References:

1. B. Ballesteros Katemann, A. Schulte, *et al.*, *Electrochem. commun.* **4**, 134–138 (2002).
2. D. A. Dean, T. Ramanathan, *et al.*, *J. Electrostat.* **66**, 165–177 (2008).
3. A. Pakiet, J. Kobiela, *et al.*, *Lipids Health Dis.* **18**, 29 (2019).

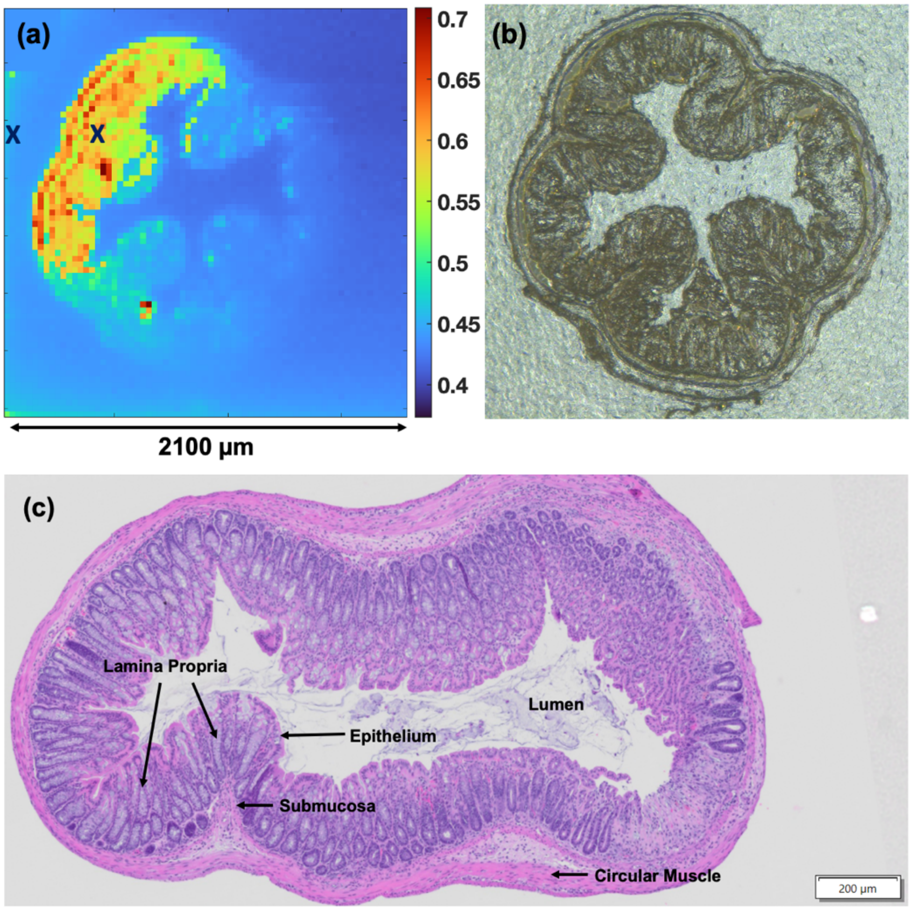


Figure 1. **(a) AC-SECM Tan δ map of mice colon section. (b) Bright field image of the same colon section before deparaffinization and imaging. (c) H&E stained section derived from the same mice colon.**

Applying Atom-Defined Building Tools to Make Quantum Sensing Devices

Robert Wolkow[1*]

[1] Department of Physics, University of Alberta, Edmonton, Alberta, Canada
* Corresponding author: rwolkow@ualberta.ca

After 3 decades of preparation, tools and procedures for reproducible fabrication of atom-perfect silicon structures have matured to a point where it has now become possible to build proto-devices while also planning viable atom-scale manufacturing. In the beginning, device complexity and production rates will be low while manufacturing costs are high, challenges that must be offset by the high value of select initial products. Inherent attributes including ultra-high speed, ultra-small size/weight/power, variance-free manufacture and routine access to some quantum effects are waiting to be harnessed.

A glimpse of our current capabilities will be shown by examples including structures we can make, unique electronic properties of those, chemical and electromagnetic sensing capabilities and fabrication automation through machine learning.

Near term device objectives such as a quantum metrological current standard, an unusually high temperature capable quantum metrology-based standard thermometer, and a uniquely portable, due to low power consumption, quantum random number generator will be mentioned.

Collaborative work with Professor Konrad Walus, EE, UBC, that shows the unprecedented low power consumption of binary and analog atom-defined silicon circuitry will be briefly sketched.

Pulsed Force Kelvin Probe Force Microscopy for < 10 nm Contact Potential Mapping in Ambient Conditions

Devon Jakob[1], and Xiaoji G. Xu[1*]

[1] Department of Chemistry, Lehigh University, 6 E. Packer Ave., Bethlehem, PA, 18015, United States
[*] Corresponding Author: xgx214@lehigh.edu

Measurement of the contact potential difference (CPD) and work functions of materials is crucial in the development and study of electronically active materials and devices. Kelvin probe force microscopy (KPFM), an imaging technique based on atomic force microscopy, is a robust and popular tool for CPD and work function mapping at the nanoscale. However, the conventional KPFM variants are typically limited in their spatial resolution to 30 – 100 nm under ambient conditions. The continuingly decreasing size and increasing complexity of photoactive materials, semiconductor devices, and related materials present challenges in uncovering their important electrical properties through KPFM. In this talk, a new paradigm of contact potential measurement in KPFM is presented. The new imaging technique, pulsed force Kelvin probe force microscopy (PF-KPFM), is developed and can reliably obtain CPD and work function images with ~ 10 nm spatial resolution on a wide range of samples.

The operating principle of PF-KPFM deviates from the fundamental paradigm of all current KPFM techniques, as there is no requirement of external oscillating voltage to produce the KPFM signal. As a result, PF-KPFM can avoid many of the intrinsic limitations associated with other KPFMs which limit their achievable spatial resolution, most notably the requirement for lift mode and the stray capacitance effect. Here, we explain the operating principle of PF-KPFM, how it compares to conventional frequency-modulated KPFM (see **Figure 1a-d**), and present results on several materials to exemplify the robustness of our technique. In particular, we show that PF-KPFM is suitable for probing the interfaces between metals and semiconductors, for probing individual ferroelectric domains and boundaries on ferroelectric materials (see **Figure 1e-f**), and for imaging nanoscale perovskite domains. From these measurements, new insights into the nanoscale electrical properties are established.

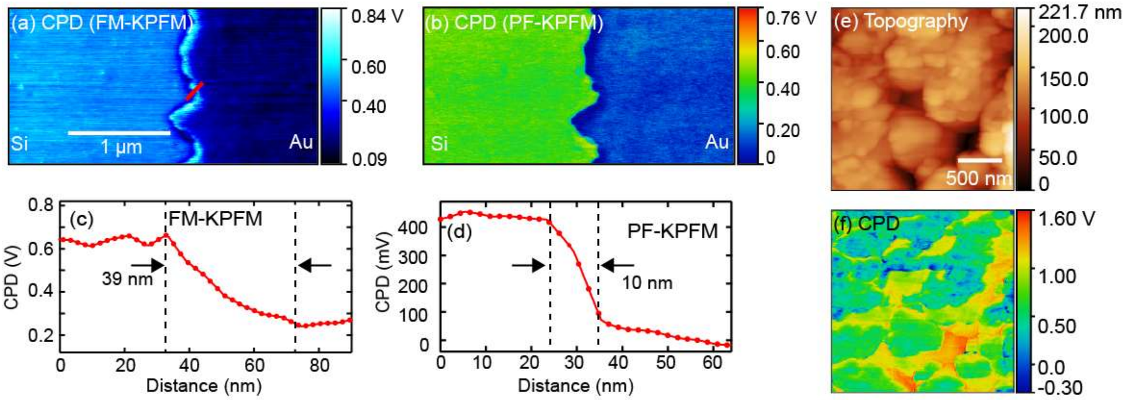


Figure 1. Comparing the spatial resolution of FM-KPFM and PF-KPFM on a standard KPFM sample composed of silicon (on the left) and gold (on the right) **(a)** FM-KPFM on the sample. **(b)** PF-KPFM over the same area. **(c)** The spatial resolution for FM-KPFM is estimated at 39 nm, found from the signal cross-section marked by the red line in (a). **(d)** The spatial resolution of PF-KPFM over the same area is estimated to be 10 nm. **(e)** The topography of a $BaTiO_3$ ferroelectric sample. **(f)** PF-KPFM measurement on the region, showing the heterogeneity of the contact potential over nanoscale domains.

Towards the atomic scale readout of single acceptor states in p-doped Si

Taras Chutora[1], Christopher Leon[1], Roshan Achal[1], Max Yuan[1]*, Furkan Altincicek[1], Lucian Livadaru[1], Jason Pitters[1], Robert Wolkow[1]

1 Department of Physics, University of Alberta, Edmonton, Alberta, Canada
* Corresponding Author: myuan1@ualberta.ca

Single acceptor dopants in Si along with dangling bonds are enabling technologies for atomic scale charge and spin-based devices.[1] Additionally, recent advances in hydrogen lithography have enabled the patterning of quantum dot based circuit elements with atomic precision.[2] We engineered a single acceptor coupled to a dangling bond wire on highly doped p-type H-Si(100) and characterized its electronic properties with scanning tunneling spectroscopy. The coupled entity has an electronic structure that behaves as a conductive wire from which the charge state of the dopant can be accessed and has a complex dependence on the dangling bond wire length. In addition, dI/dV mapping reveals features reminiscent of charging rings that are centered over the dopant and overlap with the wire.[3] This overlap varies with electric field and its tunability may augment the functionality of dangling bond based quantum devices.

References:

[1] A. Laucht et al., "Roadmap on quantum nanotechnologies", *Nanotechnology*, vol. 32, no. 16, p. 162003, 2021. Available: 10.1088/1361-6528/abb333
[2] T. Huff et al., "Binary atomic silicon logic", *Nature Electronics*, vol. 1, no. 12, pp. 636-643, 2018. Available: 10.1038/s41928-018-0180-3
[3] N. Turek, S. Godey, D. Deresmes and T. Mélin, "Ring charging of a single silicon dangling bond imaged by noncontact atomic force microscopy", *Physical Review B*, vol. 102, no. 23, 2020. Available: 10.1103/physrevb.102.235433

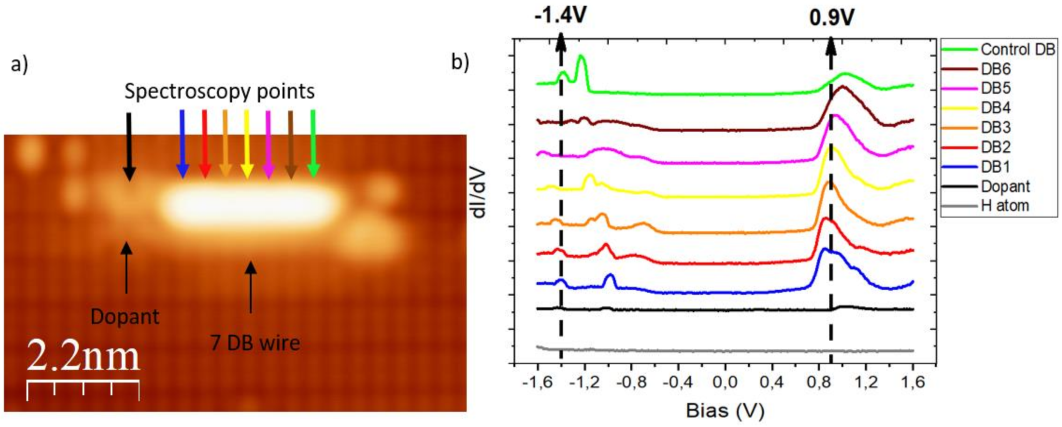


Figure 1. a) (1.8 V 50 pA) STM image of a 7 dangling bond wire fabricated next to a subsurface Boron dopant on highly doped P type H-Si (100) with individual spectroscopy points denoted by arrows. **b)** dI/dV spectroscopy taken over the dopant and wire shown in a), exhibiting previously unobserved peaks at -1.4 V and 0.9 V.

