

# Linz Winter School 2015

**Date:** Tue, Jan 27-2015 until Thu, Jan 29-2015

**Location:** JKU Life Science Center Upper Austria, Institute for Biophysics  
Gruberstraße 40, 4020 Linz, Austria

## Program

### Tuesday, Jan 27: Morning Talk Session

(Chair: Peter Hinterdorfer)

Institute for Biophysics, Gruberstraße 40, Seminar-Room, Basement

- |                      |   |
|----------------------|---|
| <b>08.30</b>         | Meeting point at Julius Raab Heim, Lobby,<br>Julius Raab Straße 10, 4040 Linz and transfer to Gruberstraße by<br>tram/bus |
| <b>09.15 – 09.30</b> | Registration  |
| <b>09.30 – 09.45</b> | Welcome & Introduction  |
| <b>09.45 – 10.15</b> | <i>Molecular Recognition Force Microscopy/Spectroscopy</i><br>Peter Hinterdorfer  |
| <b>10.15 – 10.45</b> | <i>High-Speed AFM</i><br>Johannes Preiner   |
| <b>10.45 – 11.15</b> | <b>Coffee Break</b>   |
| <b>11.15 – 11.45</b> | <i>Functionalization of AFM tips with native proteins or with His6-tagged<br/>fusion proteins</i><br>Hermann J. Gruber    |
| <b>11.45 – 12.15</b> | <i>Force Spectroscopy Experiments and Analysis</i><br>Andreas Karner  |
| <b>12.15 – 12.35</b> | <i>The Physics of TREC imaging</i><br>Sandra Posch  |
| <b>12.35 – 12.50</b> | <i>AFM Functional Imaging on Melanoma Cells</i><br>Lilia A. Chtcheglova   |
| <b>12.50 – 14.00</b> | <b>Lunch Break</b><br><b>Mensa “OÖ Gebietskrankenkasse”</b>   |

## **Tuesday, Jan 27: Afternoon Talk Session**

(Chair: Gerald Kada)

Institute for Biophysics, Gruberstraße 40, Seminar-Room, Basement

- 14.00 – 14.30**      *Introduction round of attendees*
- 14.30 – 14.50**      *Application of combined AFM and fluorescence microscopy:  
Localization of cellular membrane receptors and stimulation of T cells*  
Rong Zhu
- 14.50 – 15.10**      *Characterization of bacterial surfaces by scanning probe microscopy*  
Yoo Jin Oh
- 15.10 – 15.40**      *Introduction to AFM with Keysight Technologies*  
Gerald Kada
- 15.40 – 16.10**      **Coffee Break**
- 16.10 – 16.25**      *Scanning Microwave Microscopy: nanoscale complex impedance  
imaging, modeling, and biological applications*  
Enrico Brinciotti
- 16.25 – 16.55**      *Single Molecule Biology - Studying Movements and Meetings within the  
Plasma Membrane*  
Gerhard Schütz
- 16.55 – 17.25**      *STIM1 couples to ORAI1 via an intramolecular transition into an  
extended conformation*  
Martin Muik
- 17.25**              *Arrangement of 10 groups for the demo session on Wednesday and of 4  
groups for the CBL Demo Session on Thursday*

## **Wednesday, Jan 28: Demo Session**

Institute for Biophysics, University of Linz, Gruberstraße 40: 4<sup>th</sup> and 1<sup>st</sup> floor

**09.15** *Meeting point, Institute for Biophysics, Gruberstraße 40,  
4<sup>th</sup> floor, Seminar Room (449)*

**09.30 – 13.00 and 14.30 – 17.00** *Demos*

Attendees will be divided into groups of 3-4 participants and switched to the next demo every 30 min.