MFM Study of Magnetic Skyrmions in NiMnIn and NiMnGa Alloys

Lanping Yue*, Wenyong Zhang, Xingzhong Li, and David J. Sellmyer

Nebraska Center for Materials and Nanoscience, University of Nebraska, Lincoln, NE 68588, USA

* Corresponding author: lyue2@unl.edu

Magnetic skyrmions with nanoscale topological spin textures have attracted much interest due to their potential applications in information technologies, such as spintronics and microwave oscillators [1, 2]. A nonequilibrium rapid-quenching method has been used to produce polycrystalline NiMnGa and NiMnIn samples. Our recent researches [2,3] found that both alloys are chemically and morphologically similar but crystallographically and physically very different. NiMnGa crystallizes in a uniaxial Ni_2In-type hexagonal structure, whereas NiMnIn can be interpreted as the composite patterns of the full-Heusler compound with intermetallic phases. The topological Hall effect is smaller in NiMnIn than in NiMnGa but occurs in much lower magnetic fields. In this work, the magnetic skyrmions in NiMnIn and NiMnGa alloys were imaged by Bruker Dimension Icon magnetic force microscopy (MFM) at room temperature (RT). All MFM images were acquired in LiftMode with a lift height of 20 nm under the external out-of-plane magnetic fields from 0 up to 2.2 kOe, respectively. The optimized high-spatial-resolution MFM tip with high coercivity and low magnetic moment was used for MFM imaging. The MFM images (Fig. 1) reveal the presence of bubble-type skyrmion spin structure changing with the increase of the external magnetic fields in both NiMnIn and NiMnGa alloys. Since NiMnIn needs smaller stabilization fields (0.02–0.5 kOe) at RT in comparison with NiMnGa, it may be of interest for spintronic applications. Topological Hall-effect contribution corresponding to skyrmion spin structures were observed in the grains of the major phase, which were supported by MFM studies.

This work is supported by NSF-DMREF: SusChEM (Award Number: 1729288). The research was performed in the Nebraska Nanoscale Facility: National Nanotechnology Coordinated Infrastructure and the Nebraska Center for Materials and Nanoscience (and/or NERCF), which are supported by the National Science Foundation under Award ECCS: 2025298, and the Nebraska Research Initiative.

References:

[1] B. Balasubramanian *et al*., *Phys. Rev. Lett.* **124** (2020) 057201.

[2] W-Y Zhang *et al*., *Appl. Phys. Lett.* **115** (2019) 172404.

[3] X-Z Li *et al*., *Microsc. Microanal*. **26** (2020) 276.

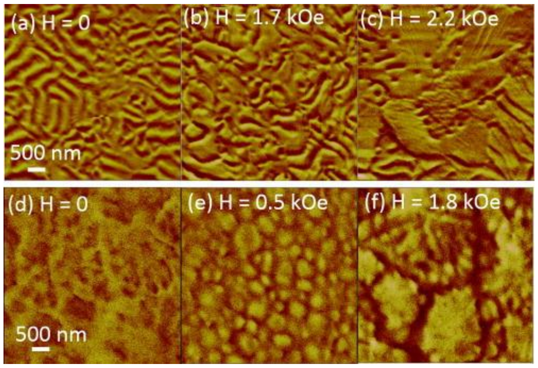


Figure 1. MFM images measured at various fields for NiMnGa (a)–(c) and NiMnIn (d)–(f).

Nanoscale Ferroelectric Characterization with Advanced Multi-Frequency Scanning Probe Microscopies

Qibin Zeng and Kaiyang Zeng
Department of Mechanical Engineering
National University of Singapore
9 Engineering Drive 1, Singapore 117576
mpezk@nus.edu.sg

Real-space investigation of nanoscale ferroelectricity is of great importance for understanding the fundamental physics of ferroelectric phenomena. During the past decades, the nanoscale ferroelectric studies have been widely relied on an important scanning probe microscopy (SPM) technique invented in 1992, i.e., the Piezoresponse Force Microscopy (PFM). However, the intensive study of the conventional PFM has revealed a growing number of concerns and limitations which are largely challenging its validity and application, and at the same time, making the further studies of ferroelectricity encounter significant bottlenecks. In our recent studies, two advanced multi-frequency SPM techniques have been introduced to significantly improve the nanoscale ferroelectric measurement,, i.e., the **Heterodyne Megasonic Piezoresponse Force Microscopy (HM-PFM)**[1, 2] and **Non-Contact Heterodyne Electrostrain Force Microscopy (NC-HEsFM).**[3] HM-PFM is based on the conventional instrument architecture of PFM, but uniquely uses 10^6 to 10^8 Hz high-frequency excitation and heterodyne method to measure the piezoelectric strain at nanoscale. It is found that HM-PFM can unambiguously provide standard ferroelectric domain and hysteresis loop measurements, and an effective domain characterization with excitation frequency up to ~110 MHz has been demonstrated. Most importantly, owing to the high-frequency and heterodyne scheme, the contributions from both electrostatic force and electrochemical strain can be significantly minimized in the HM-PFM measurements. Furthermore, a special technique, named **difference-frequency piezoresponse frequency spectrum (DFPFS)** measurement, is developed on HM-PFM and a distinct DFPFS characteristic is observed on the materials with piezoelectricity. [1, 2]

In contrast to conventional PFM and HM-PFM, NC-HEsFM unprecedently utilizes a newly-designed force sensor and heterodyne detection scheme to measure the nanoscale piezoelectric strain, and for the first time, achieves the long-awaited goal of non-contact, electrostatic force minimized ferroelectric characterization. It has been unambiguously demonstrated that NC-HEsFM can perform ideal and high-resolution ferroelectric domain mapping, standard ferroelectric hysteresis loop measurement and controllable domain manipulation, and at the same time, operate on multiple high eigenmodes. With using the newly-designed force sensor and heterodyne detection method, NC-HEsFM shows an unprecedented capability in achieving real non-contact yet non-destructive ferroelectric characterization with significantly minimized electrostatic force effect. Meanwhile, the application of the new force sensor makes NC-HEsFM highly compatible for high-vacuum and low-temperature environments, thus combining the advantages of non-contact operation and electrostatic force minimization, NC-HEsFM is expected to reach the ultimate goal of atomically resolved ferroelectric characterization.[3] In brief, we believed that both the newly-developed HM-PFM and NC-HEsFM can be extensively used in a variety of future ferroelectric or piezoelectric studies, especially those research topics where using conventional PFM is highly controversial or challengeable.

References

[1] Zeng, K.; Zeng, Q. System and Method for Scanning Probe Microscopy. *SG Appl. NO. 10202003740V*, **2020**.

[2] Zeng, Q.; Wang, H.; Xiong, Z.; Huang, Q.; Lu, W.; Sun, K.; Fan, Z.; Zeng, K., Nanoscale Ferroelectric Characterization with Heterodyne Megasonic Piezoresponse Force Microscopy. *Adv. Sci.* **2021,** 2003993, DOI: 10.1002/advs.202003993.

[3] Zeng, K.; Zeng, Q. Methods and Apparatus for Non-Contact Detection of Surface Strain on Atomic Force Microscopy Based Technique. *SG Appl. NO. 10202100483P*, **2021**.

Applications of atomic force microscopy imaging system: biophysical assessment and single molecular recognition on single cells

Anhong Zhou*, Wei Zhang, Yangzhe Wu, and Lifu Xiao

Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, Utah 84322, U.S.A..
* Corresponding author: Anhong.Zhou@usu.edu

Atomic force microscopy (AFM) has become a powerful tool that can provide nanoscale topography image and quantitatively measure biophysical properties of the cells in a native circumstance. In the work, we report our recent applications of AFM in monitoring the alternation of biophysical properties with drug-treated or toxic agent-treated cells and imaging the distribution of individual molecules on single cells. *In situ*, real time tracking of topography and nanomechanics of antimycobacterial drugs treated *Mycobactetium JLS* demonstrated that the progress of bacterial division was inhibited and the decreases of biomechanical properties including adhesion force, bacterial spring constant, and Young's modulus, were closely related to cell shape shrinkage [1]. AFM was also applied to analyze the relationship between biophysical properties and functions of endothelial cells exposed to diesel exhaust particles (DEPs). Our AFM results revealed that DEPs could alter membrane nanostructures and cytoskeleton components in a dosage- and a time-dependent manner, suggesting that DEP exposure triggers important biochemical and biophysical changes that would negatively impact the pathological development of cardiovascular diseases [2]. In single molecule imaging technique, or called topography and recognition (TREC) imaging, was applied to simultaneously obtain highly sensitive and specific molecular recognition images and high-resolution topographic images of epidermal growth factor receptor (EGFR) on single breast cancer cells. This TREC imaging technique can be used as a highly sensitive imaging tool to study different cell surface receptors at molecular level in physiological and pathological studies [3]. In conclusion, the alternation of biophysical properties and dynamic distribution of single molecules revealed by AFM can provide a novel perspective to understand the cellular activities supplemented with other regular techniques.

References:

[1] Wei Zhang, Fangjun Lin, Yan Lin, Han Zhang, Timothy A. Gilbertson, Anhong Zhou, PNAS, (2020), 117 (7) 3518-352.
[2] Yangzhe Wu, and Anhong Zhou, Chem.Comm. (2009), p. 7021-7023.
[3] Yangzhe Wu, Tian Yu, Timothy A. Gilbertson, Anhong Zhou, Hao Xu, and Kytai Truong Nguyen, Plos One. 7 (2012), e36885.
[4] Lifu Xiao, Qian Chen, Yangzhe Wu, Xiaojun Qi, and Anhong Zhou, Biochimica et Biophysica Acta. 1848 (2015) p.1988–1995.

Force spectra reveal location and dynamics of two ligand binding sites in dopamine transporter

Rong Zhu[1*], Walter Sandtner[2#], Thomas Stockner[2#], Alexander Heilinger[1], Marion Holy[2], Oliver Kudlacek[2], Linda Wildling[1], Kusumika Saha[2], Anna Sophie Fröhlich[1], Michael Bindl[1], Paraskevi Tziortzouda[1], Anna Haider[1], Julia Gobl[1], Saanfor Hubert Suh[1], Jawad Akbar Khan[2], Andreas Ebner[1], Hermann J. Gruber[1], Michael Freissmuth[2], Amy Hauck Newman[3], Harald H. Sitte[2] and Peter Hinterdorfer[1*]

[1.] Institute of Biophysics, Johannes Kepler University Linz, Austria.
[2.] Institute of Pharmacology and Gaston H. Glock Research Laboratories for Exploratory Drug Development, Center of Physiology and Pharmacology, Medical University of Vienna, Austria
[3.] Medicinal Chemistry Section, Molecular Targets and Medications Discovery Branch, National Institute on Drug Abuse-Intramural Research Program, Baltimore, USA
* Corresponding author: Rong.Zhu@jku.at; Peter.Hinterdorfer@jku.at
[#] These authors contributed equally.

X-ray structures of the serotonin transporter (SERT) revealed two ligand binding sites, whereas the crystal structures of its close homologue, the dopamine transporter (DAT) showed only one. We designed force sensors with various DAT ligands and measured their interaction forces with wild type and a series of mutated DAT, from which two distinct populations of unbinding strengths and off-rates were detected. The population of high-force was abolished by the mutation S422A, V152I, or by substitution of Na^+ with K^+ or $NMDG^+$. In contrast, mutation G386H, acetylation of lysine residues K92 and K384, or protonation of the histidine residue H477 reduced the low-force population. Our results reveal the existence of a second ligand binding site in DAT and provide information on its position and kinetics. The data of quantitative force measurements explain why the second ligand binding site might escape detection in crystals of DAT.

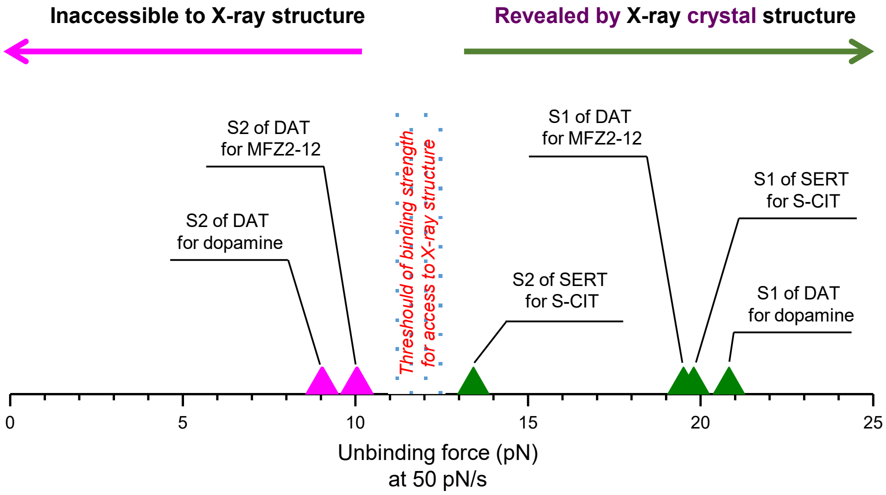


Figure 1. Binding strength might determine whether a binding site can be revealed by the X-ray crystal structure. We speculate that threshold for binding strength has to be overcome, which is presumably between 10.1 and 13.4 pN at force loading rate of 50 pN/s, to allow for detection of an occupied binding site by X-ray crystallography.

Validation of a New Theoretical Model for Contact Resonance Atomic Force Microscopy using Long, Massive Tips

Nadav Zimron-Politi[1] and Ryan C. Tung[1*]

[1.] University of Nevada, Reno, Department of Mechanical Engineering, Reno, NV USA
* Corresponding author: rtung@unr.edu

In this work, we present an experimental validation of a new theoretical model [1] for Contact Resonance Atomic Force Microscopy (CR AFM) [2] which incorporates the dynamic effects of long, massive tips. Long, massive tips have been used in the so-called “trolling” mode [3] AFM to measure properties of polymers and living cells. The use of a long sensing tip assures the micro-cantilever beam body is out of the liquid, therefore reducing hydrodynamic forces on the sensor body which results in a high quality factor, and improves the measurements.

To experimentally incorporate tip mass and rotational inertia, a glass bead is bonded to a conventional atomic force microscopy cantilever. The bead is bonded to the topside of the sensor cantilever, in order to maintain scanning capabilities and reduce adhesive forces. The freely vibrating flexural and torsional modes of the cantilever, along with the theoretical model in Ref. [1] are used to estimate the inertial properties of the massive tip. Using this information, sample elastic properties are then estimated from the in-contact resonance frequencies of the system for two different materials. The results are then compared with measurements performed using a similar cantilever on the same samples, without any cantilever modifications. We use several set-point force measurements, at different vibrational eigenmodes in order to optimize the experimental setup and use the most sensitive eigenmode for each material sample. We show that the new theoretical model successfully captures the effects of the rotational inertia and mass of the tip.

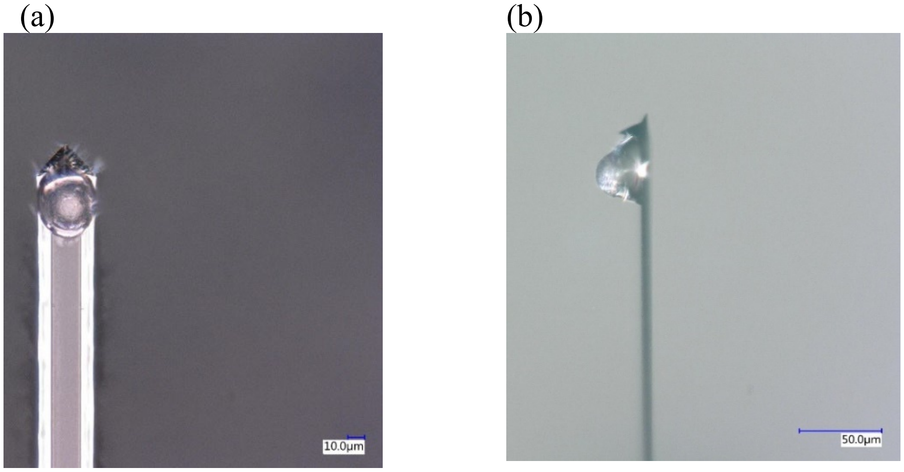


Figure 1. (a) Top view. (b) Side view. Glass bead bonded to cantilever beam. Pictures taken using a Keyence microscope, with bead initially glued to tip side

[1] T. Jaquez-Moreno et al., Sensors 19.22 (2019): 4990.
[2] U. Rabe et al., Ultrasonics 38.1-8 (2000): 430-437.
[3] M. Minary-Jolandan et al. Nanotechnology 23.23 (2012): 235704.

Force Volume Data acquired by Tuning Fork AFM at Cryogenic Temperatures by using Intermodulation Products

Marco Zutter[1*], Daniel Platz[2] and Thilo Glatzel[1]

[1.] Department of Physics, University of Basel, Klingelbergstr. 82, 4056 Basel, Switzerland
[2.] TU Wien, Institute of Sensor and Actuator Systems, Gußhausstraße 27-29, 1040 Vienna, Austria
* Corresponding author: marco.zutter@unibas.ch

In this work we present a new method of acquiring force volume data in a tuning fork based Atomic Force Microscope (AFM) using mixing products of different drive tones. The measurement of force volume data is generally very time demanding and can easily take more than 10h for a reasonable resolution [1]. We addressed this limitation by combining our low temperature (4.8 K) tuning fork AFM with two more advanced measurement methods [2,3].

This new method makes use of a Multifrequency Lock-In Amplifier (MLA, Intermodulation Products AB) detecting up to 32 intermodulation products which are generated due to a perturbation by a nonlinear tip-surface interaction. For the measurement the tuning fork is mechanically excited near resonance (f_{res}=23.7 kHz) with an amplitude of A_{res}=100 pm while the tip-sample distance is modulated (A_{mod}=500 pm) by a second drive applied to the dither-piezo at a low frequency (f_{mod}=5 Hz). With these two drives the individual frequencies of the mixing products in the response signal are defined, allowing a lock-in measurement to acquire amplitude and phase of the mixing products around f_{res}. To measure a full force volume data-set in a scan area of 3x3 nm^2 (64x64pixel) the tip is kept at constant height close to the surface inside the non-linear force regime and the tip is positioned 200 ms on each pixel resulting in a total measurement time of 30 min. With the data acquired in this way both the conservative and dissipative force quadratures at every pixel of the scan have been reconstructed and compared to traditional z-spectroscopies analysed with the Sader-Jarvis method.

References:
[1] S. Fremy, S. Kawai, R. Pawlak, Th. Glatzel, A. Baratoff, E. Meyer, Nanotechnology **23**, 055401 (2012)
[2] D. Platz, E.A. Tholén, D. Pesen, D.B. Haviland, Appl. Phys. Lett. **92**, 153106 (2008)
[3] S. Kawai, S. Hafizovic, Th. Glatzel, A. Baratoff, E. Meyer, Phys. Rev. B85, 165426 (2012)

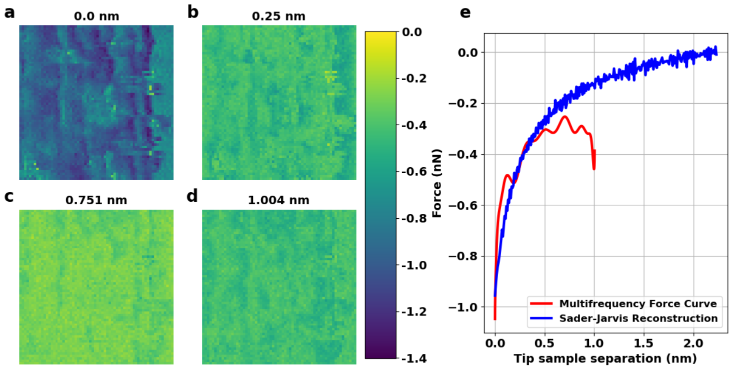


Figure 1. **a-d:** Cuts through the three dimensional force volume data at different distances from the closest approach. **e:** Comparison of a z-spectroscopy force curve using the Sader-Jarvis reconstruction (blue) and a intermodulation products force curve (red) from the force volume data.