**Lunch break from 13.00 – 14.30 at Mensa “OÖ Gebietskrankenkasse”.**

**Coffee break from 11.00 – 11.30 and 15.30 – 16.00 in the Seminar-Room 449, 4<sup>th</sup> floor.**

- DEMO-1:** Self Sensing (Room 415)  
*(Michael Leitner/ Boris Buchroithner)*
- DEMO-2:** Tip Functionalization (Room 422)  
*(Andreas Ebner/ Barbara Hartl)*
- DEMO-3:** Single Molecule Force Spectroscopy (Room 413)  
*(Sandra Posch/ Anny Fis)*
- DEMO-4:** Topography and Recognition Imaging (TREC) (Room 417)  
*(Rong Zhu/ Melanie Köhler)*
- DEMO-5:** High Speed AFM (Room 403)  
*(Johannes Preiner/ Andreas Karner)*
- DEMO-6:** Bacterial Imaging (Room 416)  
*(Yoo Jin Oh/ Markus Rech)*
- DEMO-7:** High frequency imaging of nanoscale structures (Room 405)  
*(Silviu Sorin Tuca/ Giorgio Badino)*
- DEMO-8:** Combined Optical and Force Microscope (Room 406)  
*(Lilia Chtcheglova/ Szymon Prauzner-Bechcicki)*
- DEMO-9:** Association kinetics between receptor and ligands using Quartz Crystal Microbalance (QCM) (Room 415)  
*(Lukas Traxler)*
- DEMO-10:** Patch Clamp and FRET Microscopy (1<sup>st</sup> floor Room 101 and 102)  
*(Martin Muik/ Rainer Schindl)*

**19.00**

### **Social Event**

Meeting point “Weinstadt”

Tram station: St. Magdalena (tram number 1 or 2)

Griesmayrstraße 18, 4040 Linz

## **Thursday, Jan 29: Hands-on Session and CBL Demo Session**

Center for Advanced Bioanalysis (CBL), Gruberstraße 40: 3<sup>rd</sup> floor  
Institute for Biophysics, University of Linz, Gruberstraße 40: 4<sup>th</sup> floor

**09.30 – 13.00 and 14.00 – 17.00**      **Hands-on Session: attendees' samples**  
Attendees' samples investigation and practical AFM training according to time schedule displayed in the Seminar-Room 449, 4<sup>th</sup> floor.

**10.00 – 11.00 and 11.30 – 12.30**      **CBL Demo Session** (in parallel to the hands-on session) according to the time schedule below.

**Lunch break from 12.45 – 14.00 at Mensa "OÖ Gebietskrankenkasse".**  
**Coffee break from 11.00 – 11.30 and 15.30 – 16.00 in the Seminar-Room 449, 4<sup>th</sup> floor.**

### **CBL Time Schedule (4 groups)**

- 10.00**  
**10.00 – 11.00**      Meeting point CBL, 3<sup>rd</sup> floor **First and second group**  
Introduction and Demos:
- Single molecule fluorescence microscopy  
(*Jan Hesse*)
  - Soft Lithography and Microfluidic Biochips  
(*Bozhi Ji*)  
Surface Chemistry and Micro Contact Printing  
(*Thomas Haselgrübler and Roland Hager*)
- 11.30**  
**11.30 - 12.30**      Meeting point CBL, 3<sup>rd</sup> floor **Third and forth group**  
Introduction and Demos:
- Single molecule fluorescence microscopy  
(*Jan Hesse*)
  - Soft Lithography and Microfluidic Biochips  
(*Bozhi Ji*)  
Surface Chemistry and Micro Contact Printing  
(*Thomas Haselgrübler and Roland Hager*)

# **Molecular Recognition Force Microscopy/Spectroscopy**

Peter Hinterdorfer

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In molecular recognition force microscopy (MRFM), ligands are covalently attached to atomic force microscopy tips for the molecular recognition of their cognitive receptors on probe surfaces. A ligand-containing tip is approached towards the receptors on the probe surface, which possibly leads to formation of a receptor-ligand bond. The tip is subsequently retracted until the bond breaks at a certain force (unbinding force). In force spectroscopy (FS), the dynamics of the experiment is varied, which reveals a logarithmic dependence of the unbinding force from the loading rate. These studies give insight into the molecular dynamics of the receptor-ligand recognition process and yield information about the binding pocket, binding energy barriers, and kinetic reaction rates. Applications on isolated proteins, native membranes, viruses, and cells will be presented. We have also developed a method for the localization of specific binding sites and epitopes with nm positional accuracy. A magnetically driven AFM tip containing a ligand covalently bound via a tether molecule is oscillated at a few nm amplitude while scanning along the surface. In this way, topography and recognition images are obtained simultaneously.