Modeling AFM in the SMFS mode by Steered Molecular Dynamics simulations: Effects on Molecular Recognition of Protein Adsorption on Silane Monolayers

Solène Lecot[1], Yann Chevolot[1], Christelle Yeromonahos[1] and Magali Phaner-Goutorbe[1*]

[1.] Univ Lyon, ECL, INSA Lyon, CNRS, UCBL, CPE Lyon, INL, UMR5270, 69130 Ecully, France.
* Corresponding author: magali.phaner@ec-lyon.fr

Protein adsorption on surfaces is used in biosensing tools as an immobilization mean to trap the analyte to be detected. However, adsorption can lead to conformational changes in the protein structure, resulting in a loss of bioactivity. Among surfaces, self-assembled monolayers of silane molecules are widely used to functionalize SiO_2, as the surface charge and hydropathy can be tuned by using silane molecules with different the head-group charges and alkyl chain lengths. The objective of this study is to decipher the impact of streptavidin adsorption on silane monolayers on its further interactions with biotin.

Molecular Dynamics (MD) simulations are well-suited to investigate protein-surface interactions and adsorption-induced conformational changes at atomic scale. Furthermore, Steered Molecular Dynamics (SMD) simulations, that mimic Atomic Force Microscopy (AFM) experiments, provide additional information regarding forces and dynamics of individual ligand-receptor interactions.

Firstly, a MD simulation system (GROMACS – OPLS-AA) was developed and several silane monolayers were modeled. Their structural properties were qualitatively validated by FTIR and XPS experimental results. Then, streptavidin adsorption on silane monolayers and its subsequent interactions with biotin were investigated by coupling MD and SMD simulations. It was shown that adsorption-induced conformational changes in streptavidin, which depend on the type of silane molecules, induce a decrease of the streptavidin-biotin rupture force [1]. Individual streptavidin-biotin interactions were further investigated by SMD simulations at various pulling velocities, from 0.002×10^6 to 20×10^6 µm/s, to approach the pulling velocities obtained with AFM experiments. By comparison with previous experimental results and SMD rupture forces obtained without adsorption on silane monolayers [2], it was demonstrated that silane molecules with uncharged head-group and short alkyl chain allow streptavidin immobilization while keeping biotin interactions better than silane molecules with positively charged head-groups. Impact of the nature of silane molecules on the unbinding process and binding energy were also deciphered.

The same methodology was applied to another complex, including the cell receptor ACE2 and the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Contrary to the streptavidin-biotin complex, it was demonstrated that ACE2 adsorption on mixed silane monolayers induces an increase in the rupture force of spike protein RBD, indicating a reinforcement of protein-protein interactions.

References:
[1] S. Lecot, Y. Chevolot, M. Phaner-Goutorbe and C. Yeromonahos, J. Phys. Chem. B, **124** (2020), p.6786-6796.
[2] F. Rico, A. Russek, L. Gonzalez, H. Grubmüller and S. Scheuring, PNAS, **116** (2019), p.6594-6601.

Fabricating Protein 2D periodic architectures at Solid-Liquid Interfaces and *In-situ* Visualizing the Emergence of Order with High-Speed AFM

Shuai Zhang, [1,2]* Harley Pyles,[3,4] Ben Legg[2], Robbie Sadre,[5] Maxim Ziatdinov[6], Christopher J. Mundy[2], Sergei V. Kalinin,[6] Oliver Rübel,[5] David Baker,[3,4,7,] Jim, De Yoreo[1,2]

[1] Materials Science and Engineering, University of Washington, Seattle, WA, USA
[2] Physical Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA
[3] Department of Biochemistry, University of Washington, Seattle, WA, USA
[4] Institute for Protein Design, University of Washington, Seattle, WA, USA
[5] Computational Research Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
[6] The Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831
[7] Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA

* Corresponding author: zhangs71@uw.edu

In the last few decades, inspired by nature, various approaches have been developed for building novel supramolecular nanomaterials. However, progress in designing bio-hybrid materials on inorganic surfaces in a pre-designed manner lags behind the achievements of protein engineering and self-assembly in bulk solution. Additionally, it remains unclear how chemical/sequence information and intermolecular interactions, including electrostatic interactions, dipole-dipole interactions, and desolvation effects, define the energy landscapes across which hierarchy develops.

Recently, we programmed *de novo* protein interfaces matching to the (001) face of muscovite mica. Our results show that the protein nanorods formed 2D anisotropic liquid crystal (LC) phases with different concentrations of electrolyte.[1] We then used high-speed AFM (~1 frame/s) to record the evolution of the protein 2D periodic architectures *in-situ* from isolated monomers to the anisotropic 2D LC with smectic order. The analysis showed that protein mobility increases dramatically with KCl concentration, and there is a thermodynamically dominant orientation. Besides, at 10 mM KCl, the rotational dynamics of the proteins between directions at local energy minima have two distinct mechanisms. Transitions between adjacent orientational states appear to follow in-plane Brownian motion, biased by the angle-dependent energy landscape, to produce rates that are exponential in the energy-barriers between states. In contrast, transitions between more distant angular states occur by high-energy Levy-flight transitions, with rates that are decoupled from energy-barrier heights.

Moreover, at high KCl concentrations, order develops from an initial 2D liquid phase through the formation of fluctuating clusters whose average size increases exponentially with time. The increased mobility promotes the formation of the ordered LC phases. The known effects of high salt concentration, both screening the charges of proteins and structuring the interfacial hydration layers, likely play decisive roles in achieving order. Numerous evidences imply that entropic interactions drive the assembly process. However, designed interactions introduce enthalpic terms that modify the phase diagram. The findings provide insight into the remarkable diversity of self-assembled architectures adopted by these protein nanorods that extend well beyond what was expected from the initial design.

[1] Pyles, H., Zhang, S., De Yoreo, J. J. & Baker, D. Nature 571 (2019), 251-256.

Isomorphic contact resonance: demonstration of a new contact resonance force microscopy technique for more direct material properties measurements

Lawrence H. Robins[1*], Matt D. Brubaker[2], Ryan C. Tung[3] and Jason P. Killgore[4]

[1.] Applied Chemicals and Materials Division, NIST, Boulder, Colorado, USA
[2.] Applied Physics Division, NIST, Boulder, Colorado, USA
[3.] Department of Mechanical Engineering, University of Nevada – Reno, Reno, Nevada, USA
* Applied Chemicals and Materials Division, NIST, Boulder, Colorado, USA

We introduce isomorphic contact resonance force microscopy (iso-CRFM), a new CR imaging technique that acquires data at constant frequency and contact stiffness throughout the scan area. Constant frequency is obtained by acquiring a force versus distance curve at each pixel, such that a preselected target frequency is reached somewhere in the force curve. As a consequence, the cantilever maintains an invariant vibrational shape and a constant environmental damping, thus simplifying interpretation of amplitude and quality factor image contrast. Advantages of iso-CR are demonstrated by presenting iso-CRFM (mechanically driven) and iso-CR piezoresponse force microscopy (iso-CR-PFM, electrically driven) images of a piezoelectric AlN thin film containing nanoscale Al-polar and N-polar domains. The domain structure is revealed by iso-CR-PFM phase imaging, which shows nearly 180° contrast between domains of opposite polarity. The PFM amplitude and Q-factor images also show domain contrast, which decreases with increasing CR frequency. Further, the difference between the iso-CR-PFM and iso-CRFM results decreases with increasing frequency. These frequency-dependent effects, summarized in Figure 1, are ascribed to frequency-dependent electrostatic artifacts in the measured PFM signals. Additional experiments were performed to examine the effect of varying the DC tip voltage (and thus varying the strength of the electrostatic force) on the iso-CR-PFM amplitude, Q factor, and other measurable parameters. We conclude that the iso-CR capability to control the CR frequency across multiple excitation schemes helps elucidate the origin of the amplitude and Q-factor image contrast.

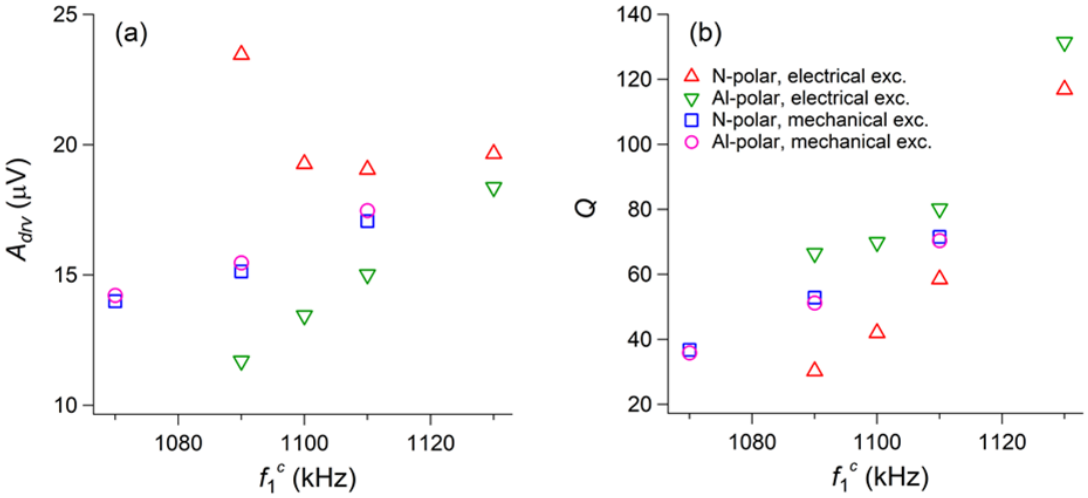


Figure 1. (a) Dependence of median amplitude (A_{drv}) on CR frequency (f_1^c) for electrical excitation in N-polar domains (triangles), electrical excitation in Al-polar domains (inverted triangles), mechanical excitation in N-polar domains (squares), and mechanical excitation in Al-polar domains (circles) of an AlN thin film. (b) Dependence of median Q-factor (Q) on f_1^c, with same labeling of data points as in (a).

Automated Structure Discovery in Atomic Force Microscopy

Adam S. Foster[1,2*]

[1.]Department of Applied Physics, Aalto University, Helsinki Finland
[2.]WPI Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Kanazawa, Japan.
* Corresponding author: adam.foster@aalto.fi

Atomic force microscopy (AFM) with molecule-functionalized tips has emerged as the primary experimental technique for probing the atomic structure of organic molecules on surfaces [1]. Most experiments have been limited to nearly planar aromatic molecules, due to difficulties with interpretation of highly distorted AFM images originating from non-planar molecules [2]. Here we develop a deep learning infrastructure that matches a set of AFM images with a unique descriptor characterizing the molecular configuration, allowing us to predict the molecular structure directly in a few seconds on a laptop [3]. We apply this methodology to resolve several distinct adsorption configurations and conformations of molecules based on low-temperature AFM measurements. In general, we find high success rates in predicting the atomic and chemical structure of molecules. This approach opens the door to apply high-resolution AFM to a large variety of systems for which routine atomic and chemical structural resolution on the level of individual objects/molecules would be a major breakthrough. We also look at applications of similar approaches to the imaging of biomaterials and AFM imaging in liquids.