# High-speed AFM

Johannes Preiner

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The function of proteins is determined by their structure and is generated when changing it dynamically. These nano-mechanical dynamics cannot be captured by available techniques such as X-ray crystallography, NMR spectroscopy, and electron or optical microscopy, since they are either ensemble techniques, carried out under non-physiological (vacuum) conditions, or do not have the proper spatial resolution. With commercially available AFMs, only very slow processes could be recorded, since it requires minutes to form an acceptable image at high spatial resolution, far beyond the timescale at which biological processes occur. Recent developments on tapping mode AFM, conducted in the lab of T. Ando (reviewed in (1, 2)) have led to an AFM capable of recording images of 100x100 pixel<sup>2</sup> within 40 ms at sub-nanometer resolution in aqueous solutions. In order to achieve this high scan rate, various parts involved in an AFM such as cantilevers, scanners, and electronic devices had to be modified to obtain efficiently small response times of every single component. In this way it is possible to generate movies consisting of many successive images (40 ms-intervals) of biological specimens and their dynamics(3, 4, 5).

Besides the technical aspects of HS-AFM, this lecture will give an overview on HS-AFM experiments conducted in our lab, especially on the interaction of antibodies with antigenic epitopes on bacterial and viral surfaces (6). Antibodies, also named Immunoglobulins are key for the immune system. Via their Fab arms IgGs can bind two neighboring epitopes resulting in higher avidity and slower dissociation as compared to monovalent Fabs. We demonstrate that IgG molecules do not remain stationary on surfaces of regularly spaced epitopes but exhibit “bipedal” random walking. Their mobility depends on symmetry and spacing of the antigens; monovalent Fabs do not move. We identified steric strain as the main reason for short-lived bivalent binding. On collision, the randomly walking antibodies form transient clusters. Such aggregates might serve as docking sites for the complement system and/or phagocytes.

- [1] Ando T, *et al.* (2007) High-speed atomic force microscopy for observing dynamic biomolecular processes. *J Mol Recognit.*
- [2] Ando T, Uchihashi T, & Fukuma T (2008) High-speed atomic force microscopy for nano visualization of dynamic biomolecular processes. *Progress in Surface Science* 83(7-9):337-437.
- [3] Kodera N, Yamamoto D, Ishikawa R, & Ando T (2010) Video imaging of walking myosin V by high-speed atomic force microscopy. *Nature* 468(7320):72-76.
- [4] Uchihashi T, Iino R, Ando T, & Noji H (2011) High-speed atomic force microscopy reveals rotary catalysis of rotorless F1-ATPase. *Science* 333(6043):755.
- [5] Shibata M, Yamashita H, Uchihashi T, Kandori H, & Ando T (2010) High-speed atomic force microscopy shows dynamic molecular processes in photoactivated bacteriorhodopsin. *Nat Nano* 5(3):208-212.
- [6] Preiner J, *et al.* (2013) Antibody movement on regular antigen clusters: Fab arms are made for walking. *submitted.*

# Functionalization of AFM tips with native proteins or with His<sub>6</sub>-tagged fusion proteins

Doris Sinwel<sup>a</sup>, Martina Rangl<sup>a</sup>, Rong Zhu<sup>a</sup>, Elena Pohl<sup>b</sup>, Robert Tampé<sup>c</sup>, Andreas Ebner<sup>a</sup>, Michael Rexach<sup>d</sup>, Peter Hinterdorfer<sup>a</sup>, and Hermann Gruber<sup>a\*</sup>

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Flexible attachment of a probe molecule (e.g. an antibody) to the AFM tip converts the tip into a biospecific sensor by which can localize and identify cognate target molecules on the sample surface when using dynamic force microscopy modes. Moreover, the biophysical details of the interaction between a tip-bound ligand molecule and complementary receptor molecules on the sample surface can be studied by force spectroscopy. Best results were obtained if the probe molecules were tethered to the apex of the AFM tip via a single polyethylene glycol chain (PEG) with a maximal length of 6-10 nm. The standard procedure requires four steps: (i) generation of NH<sub>2</sub> groups on the tip surface, (ii) reaction of the NH<sub>2</sub> groups with one end of a PEG chain, (iii) generation of SH groups on the protein, and (iv) reaction of the SH group with the free-tangling end of the PEG chain.<sup>1</sup>

Step (iii) is inconvenient and it requires considerable amounts of protein. Therefore, a new heterobifunctional linker (aldehyde-PEG-NHS) was devised which allowed coupling native proteins via their endogenous NH<sub>2</sub> groups (antibodies have 80-90 NH<sub>2</sub> groups per molecule<sup>2</sup>). As a consequence, minute amounts of protein are sufficient for tip functionalization by this method.<sup>2</sup> It can also be used for attachment of small molecules, as exemplified by a commercial NH<sub>2</sub> derivative of ATP.<sup>3</sup> In spite of this significant improvement, the new linker aldehyde-PEG-NHS has adverse aspects: (1) It must be used at high concentration in order to suppress crosslinking of adjacent NH<sub>2</sub> groups on the tip surface by the two ends of the linker, and (2) its synthesis is inconvenient and has low yield. Meanwhile, all problems are solved with a new linker (acetal-PEG-NHS) which is easy to use for tip functionalization with unmodified (native) proteins.<sup>4</sup>

Due to the abundance of NH<sub>2</sub> groups on proteins, the above method is widely applicable, the only disadvantage being the random attachment site of the linker on the protein. We now present a novel method for firm attachment of His<sub>6</sub>-tagged proteins to AFM tips. The aminosilanized tip is reacted with maleimide-PEG-NHS, subsequently tris-NTA is attached to the free-tangling end of the PEG chain on the AFM tip. In the presence of 200 μM Ni<sup>2+</sup>, the His<sub>6</sub> tag is stably bound to tris-NTA via three Ni<sup>2+</sup> ions on the time scale of a day, even in presence of EDTA, providing for the easiest method of AFM tip functionalization with engineered proteins. It has extensively been used to study the interaction of nuclear shuttle proteins by force spectroscopy.

[1] Kamruzzahan et al. (2006) *Bioconjugate Chem.* 17, 1473-1481.

[2] Dorner et al. (1967) *J. Exp. Med.* 125, 823-831.

[3] Ebner et al. (2007) *Bioconjugate Chem.* 18, 1176-1184.

[4] Wildling et al. (2011) *Bioconjugate Chem.* 22, 1239-1248.

[5] Rangl et al. (2013) *Angew. Chem.* 52, 10356-10359.

# Force spectroscopy experiments and analysis

Andreas Karner

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In force spectroscopy experiments, the binding of ligands immobilized on AFM tips toward surface bound receptors (or vice versa) is studied by applying force to the receptor-ligand complex until the bond breaks at a measurable rupture force<sup>1</sup>. The interaction forces of such ligand-receptor pairs are measured in force-distance cycles using a ligand carrying tip and a target surface with firmly attached receptor molecules. As a typical example, a force distance cycle of tip bound human rhinovirus serotype 2 and its binding partner a very-low density lipoprotein receptor fragment is shown in Figure 1. At a fixed lateral position, the tip vertically approaches the surface and is subsequently retracted. During this cycle, the cantilever deflection which is direct proportional to the force is continuously measured and plotted versus piezo movement. The characteristic nonlinear force–distance profile is determined by the elastic properties of the flexible tether, whereas the strength of the interaction (termed “rupture force”) is governed by the type of receptor-ligand pair. The specificity of ligand-receptor binding is usually demonstrated by blocking experiments with free ligands injected into the solution to block the receptor sites on the surface.

When ligand-receptor binding is viewed on the single-molecule level, the average bond lifetime at zero force is given by the inverse of the kinetic off-rate constant. Therefore, ligands will dissociate from receptors even without any force applied to the bond, driven by thermal impulses. This stochastic nature results in a distribution of rupture forces (Figure 2). If molecules are pulled apart very fast, the bond will resist and require measurable force for detachment. In the thermal activation model, the dissociation rate of a complex in solution is described by an Arrhenius dependency on the barrier height. A force acting on a binding complex deforms the interaction energy landscape and lowers the activation energy barrier. This description together with the stochastic description of the unbinding process predicts the rupture force distribution. The maximum of each force distribution (termed “unbinding force”) scales linearly with the logarithm of the loading rate<sup>2</sup> (Figure 3), i.e. the effective force increase, which can be deduced as  $df/dt$  at rupture, being equal to the product of pulling velocity and effective spring constant. In force spectroscopy experiments, the variation in the pulling speed applied to specific ligand-receptor bonds will lead to detailed structural and kinetic information of the interaction. Length scales of energy barriers are obtained from the slope of the spectroscopy plot (i.e. force versus loading rate) and extrapolation to zero forces yields kinetic off-rate for the dissociation of the complex in solution.