[1] N. Pavliček and L. Gross, Nat. Rev. Chem. **1**, 0005 (2017).
[2] P. Jelinek, J. Phys.:Condens. Mat. **29**, 343002 (2017).
[3] B. Alldritt, P. Hapala, N. Oinonena, F. Urtev, O. Krejci, F. F. Canova, J. Kannala, F. Schulz, P. Liljeroth, and A. S. Foster, Sci. Adv. **6** (2020)

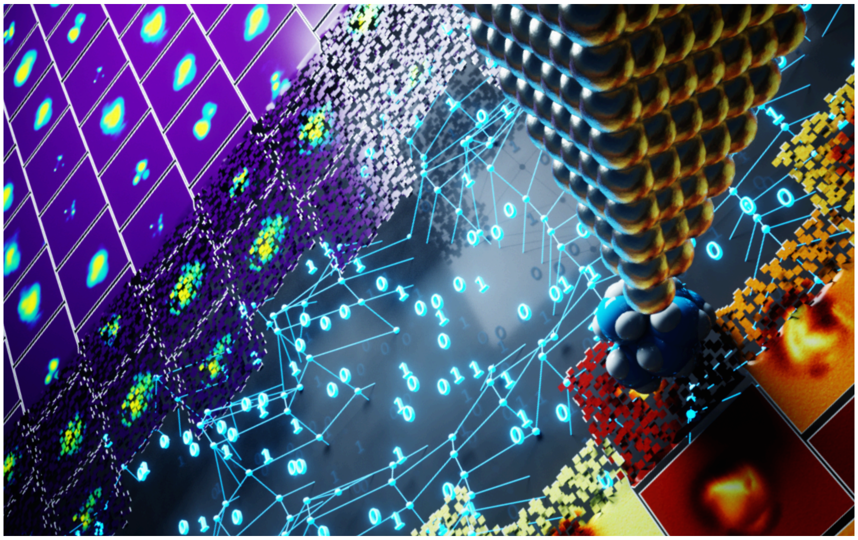


Figure 1. Schematic showing the conversion of AFM images via a convolutional neural network into *descriptors* used to identify molecular structure.

Few-cycle Regime Atomic Force Microscopy

Enrique A. López-Guerra[1], Suhas Somnath[2], Santiago D. Solares[1], Stephen Jesse[3], Gabriele Ferrini[4*]

[1.] Department of Mechanical and Aerospace Engineering, The George Washington University, Washington, DC, 20052, USA.
[2.] National Center for Computational Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 37831, USA.
[3.] Center for Nanophase Materials Sciences and Institute for Functional Imaging of Materials, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 37831, USA.
[4.] Interdisciplinary Laboratories for Advanced Materials Physics and Dipartimento di Matematica e Fisica, Università Cattolica del Sacro Cuore, I-25121, Brescia, Italy.
* Corresponding author: gabriele.ferrini@unicatt.it

Traditionally, dynamic atomic force microscopy (AFM) techniques are based on the analysis of the quasi-steady state response of the cantilever deflection in terms of Fourier analysis. Here we describe a technique that instead exploits the often disregarded transient response of the cantilever through a relatively modern mathematical tool, which has caused important developments in several scientific fields but that is still quite unknown in the AFM context: the wavelet analysis [1].

Wavelet analysis allows us to localize the time-varying spectral composition of the initial oscillations of the cantilever deflection when an impulsive excitation is given (as in the band excitation method), a mode that we call the *few- cycle regime*. We show that this regime encodes very meaningful information about the tip-sample interaction in a unique and extremely sensitive manner.

We exploit this high sensitivity to gain detailed insight into multiple physical parameters that perturb the dynamics of the AFM probe, such as the tip radius, Hamaker constant, sample's elastic modulus and height of an adsorbed water layer. We validate these findings with experimental evidence and computational simulations and show a feasible path towards the simultaneous retrieval of multiple physical parameters. These techniques applies also to samples that demand imaging in liquid native environments and also to highly vulnerable samples whose compositional mapping cannot be obtained through standard tapping imaging techniques [2].

[1] López-Guerra, E. A., Somnath, S., Solares, S. D., Jesse, S., & Ferrini, G. (2019). Few-cycle Regime Atomic force Microscopy. *Scientific reports, 9*(1), 1-10.
[2] López-Guerra, E. A., Banfi, F., Solares, S. D., & Ferrini, G. (2018). Theory of Single-Impact Atomic Force Spectroscopy in liquids with material contrast. *Scientific reports*, *8*(1), 1-16.

Study of molecular coatings on nanoparticles with Ringing mode

N. Makarova[1], B. Peng[2], S. Peerzade[2], M.E. Dokukin[1,3], I. Sokolov[1,2 *]

[1.] Department of Mechanical Engineering, Tufts University, Medford, MA 02155, USA
[2] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA
[3] Sarov Physical and Technical Institute, National Research Nuclear University, Sarov, 607186 Russia
* Corresponding author: Igor.Sokolov@Tufts.edu

The molecular coating of nanoparticles is important for particle functionalization. Direct imaging of the molecular coating on the nanoparticle's surface with a sufficiently high lateral resolution is challenging. Recently introduced Ringing mode of AFM allows for simultaneous imaging of several maps of physical properties of a sample surface [1, 2], including imaging the height of the molecular coat. To the best of our knowledge, there is no other technique capable of measuring the presence of a thin layer of organic molecules on organic nanoparticles with a high-resolution. Here we demonstrate that Ringing mode allows imaging of PEG on mesoporous silica with the lateral resolution down to ~1 nm. Furthermore, PEG coating on cellulose acetate particles can also be imaged in Ringing mode (though it is hard to estimate the resolution because of a rather smooth coating of cellulose acetate particles).

It is interesting to note that there is no one-to-one correspondence between the adhesion and the size of the molecular coating on the particle surface [3]. Figure 1 shows an example of simultaneous imaging of adhesion, neck height, and the size of the molecular coat (disconnection distance). While some correlation between the adhesion and neck height can be seen, heterogeneity of distribution of the neck height is much richer than the adhesion map. The distribution of the height of the molecular coat is substantially different from the neck height. These images indicate a high complexity of the formation of the molecular coat on nanoparticles. The mechanism of formation of the coating layer is not well understood. Therefore, Ringing mode may be instrumental in the understanding of the formation of such a layer.

References:
[1] M.E Dokukin, and I. Sokolov, Scientific Reports, **7** (2017), p. 11828.
[2] I. Sokolov, and M.E. Dokukin, Nanoscale Imaging: Methods and Protocols, **1814 (**2018) p. 469.
[3] Makarova, N., Peng, B., Peerzade, S., Dokukin, M., & Sokolov, I. (2020). Microscopy and Microanalysis, 26(S2), 3136-3138.

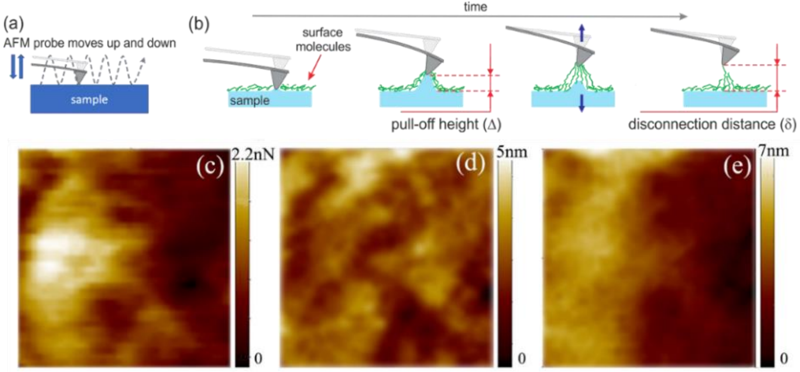


Figure 1. a) Schematics of the motion of the AFM probe during the imaging; the probe oscillates in a sub-resonance mode. (b) graphical definition of the meaning of two channels imaged in Ringing mode: the pull-off neck height and disconnection distance (height of the molecular coat). 70x70 nm^2 area of a cellulose acetate nanoparticle imaged in Ringing mode. Three channels recorded simultaneously are shown: (c) adhesion force, (d) neck height, (e) disconnection distance.

Visualizing Hydration Layers in Alkane-Mica Interfaces by Three-Dimensional Atomic Force Microscopy

Simone Benaglia[1*], Manuel R. Uhlig[1], Ricardo Garcia[1]
[1.] Instituto de Ciencia de Materiales de Madrid, CSIC, c/ Sor Juana Ines de la Cruz 3, 28049 Madrid, Spain
*Corresponding author: simone.benaglia@csic.es

It is well known that strong attractive interactions between water molecules and hydrophilic surfaces give rise to hydration layers. In a similar way, nonpolar liquids such as hydrocarbons readily form solvation layers at graphitic and other organic surfaces. Solid-liquid interfaces (SLIs) formed by liquids and solids with weak interactions between each other are sensitive to trace amounts of solutes and changes in the environmental conditions [1,2]. This is relevant in the context of organic liquids in contact with hydrophilic materials; at their surface, trace amounts of water can diffuse and condensate, leading to the formation of a nanometer-thick film [3]. Up to now, we lack a nanoscale understanding of this film.

3D-AFM is a novel advanced AFM technique which allows the visualization of SLIs with sub-nm resolution [4-5]. We performed 3D-AFM experiments to map the SLI of nonpolar organic media (n-alkanes) with hydrophilic and hydrophobic surfaces. We show how the presence of trace amounts of water dominates the interface with hydrophilic materials and forms up to three individual hydration layers. Through direct visualization of the wetting film, we reveal up to three individual hydration layers. Upon molecular sieving the majority of water is removed, and the solvent molecules can flatly adsorb on the mica surface, forming solvation layers. In contrast to that, hydrophobic surfaces and hydrocarbons are characterized by a high affinity which makes their SLI insensitive to trace amount of solutes despite their presence in the bulk liquid. This allows the organization of solvent molecules through solvation layers. The described mechanism provides a better understanding of the SLI at hydrophilic and hydrophobic materials and, in particular, how liquids organize at their surfaces.

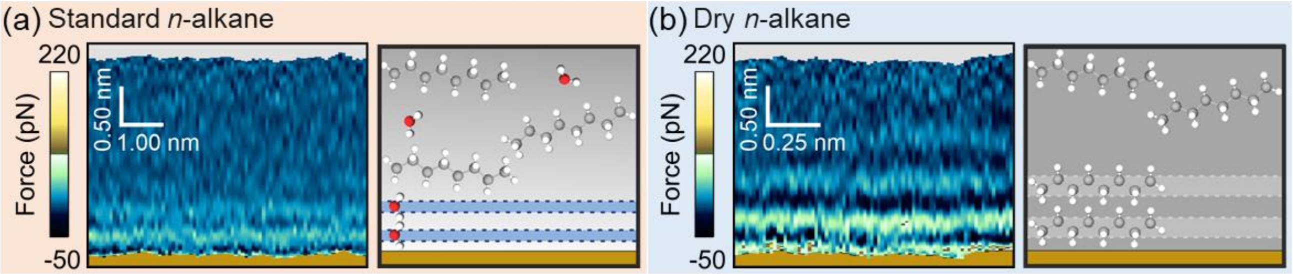


Figure 1. Solid-liquid interface of *n*-alkanes with a hydrophilic surface. (a) 3D-AFM panel and scheme of an organic solvent in equilibrium with the water environment and mica. The interface shows hydration layers. (b) 3D-AFM panel and scheme of a molecularly-sieved organic solvent and mica. The interface shows solvation layers of the organic liquid.