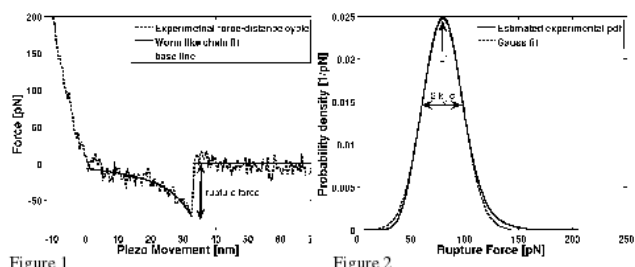


Figure 1

Figure 2

Figure 3

[1] C. Rankl, F. Kienberger, L. Wildling, J. Wruss, H. Gruber, D. Blaas, P. Hinterdorfer. Multiple receptors involved in human rhinovirus attachment to live cells. PNAS 105 (2008), 17778-83.

[2] J. Preiner, H. Janovjak, H. Knaus, D. Cisneros, A. Kedrov, F. Kienberger, D. Mueller\*, P. Hinterdorfer. Insertion and folding free energy of membrane proteins derived from single-molecule force measurements. Biophysical Journal 93 (2007), 930-7.

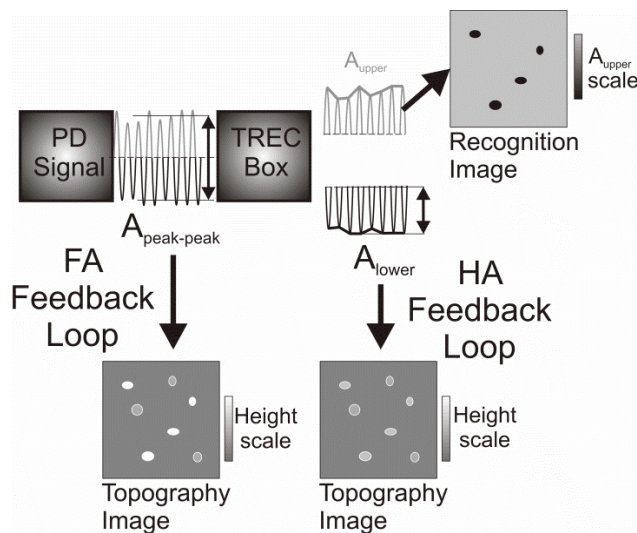


# The Physics of TREC imaging

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Simultaneous topography and recognition imaging (TREC) allows the investigation of receptor distributions on natural biological surfaces under physiological conditions[1]. Based AFM in combination with a cantilever tip carrying a ligand molecule, it enables to sense topography and recognition of receptor molecules simultaneously with nanometer accuracy [2-10]. Here, we discuss optimized handling conditions and guide through physical properties of the cantilever-tip-sample ensemble [9], which is essential for the interpretation of the experimental data gained from this technique. In contrast to conventional AFM methods TREC is based on a more sophisticated feedback loop, which enables to discriminate topographical contributions from recognition events in the AFM cantilever motion. The features of this feedback loop were investigated through a detailed analysis of topography and recognition data obtained on a model protein system. Single avidin molecules immobilized on a mica substrate were imaged with an AFM tip functionalized with a biotinylated IgG. A simple procedure for adjusting the optimal amplitude for TREC imaging is described by exploiting the sharp localization of the TREC signal within a small range of oscillation amplitudes. This procedure can also be used for proving the specificity of the detected receptor-ligand interactions. For understanding and eliminating topographical crosstalk in the recognition images we developed a simple theoretical model, which nicely explains its origin and its dependence on the excitation frequency.



**Figure 1:** The principle of TREC imaging and the two possible feedback mechanisms. In case of molecular recognition between the ligand coupled to the AFM cantilever tip and a receptor on the sample, the cantilevers oscillation signal, coming from the photodiode (PD) contains information about the samples topography (at the lower part of the oscillation, black), and information about the recognition process (at the upper part of the oscillations, grey). The recognition image is constructed from the envelope of the upper part ( $A_{\text{upper}}$ ) of the oscillation, and recognition spots on the sample are usually displayed as dark spots. Depending on the used feedback parameter, i.e.  $A_{\text{peak-peak}}$  or  $A_{\text{lower}}$ , topographical features recognized by the ligand on the tip, exhibit their true height (HA feedback loop) or an increased height (FA feedback loop), since in the latter case the feedback tries to compensate for the additional amplitude reduction in the top peaks of the oscillations due to the stretching of the linker molecule.