References:
[1] C. Cafolla and K. Voïtchovsky, Nanoscale **12**, 14504 (2020).
[2] M. R. Uhlig, S. Benaglia, R. Thakkar, J. Comer, and R. Garcia, Nanoscale **13**, 5275 (2021).
[3] F. Mugele, B. Bera, A. Cavalli, I. Siretanu, A. Maestro, M. Duits, M. Cohen-Stuart, D. Van Den Ende, I. Stocker, and I. Collins, Sci. Rep. **5**, 1 (2015).
[4] T. Fukuma and R. Garcia, ACS Nano **12**, 11785 (2018).
[5] M. R. Uhlig, D. Martin-Jimenez, and R. Garcia, Nat. Commun. **10**, 2606 (2019).

Reducing thermal noise in atomic force microscopy with intermodal coupling

Ioan Ignat[1*], Bernhard Schuster[1], Jonas Hafner[1], MinHee Kwon[1], Daniel Platz[1], Ulrich Schmid[1]

[1.] TU Wien, Gußhausstraße 27-29, 1040 Austria
* Corresponding author: ioan.ignat@tuwien.ac.at

During the last decades, a plurality of atomic force microscopy methods (AFM) have been developed which strive for increasing the sensitivity of AFM measurements. However, the thermal noise background has always been considered as a fundamental sensitivity limit. Recently, in field of nanomechanics novel multifrequency methods have been investigated to reduce the thermal noise in micromechanical resonators. In this talk, we investigate the potential use of these techniques in AFM measurements.

The mechanism of noise reduction depends solely on intermodal coupling which is enabled by the choice of excitation frequencies. By exciting a commercial AFM cantilever in vacuum at a frequency equal to the difference between the first two flexural eigenfrequencies, the first mode can be effectively cooled, corresponding to a reduction of the thermal noise temperature [1]. We reduced the effective temperature of the first flexural mode from room temperature down to 99.6 K (**Fig. 1 a, b**).

For an excitation frequency equal to the sum of the two eigenfrequencies, a squeezed state between the quadratures of the two modes is created [2], i.e. in phase space the noise ball is elongated (**Fig 1c**). By performing a measurement in rotated quadratures X_c and Y_c the thermal noise in the X_c quadrature is reduced by 49%.

Our measurements demonstrate that both cooling and squeezing are potential routes towards noise-reduced measurements with standard AFM equipment.

References:
[1] Mahboob et al. Nat. Phy. **8** (2012).
[2] Patil et al. Phys. Rev. Lett. **115**, 017202 (2015)

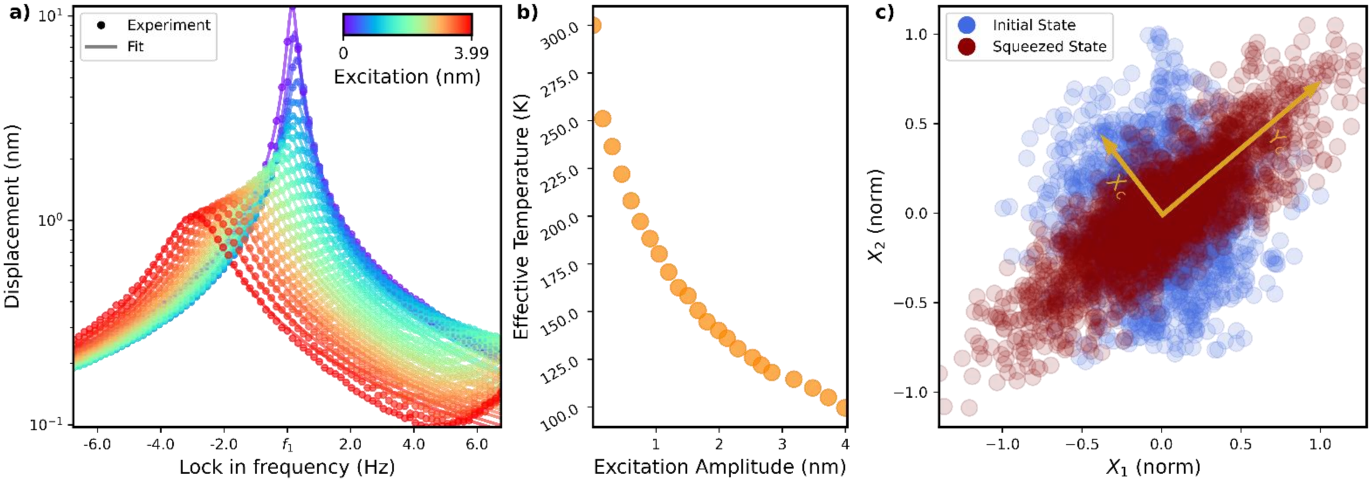


Figure 1. a) Measurement of frequency response of the first flexural mode with excitation strengths. b) The effective temperature of first mode and coupling rate w.r.t. the amplitude at the excitation frequency. c) Phase space distribution of 1st and 2nd mode quadratures in absence of an excitation (blue) and with an excitation tone at the sum frequency (red)

The Rose Petal surface, a wonder of nature: complexity on the nanoscale

Lisa Almonte[1], Carlos Pimentel[2], Enrique Rodríguez-Cañas[3], José Abad[4], Victoria Fernández[5] and Jaime Colchero[1]
[1.] CIOyN, Departamento de Física, Campus Espinardo, Universidad de Murcia, E-30100 Murcia.
[2.] Instituto Andaluz de Ciencias de la Tierra, Av. de las Palmeras, 4. E-18100 Granada.
[3.] IDiBE, Universidad Miguel Hernández, Elche, E-03202 Alicante.
[4.] Applied Physics Department, Technical University of Cartagena, E-30202 Cartagena.
[5.] School of Forest Engineering, Technical University of Madrid, E-28040 Madrid, Spain.
* Corresponding author: colchero@pum.es

Biological organisms are covered with a cuticle as outermost extracellular material and interface between organs and the environment. A precise understanding of the structure-function relation of these interfaces is still an open issue. In particular for the case of wetting, it is far from clear how nanoscale chemistry and structure determine macroscopic wetting. The rose petal combines antagonistic wetting properties: high water contact angles and high drop adhesion.

We use Atomic Force Microscopy (AFM) to simultaneously measure nanoscale topography and chemistry of (natural!) Rose Petals. We found two extraordinary features linked to their peculiar wetting properties, namely: (i) surface roughness is concentrated on the nanoscale and fractal-like, and (ii) the surface has an extreme nanoscale chemical variability, which critically defines wetting properties. While high roughness is generally accepted to be the origin of peculiar wetting (super-hydrophobicity) the role of nanoscale chemical variability is usually not really a topic of discussion; most probably because -up to now- it could not be "seen".

The combination of topographic and chemical variability is modelled with a single nanoscale wetting parameter that naturally explains the Rose Petal Effect, which has been an unsolved puzzle. The fundamental mechanism inducing the Rose Petal Effect should be universal; this work will thus trigger new research on wetting and bio-inspired functional surfaces.

Figure 1. Left: Rose Petal with small drops having high contact angle but nevertheless adhering to the petal surface. Middle: AFM images showing topography (vertical scale) and wetting properties (color of surface. blue: hydrophilic, red: hydrophobic). Image scales correspond to top image (12.5μm)^2 and 4 μm height, bottom image (2.5μm)^2 and 2μm height. Right: Wetting parameter calculated from topography and local chemistry data.

Traceable Lateral Force Calibration (TLFC) for Atomic Force Microscopy

Arnab Bhattacharjee

New University of Delaware

Efforts to reliably measure AFM lateral forces have been impeded by the difficulties in obtaining appropriate calibration standards, applying those force standards to the apex of the tip, and quantifying calibration uncertainty. Here we propose a new method, Traceable Lateral Force Calibration (TLFC), which combines the reliability of direct methods with the convenience of indirect/semi-direct methods. Like other direct methods, ours comprise three essential steps: (1) fabrication of a spring (the Traceable Reference Lever or TRL); (2) calibration of the TRL spring constant; (3) conversion of measurable TRL deflections into absolute lateral force measurements based on its pre-calibrated spring constant (TLFC method). The TRL device, a simple two-axis cantilever, is easy to design, fabricate, and directly pre-calibrate with a standard laboratory microbalance. Following pre-calibration, the TRL device becomes a convenient absolute standard for AFM lateral force measurements. This paper describes the complete method and demonstrates its primary merits, which include (1) traceability to measurement standards; (2) ease of use by outside user groups; (3) absolute measurement errors 10% for moderately stiff cantilevers (1 N/m normal stiffness); (4) robustness over a wide range of common loads, instruments, probes, and environments. While the method and proof-of-concept devices described in this paper were designed primarily for moderate to high load cantilevers (1 N/m), we discuss how a next generation of compliant TRL devices can be used with the TLFC method to reliably calibrate arbitrary AFM cantilevers (1 N/m) and forces.

High-resolution Label-free 3D mapping of pH, Oxygen and ROS of single living cells

A. Erofeev[1,2,3*], P. Gorelkin [1,4], C. Edwards[4,5], A. Majouga[1,2,6], Y. Korchev[1,5]

1. National University of Science and Technology "MISIS", Moscow, Russian Federation; 2. Lomonosov Moscow State University, Moscow, Russian Federation;3. NanoProfiling Limited Liability Company, Technopark Skolkovo, Moscow, Russian Federation 4. ICAPPIC Ltd, London, United Kingdom; 5. Department of Medicine, Imperial College London, London, United Kingdom;6. Dmitry Mendeleev University of Chemical Technology of Russia, Moscow, Russian Federation

* Corresponding author: erofeev@polly.phys.msu.ru

Nanopipettes have been used in different applications with integration into Scanning Ion Conductance Microscopy (SICM): high resolution topographical imaging of living cells, quantitative delivery of molecules to the surface of living cells. Recently, we reported on the development of a label-free pH-sensitive nanoprobe consisting of a self-assembled zwitterion-like nanomembrane at the tip of a nanopipette [1]. This platform allows for SICM feedback-controlled precise positioning of the nanoprobe to the cell surface to monitor the local pHe with high spatiotemporal resolution and high sensitivity. The probe was developed by cross-linking glucose oxidase and poly-l-lysine at the tip of a glass nanopipette, resulting in drying-mediated self-assembly of a pH-selective nanomembrane with a sensitivity higher than 0.01 units, capable of fast response times (down to ~2 ms), and a high spatial resolution (~50 nm). Additionally, nanopipette probes still hold great promises as intracellular biosensors. Here we describe the fabrication, characterization, and tailoring of carbon nanoelectrodes based on nanopipette for intracellular electrochemical recordings. We demonstrate the fabrication of disk-shaped nanoelectrodes whose radius can be precisely tuned within the range 5-200 nm. The functionalization of the nanoelectrode with platinum allowed the monitoring of oxygen consumption outside and inside of melanoma cell. These novel platinum nanoelectrodes are useful for understanding cell oxygen metabolism and can be employed to study the redox biochemistry and biology of cells, tissues and organisms. We showed that microinjury of Chara corallina internodal cells with the tip of a glass micropipette is associated with a drastic decrease in oxygen concentration at the vicinity of the stimulation site [2]. We applied the nanoelectrode to perform intracellular reactive oxygen species (ROS) measurement in cultured melanoma cells, HEK293 and LNCap cancer cell. [3-5]. A cell can withstand multiple penetrations and we measured a substantial difference between the electrochemical signal measured inside and outside the cell. We believe these results show the potential of functional nanoelectrode to probe endogenous species into cells and with further improvements they may allow the study of oxidative stress under influence of different drugs and nanoparticles [3]. The efficiency of ROS generation under flavin mononucleotide blue light irradiation was measured in single melanoma cells by a label-free technique using an electrochemical nanoprobe in a real-time control manner [4].We have shown that our new method can measure the ROS response to chemotherapy in tumor-bearing mice in real-time. ROS levels were measured in vivo inside the tumor at different depths in response to doxorubicin [5].