- [1] P. Hinterdorfer, and Y. F. Dufrene, *Nat Methods* **3** (2006).
- [2] A. Ebner *et al.*, *Chemphyschem* **6** (2005).
- [3] C. M. Stroh *et al.*, *Biophys J* **87** (2004).
- [4] C. Stroh *et al.*, *Proc Natl Acad Sci U S A* **101** (2004).
- [5] J. Tang *et al.*, *Nano Letters* **8** (2008).
- [6] A. Ebner *et al.*, *Nanotechnology* **19** (2008).
- [7] L. Chtcheglova *et al.*, *Biophys J* (2007).
- [8] L. Chtcheglova *et al.*, *Pflügers Archiv European Journal of Physiology* (2008).
- [9] J. Preiner *et al.*, *Nanotechnology* **20** (2009).
- [10] J. Preiner *et al.*, *Nano Letters* **9** (2009).

# **AFM Functional Imaging on Melanoma Cells**

Lilia A. Chtcheglova

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One of challenging tasks in molecular cell biology is to identify and localize specific binding sites on biological samples with high spatial accuracy. This routinely can be achieved by immunofluorescence techniques, however, the lateral resolution is limited by ~ 200 nm and no information about cell topography can be obtained. TREC technique permits to record AFM topography images and simultaneously localize specific binding sites with a lateral accuracy of several nm, as shown on isolated protein systems such as avidin-biotin, chromatin, and isolated RBC membranes. Contrarily to these systems, a cell surface represent a more complex composition, organization, and processing in space and time.

In the present study TREC technique was applied to B16 mouse melanoma cells in order to visualize the stress inducible heat shock protein 70 (Hsp70). Beside its normal cytosolic localization, Hsp70 is also known to be present at the extracellular leaflet of the plasma membrane of most tumor but not corresponding healthy cells. Apart from an immunostimulatory effect, a function of Hsp70 localized at the tumour cellular surface remains elusive.

# **Application of combined AFM and fluorescence microscopy: Localization of cellular membrane receptors and stimulation of T cells**

Rong Zhu<sup>1</sup>, Memed Duman<sup>1</sup>, Josef Madl<sup>1</sup>, Christian Rankl<sup>2</sup>, Gerald Kada<sup>2</sup>,  
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Combined atomic force microscopy (AFM) and fluorescence microscopy has become a powerful tool for the investigations in the micro- and nano-world, especially for studies in cellular biology. Localizing cellular membrane receptors using conventional fluorescence microscopy is fast and simple, yet the resolution is limited to ~300 nm due to the Rayleigh criterion. This limitation can be overcome by using simultaneous topography and recognition imaging (TREC), an AFM based technique to localize cellular membrane receptors with ~10 nm resolution. Here we used this technique to determine the distribution of a GPI anchor protein derived from the decay acceleration factor fused to GFP, which was reported to be a highly effective lipid raft marker. The fluorescence and the recognition images revealed micro-domains of the GPI-GFP molecules at the same location on the T24 cell membrane, showing the capability of both techniques for specific detection. The recognition image further revealed nano-domains of GPI-GFP, which is beyond the resolution of conventional fluorescence microscopy. Using the same technique, we also measured the distribution of CD4-YFP molecules on the T24 cell membrane, which revealed interesting nano-domains. Furthermore, fluorescence guided force spectroscopy showed reasonable correlation between the binding probability and the expression level of CD4-YFP on the cell membrane. At last, we used the combined setup for the study of the activation of the T cell (Jurkat cell was used here) by anti-CD3 antibody functionalized cantilever tips via monitoring the calcium concentration in the cell with the fluorescence indicator Fura-2. From the experiments, we found that the Jurkat cells are much more active in HBSS with Ca<sup>++</sup> & Mg<sup>++</sup> and with 10% FCS at 37°C than in PBS at room temperature. The cantilever tip functionalized with non-specific goat IgG did not activate the Jurkat cell, in contrast to tips containing anti-CD3 antibody. Force-distance curve measurements at different force loading rates allowed the calculation of the energy landscape of the interaction between the anti-CD3 antibody tip and the Jurkat cell. Recognition imaging provided the information of the distribution of CD3 molecules on the Jurkat cell.

# Characterization of bacterial surfaces by scanning probe microscopy

Yoo Jin Oh

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The bacterial cell wall plays a significant role in maintaining cellular structure and resisting turgor pressure. It changes during growth and division and also opens a pathway to transfer information from the outer environment into the cell, suggesting that the cell wall is dynamic and its mechanical properties are of significant importance. Biological scanning probe microscopy (BIO-SPM) is the tool of choice for detailed microbial studies because it allows for studying living microbial organisms in their natural environment at the nano-scale. As the microbial outer membrane interacts with the extra-cellular environment or other surfaces directly, characterizing its membrane structures and binding capacities provides crucial information for understanding fundamental processes such as bacterial adhesion, surface recognition, and initial attachment to abiotic or biotic surfaces. Bio-SPM is also capable of measuring the cell wall stiffness. Analyses of AFM force-indentation curves yield physical properties of the cellular surface such as Young's modulus, internal turgor pressure, and the stretching modulus of the bacteria [1]. In addition, several sample preparation methods and experimental details for high-resolution bacterial AFM imaging [2] will be given.

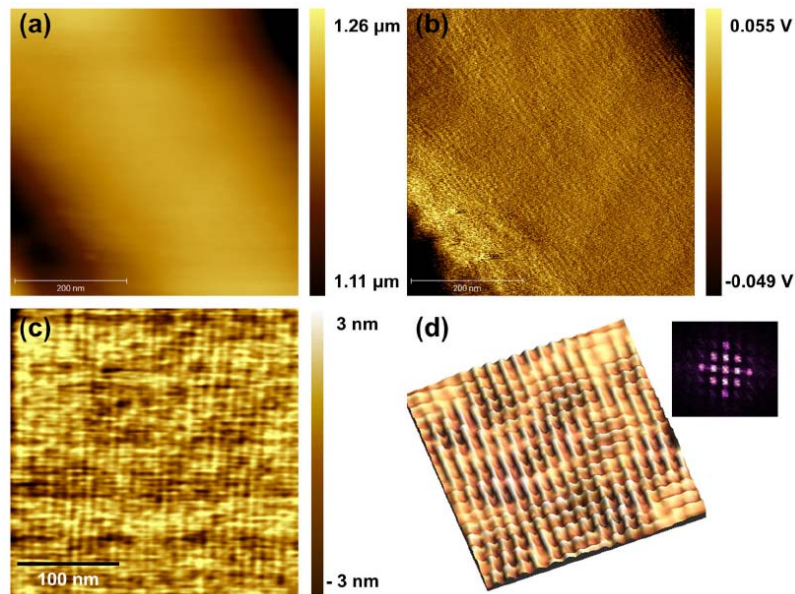


Fig.1. AFM images of *T. forsythia* wild-type bacteria: (a) Topography, (b) amplitude image, (c) magnified topographic image, and (d) reconverted FFT 3D image of topography

- [1] Y. J. Oh, Y. Cui, H. Kim, Y. Li, P. Hinterdorfer, and S. Park, *Biophysical Journal* 103, 1666 (2012)
- [2] Y. J. Oh, G. Sekot, M. Duman, L. Chtcheglova, P. Messner, H. Peterlik, C. Schäffer, and P. Hinterdorfer, *J. Mol. Recognit.* 26, 542 (2013)

# Introduction to AFM with Keysight Technologies

Dr. Gerald Kada

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*Email: Gerald\_Kada@Keysight.com*  
*Webpage: www.keysight.com/find/nano*

The unique combination of characterization and modification of molecules on solid surfaces with high resolution (nanometer and sub-nanometer scale) in all environments (air, gases, or solution) makes Atomic Force Microscopy (AFM) the technique of choice for nanotechnology measurements in biology, material science and polymer applications.

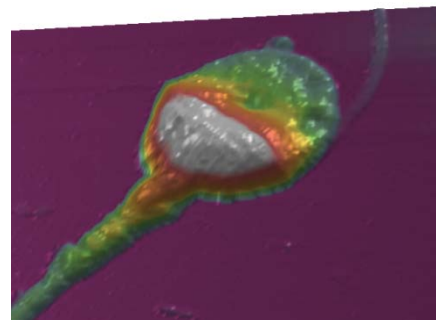
The lecture will provide an introduction to AFM measurement techniques, different operating modes and options for system setup, focusing on biological and single molecule studies.