References: [1] Y. Zhang et al., Nat. Commun 10 (2019) p. 1–9. [2] A. Alova et al., J. Exp. Bot. (2019) p. 1–13. [3] A. Erofeev et al., Sci. Rep. 8 (2018) p. 1–11. [4] R. A. Akasov et al, Sci. Rep. 9 (2019) p. 1–11. [5] A. Vanev et al., Anal. Chem. 92 (2020), 92, p. 8010–8014.

Mapping mechanical properties of living cells at nanoscale using intrinsic nanopipette–sample force interactions

Petr Gorelkin

NUST MISiS

Mechanical properties of living cells determined by cytoskeletal elements play a crucial role in a wide range of biological functions. However, low-stress mapping of mechanical properties with nanoscale resolution but with a minimal effect on the fragile structure of cells remains difficult. Scanning Ion-Conductance Microscopy (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. In this work, we have provided an alternative estimation of intrinsic force and stress and demonstrated the possibility to perform qualitative and quantitative analysis of cell nanomechanical properties of a variety of living cells. Force estimation on decane droplets with well-known elastic properties, similar to living cells, revealed that the forces applied using a nanopipette are much smaller than in the case using atomic force microscopy. We have shown that we can perform nanoscale topography and QNM using a scanning procedure with no detectable effect on live cells, allowing long-term QNM as well as detection of nanomechanical properties under drug-induced alterations of actin filaments and microtubulin. We compared QNM data obtained with low-stress SICM and peak-force AFM. Low-stress SICM gave more reproducible data for the surface layer mechanical properties of living cells. However, AFM has the advantage that it can determine the physical properties of the inner structures of the living cell such as the cell nucleus or nucleoli. We have demonstrated that our technique can be applied to QNM using the intrinsic force of sharp nanopipettes for high-resolution imaging of the mechanical properties of living cells. Comparative studies of AFM and SICM for nanomechanical characterisation of living cells were performed on PC3 cells. We observed how the mechanical properties of single cells changed in the presence of drugs acting on various parts of the cytoskeleton. Using the destabilisation of microtubules produced by MMAE, we observed a decrease in Young's modulus of the living PC3 cells. The effect of inhibition of actin polymerisation on the mechanical properties of the living PC3 cells was mainly located in the periphery. We have also shown the possibility of applying low-stress SICM for long-term nanomechanical mapping in real-time and the visualisation of the dynamic process related to the changes in the mechanical properties of the living cells. This technology is fast enough to observe relatively rapid biological events. We have also shown that it is possible to perform highly localized QNM measurements on living cell surfaces.

Attoampere Nanoelectrochemistry

Georg Gramse

Johannes Kepler University

Electrochemical microscopy techniques have extended our understanding of surface chemistry to the micrometer and even sub-micrometer level. However, fundamental questions related to charge transport at the solid-electrolyte interface, such as catalytic reactions or operation of individual ion channels, require improved spatial resolutions down to the nanoscale. A prerequisite for single-molecule electrochemical sensitivity is the reliable detection of a few electrons per second – i.e., currents in the atto-Ampere (10-18 A) range, 1000 times below today's electrochemical microscopes. Here we will report on local nano cyclic voltammetry (CV) measurements of ferrocene self-assembled monolayer (SAM) with sub atto-Ampere sensitivity and simultaneous spatial resolution 80 nm. Such sensitivity is obtained through measurements of the charging of the local faradaic interface capacitance at GHz frequencies. Nanometer-scale details of different molecular organization with a 19% packing density difference are resolved, with an extremely small dispersion of the molecular electrical properties. This was previously predicted based on weak electrostatic interactions between neighboring redox molecules in a SAM configuration. These results open new perspectives for nanoelectrochemistry like the study of quantum mechanical resonance in complex molecules and a wide range of applications from electrochemical catalysis to biophysics.

S Grall, I Alić, E Pavoni, T Fujii, S Müllegger, M Farina, N Clément and Gramse G* 2021 Attoampere Nanoelectrochemistry, Small (in review), arXiv:2011.10405

Quantitative Corrections to Voxel-scale Heterogeneity via In situ Grayscale Vat Photopolymerization in an Atomic Force Microscope

Callie Higgins

National Institute of Standards and Technology

Vat photopolymerization is a powerful additive manufacturing technique that address many applications ranging from personalized medicine to large-scale manufacturing. Unfortunately, these printing processes introduce micrometer-scale anisotropic inhomogeneities due to the resin absorptivity, diffusivity, reaction kinetics, and swelling during the requisite photoexposure. Previously, it has not been possible to characterize high-resolution mechanical heterogeneity as it develops during the printing process. By combining vat photopolymerization additive manufacturing with atomic force microscopy (AFM) in a hybrid instrument, heterogeneity of a single, in situ printed voxel is not only characterized and quantified for the first time, but also an as-printed modulus-informed corrective algorithm is applied to fabricate homogeneous voxels. Our results demonstrate the complex properties of printed voxels at all relevant stages of the printing process including in resin and after post-processing (both after rinse and after secondary cure) using a state-of-the-art fully-calibrated cantilever ensuring the most quantitative measurement of vat photopolymerized single-voxel mechanical properties to date. Notedly, though we measured substantial creep behavior in the as-printed, in situ voxels, this behavior was drastically reduced after the samples were removed from the resin and washed, which could adversely affect the final part fidelity in a layer-by-layer printing process. By combining AFM with vat photopolymerization, this instrument equips researchers with a tool to develop rich insight into resin development, process optimization, and fundamental printing limits.

Automated and Autonomous Experiment in Scanning Probe Microscopy: If, Why, When, and How

Sergei Kalinin

ORNL

Machine learning and artificial intelligence (ML/AI) are rapidly becoming an indispensable part of physics research, with domain applications ranging from theory and materials prediction to high-throughput data analysis. In parallel, the recent successes in applying ML/AI methods for autonomous systems from robotics through self-driving cars to organic and inorganic synthesis are generating enthusiasm for the potential of these techniques to enable automated and autonomous experiment (AE) in imaging. In this presentation, I will discuss challenges and opportunities of AE in Scanning Probe Microscopy, ranging from feature discovery to controlled intervention and physics discovery. Using Piezoresponse Force Microscopy as an example, I will illustrate the deep learning workflows for the semantic segmentation of the data, including automated discovery of the ferroelectric and ferroelastic domain walls. I will further discuss the unsupervised and physics-based learning from the data. The special emphasis is made on the rotationally invariant variational autoencoders that allow to disentangle rotational degrees of freedom from other latent variables in imaging and spectral data. The analysis of the latent space of autoencoders further allows establishing physically relevant transformation mechanisms in complex domain structures. Extension of encoder approach towards establishing structure-property relationships in ferroelectrics will be illustrated. I further discuss the strategies based on Gaussian Processes for automated experiment, and demonstrate some initial results for AE in PFM including FerroBOT and image-based exploration of complex domain structures.

On the Comparison of Dynamic AFM Modes to Quantitatively Map the Mechanical Properties of Polymeric Materials

Phillipe Leclère

University of Mons (UMONS), Laboratory for Chemistry of Novel Materials, B 7000
Mons, Belgium

Over the past few decades (nano)composites and functional polymer blends have replaced metals in many applications from aerospace to sports gear, from automobiles to wind turbines, and from circuit boards to civil structures such as bridges and buildings. With these novel materials impacting every part of our lives, they have become ubiquitous. Mechanical property mapping can provide critical insights into the fundamental processes at the local scale that lead to deformation phenomena in these materials. The relatively recent development of dynamic mechanical scanning probe microscopies allows measuring mechanical properties of materials, providing well-adapted, fast, and versatile methods for mapping these properties. However, there is a lack of quantitative measurements, such as the elastic modulus or the viscoelastic properties (storage and loss modulus, loss tangent). In this work, we systematically compare the performances of the most promising (recent) methodologies based on multifrequency SPM (namely HarmoniX, Bimodal AFM, Contact Resonance AFM, Intermodulation AFM, and nano Dynamic Mechanical Analysis AFM) to more classical approaches. For instance, by considering as model systems, poly(styrene) - poly(ε- caprolactone) polymer blends, we propose adapted protocols for the data analysis, expecting to help the scientific community to better understand the key parameters in the optimization of the behavior of materials not only for fundamental aspects but also for industrial applications. In this context, Machine Learning (ML) has been perceived as a promising tool for the design and discovery of novel materials for a broad range of applications. In this talk, we will discuss computational methods and ML algorithms dealing with data clustering (such as K-Means or Automatic Gaussian Mixture Model) that can be used to detect the different domains and (inter)phases in materials (by partitioning the recorded data (i.e. the observables) into clusters according to their similarities. Additionally, based on the Tabor coefficient calculation, we will also propose some protocols that can be easily implemented to rapidly determine which mechanical model(s) can be applied to obtain the quantitative mapping of the mechanical properties for each local domain or phase. This algorithmically driven approach will enable analyze materials with more complex architectures and/or other properties (such as electrical ones), opening new avenues of research on advanced materials with specific functions and desired properties leading to the creation of functional and more reliable structural materials. In a step forward, we analyzed in-depth the nanomechanical properties of other polymer-based materials (such as industrial polymer blends, block copolymers, multifunctional nanocomposites, polymer-plasma thin films, hydrogels for cell culture, and bio glue coatings (obtained from recombinant adhesive proteins of sea stars), for which conventional analysis are not "technically" feasible.

A novel method of measurement of tracer diffusion in nanoconfined liquids

Shivprasad Patil

Indian Institute of Science Education and Research, Pune

The flow of water confined to nanometer-sized pores is central to a wide range of subjects from biology to nanofluidic devices. Despite its importance, a clear picture about nanoscale fluid dynamics is yet to emerge. Water permeation through hydrophobic channels, such as nanotubes, is five orders larger than expected from conventional fluid theory[1]. On the other hand, the measured viscosity through hydrophilic nanochannels is 30 percent larger than bulk water[2]. The viscosity measurement of nanoconfined water by independent means, such as Atomic force Microscopy (AFM) and Surface Force apparatus (SFA) have resulted in contradictory findings. In recent years we have observed that water under confinement exhibits nonlinear rheological response such as viscoelasticity and shear thinning [3-6]. In micro-rheology, it is also possible to determine the parameters governing viscoelasticity of the medium by measuring diffusion of tracer dye molecules through it. FCS is one of the methods to measure diffusion and can be used to measure viscoelastic response. There are many technical challenges in performing FCS measurements on nanoconfined liquids. In this talk, I will present a new instrument developed in our lab to measure diffusion in nanoconfined liquids[7]. It is a combination of Fluorescence correlation spectroscopy and Atomic Force Mivroscopy. In this instrument we have optical access to the confined region to measure diffusion in water under confinement. I will present development of the instrument, its capability and measurement of diffusion of a standard dye in nanoconfined water [7]. A careful analysis of the data will further allow development of microrheology-like tool to measure non-Newtonian response which is complementary to the standard rheology previously developed in our lab [4-6].

References:

1. M. Majumder, N. Chopra, R. Andrews, and B. J. Hinds, Nature 438, (2005) 44.

2. N. R.Tas, J. Haneveld , H. V.Jansen, M. Elwenspoek, and A. van den Berg, Appl. Phys. Lett. 85, (2004) 3274.

3. S. H. Khan, G. Matei, S. Patil, and P. M. Hoffmann, Phys. Rev. Lett. 105, (2010) 1061011.

4. K. Kapoor, V. Kanwade, V. Shukla, and S. Patil, Rev. Sci.Instrum. 84, (2013) 025101.

5. K.Kapoor, A. Sekhon and S. Patil, Phys. Rev. E 89, (2014) 0130041.

6. A. Sekhon, VJ. Ajith and S. Patil J. Phys. Condens. Matter 29, (2017) 205101. 7. VJ. Ajith and S. Patil, Rev. Sci. Instrum. 91, (2020) 013702

Scanning Kelvin Probe Microscopy Reveals That Ion Motion Varies with Dimensionality in 2D Halide Perovskites

Justin Pothoof

University of Washington

We study ion migration in 2D lead halide perovskites of varying dimensionality using scanning Kelvin probe microscopy (SKPM). We perform potentiometry on micrometer-scale lateral junctions in the absence of injected charge, and we compare how ion motion varies between prototypical two-dimensional n-butylammonium lead iodide perovskites (BA2PbI4, n = 1), and methylammonium-incorporated quasi-2D perovskites (BA2MA3Pb4I13, ~⟨n⟩ = 4) under the effects of illumination and temperature. We attribute the observed slow dynamics to relaxation of the bias-induced ionic charge distributions at different temperatures, and we extract the activation energies associated with the ionic motion in each case. Finally, we propose an explanation for these phenomena by hypothesizing that ion motion in purely-2D BA2PbI4 perovskite films is dominated by paired halide and halide vacancy, whereas for quasi-2D BA2MA3Pb4I13 perovskites, the ion motion is a combination of both halide and methylammonium (vacancy) migration. These data show that dimensionality in these systems plays a critical role in ion dynamics.

Getting to Zero - Quantitative Electromechanical Atomic Force Microscopy

Roger Proksch

Asylum Research

Since the very early days of atomic force microscopy (AFM), voltage modulation (VM) of AFM has been used to try to quantify a host of electronic, electrochemical and electromechanical functionalities across nanometer length scales. The critical importance of such information has resulted in the development of a plethora of VM-AFM techniques for exploration of either long or short-range forces. Of relevance for ferroelectrics, piezoresponse force microscopy (PFM) imaging and associated spectroscopies have effectively opened the door to the exploration of nanoscale ferroelectric properties. The rise of PFM, however, has also brought about claims of ferroelectricity in materials which were subsequently thought to be not ferroelectric,[1] even unlikely materials such as soda-lime glass. Explanations for the origins of these unexpected nanoscale phenomena have not been in short supply, including new material properties, surface-mediated polarization changes and/or spatially resolved behavior that is not present in bulk measurements. At the same time, it is well known that VM-AFM measurements are susceptible to numerous forms of crosstalk and despite efforts within the AFM community, a global approach for quantitative, crosstalk-free techniques remains elusive. In an effort to understand the true origins of the measured VM-AFM signals we demonstrate the presence of hysteretic ("false ferroelectric") long-range interactions between the sample and cantilever body and show that these are intrinsic to traditional VM-AFM detection methods. However, we show that with interferometric displacement sensor (IDS) [2] it is possible to separate the true tip motion from the cantilever dynamics. Using the IDS we have established a rapid and simple flagging routine of false piezo and ferroelectric responses and are able to demonstrate fully quantitative and repeatable nanoelectromechanical characterization. We attribute a lot of the observed unexpected hysteretic behavior to surface water, since it is ubiquitous in ambient conditions for even mildly hydrophilic surfaces and may explain the plethora of behaviors discussed above. Finally, we demonstrate that through using the interferometric approach allows for putting of quantitative limits on the electromechanical sensitivity. For example, we were able to define a $d_eff \leq 40$fm/Volt for soda-lime glass [3] using interferometry that is much smaller (albeit still not zero) than other VM-AFM measurements. These quantitative measurements are critical for a wide range of new devices ranging from mems actuators, memristor devices, energy storage and dynamic computer memory.

References

[1] Vasudevan, Rama K., et al. Ferroelectric or non-ferroelectric: Why so many materials exhibit "ferroelectricity" on the nanoscale. Applied Physics Reviews 4.2 (2017): 021302.

[2] Labuda, Aleksander, and Roger Proksch. Quantitative measurements of electromechanical response with a combined optical beam and interferometric atomic force microscope. Applied Physics Letters 106.25 (2015): 253103.

[3] Liam Collins,Yongtao Liu, Olga S. Ovchinnikova and Roger Proksch, "Quantitative Electromechanical Atomic Force Microscopy", ACS Nano 13, 8055 (2019).

AI in SPM: Recent progress, challenges and the coming wave of automation

Rama Vasudevan

Oak Ridge National Laboratory

The use of Artificial intelligence (AI) methods in scanning probe microscopy is steadily increasing, facilitating everything from improved signal resolution [1] to model fitting [2] to image segmentation and adaptive sampling [3]. With the employment of these methods, large opportunities and pressing challenges remain as the path towards increasing automation becomes clearer. In this talk, I will discuss our recent work of applying AI and other statistical algorithms to improve SPM methods. I will briefly showcase our work on improving signal acquisition and inference, via Bayesian methods, as well as the use of deep neural networks and statistical methods to improve spectral fitting. I will then discuss the use of more advanced active learning and Bayesian optimization algorithms to facilitate automated experiments, enabling more data to be efficiently captured, reducing time and potentially expanding the suite of materials for which longer spectroscopies become viable. Finally, I will discuss how we can expand beyond simple efficiency gains to true scientific discovery, via use of advanced AI methods based on reinforcement learning and curiosity-based rewards. Challenges to implement these methods include the need for ‘simulation gyms’, compute power both at the edge and offline, and appropriate hardware and software links. This work was conducted at the Center for Nanophase Materials Sciences, a US DOE Office of Science User Facility.

References

[1] Somnath et al. Nat Commun. 9, 513 (2018)

[2] Vasudevan et al. J. Appl. Phys. 128, 054105 (2020)

[3] Ziatdinov et al. J. Appl. Phys. 128, 055101 (2020).

Nonlinear detachment dynamics in contact resonance atomic force microscopy

Ryan Wagner

Purdue

Dynamic AFM techniques in which deflection feedback is used to maintain a constant average force between the AFM tip and sample can measure viscoelastic, piezoelectric, and electrochemical properties. In particular, piezoresponse force microscopy's ability to characterize ferroelectric materials is becoming increasingly important as a tool for new material discovery and quality control due to interest in integrating ferroelectric materials into next generation computing platforms. In all these AFM techniques increasing the amplitude of the driving signal is desirable because it improves measurement signal to noise. It is known that there are limits to this amplitude increase as nonlinearity begins to affect the results at extreme amplitudes; however, the precise mechanics of this phenomenon remain poorly explored. In this work, we model the nonlinear dynamic process of increasing drive amplitude to the point where the AFM tip loses contact with the sample. We then capture both the nonlinear softening process and reduction in maximum first harmonic photodiode signal after loss of permanent contact with the sample. The model is validated with a comparison to contact resonance AFM measurements. Ultimately, our exploration of the developed model provides great insight into the upper limits of drive amplitude in dynamic permanent contact AFM and reveals signatures in experiments warning that this limit is exceeded.

Machine Learning to Classify and Correlate AFM Phase Images of polymers

Dalia Yablon

SurfaceChar

Machine learning has been applied to classify and correlate AFM phase images of impact copolymer materials (ICP). A series of 6 ICP materials were synthesized, where each ICP comprises of a polypropylene matrix, ethylene propylene rubber (EPR), and ethylene inclusions within the rubbers. The various ICPs differ in their bulk mechanical properties and their microstructure, namely, the morphology, size, and density of the EPR and its inclusions. Machine learning models were built around 10um x 10um AFM phase images of each of these ICP materials. Our models successfully classified binary and ternary combinations of some ICP's, but not others. This points to real and meaningful differences in the microstructure of some of the ICP materials. At the same time, these results indicate that other ICP's are indistinguishable in their microstructure, despite some their bulk mechanical properties being significantly different. The ICP phase images were also correlated with bulk mechanical properties. The correlation of the microstructure in the AFM image was strongest with the plastic properties of yield strength and ultimate elongation percentage. The AFM images showed poor correlation with elastic properties such as flexural modulus as well as the Notched Izod RT test.

Designed Interfaces Between Proteins and Inorganic Crystals for Templated Assembly and Co-Assembly

Sakshi Yadav

Pacific Northwestern National Laboratory

Previously (1Pyles, H., Zhang, S., De Yoreo, J.J., Baker, D.; Nature 2019) we have shown we could use Rosetta to design proteins that exhibited a lattice match to mineral surfaces1. We discovered that we could exploit those interactions to generate a variety of ordered 2D phases (micrometer-long wires and extensive honeycomb arrays) that were strongly dependent on electrolyte type and concentration and several other factors. Comparison to Monte Carlo simulations of non-interacting colloidal rods demonstrates that these phases are not predicted and thus must result from the competition between the designed interfaces (protein-protein and protein-substrate) and the colloidal forces, while machine learning analysis shows that the orientation dependent energy landscape is both complex and also dependent on electrolyte type and concentration. Moving beyond a system of homogeneous protein building blocks, we are now using the power of co-assembly to both investigate the role of complementarity and frustration in defining order and to generate higher-order assemblies.