Examples will demonstrate **IMAGING** of biological species ranging from cells to small molecules, **PROBING** single molecule properties like recognition or protein unfolding, and **COMBINATION** with other complementary techniques like fluorescence microscopy or nearfield microwaves.

1. Controlled conditions operation in air and solution: both controlled temperature (heating and cooling), controlled environment (inert or harsh, air or buffer), controlled imaging forces (MACmode) and higher harmonics imaging.
2. Topography and recognition: We will also present recent advances in the unique AFM technology called TREC, a technique that enables measuring real-time, simultaneous Topography and RECOgnition in a single scan.
3. Single Molecule Force Spectroscopy: from intermolecular forces to protein unfolding
4. AFM in combination with inverted light microscopy for simultaneously gathering of light, fluorescence, and probe data of living cells.
5. Scanning *nearfield* Microwave Microscopy (SMM) and its potential in biology



7500 AFM



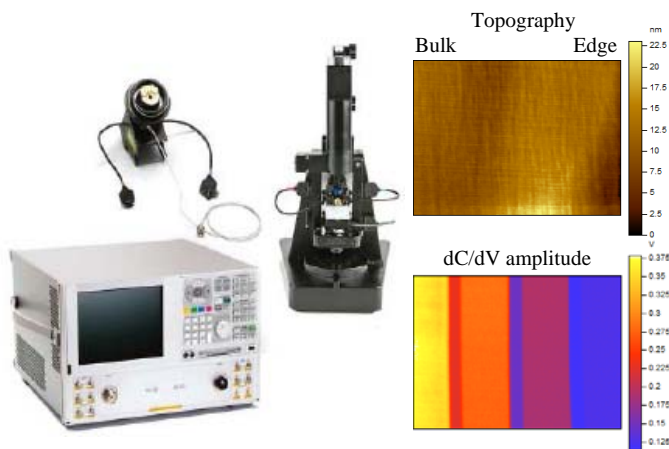
Sperm cell

# Scanning Microwave Microscopy: nanoscale complex impedance imaging, modeling, and biological applications

Enrico Brinciotti

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Scanning Microwave Microscopy (SMM) is a recent development in nanoscale imaging technique that combines the lateral resolution of Atomic Force Microscopy (AFM) with the high measurement precision of microwave analysis at GHz frequencies. It consists of an AFM interfaced with a Vector Network Analyzer (VNA). SMM allows measuring complex materials properties for nano-electronics, materials science, and life science applications with operating frequencies ranging between 1 MHz and 20 GHz. Here we present the basic working principles of SMM and its advanced applications. In particular, the capabilities of the SMM include: calibrated capacitance and resistance measurements with a noise level of 1 aF [1]; a 2D mapping workflow to acquire roughly 20.000 C-V curves during one image [3]; calibrated complex impedance imaging of semiconductor [2], dielectric [6, 7], and biological [8] samples; point wise C-V (capacitance-voltage) spectroscopy curves allowing for oxide quality characterization, interface traps, and memory effects of novel materials. Recently, calibrated complex impedance images of cells and bacteria have been obtained with the SMM [8]. Experimental investigations are complemented by finite element radio-frequency modelling using the 3D architecture of the probe and the sample, done with the Keysight software EMPro [4, 5, 9].



## Left panel:

*SMM experimental setup. The AFM is interfaced with a Vector Network Analyzer (VNA), probing the electromagnetic properties of the sample under test.*

## Right panel:

*Topography and dopant density (dC/dV) image of a semiconductor dopant sample with different dopant concentrations for quantitative and calibrated measurements.*

## References:

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# Single Molecule Biology - Studying Movements and Meetings within the Plasma Membrane

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Current scientific research throughout the natural sciences aims at the exploration of the *Nanocosm*, the collectivity of structures with dimensions between 1 and 100nm. In the life sciences, the diversity of this Nanocosm attracts more and more researchers to the emerging field of Nanobiotechnology. In my lecture, I will show examples how to obtain insights into the organization of the cellular Nanocosm by single molecule experiments. Our primary goal is an understanding of the role of such structures for immune recognition. For this, we apply single molecule tracking to resolve the plasma membrane structure at sub-diffraction-limited length-scales by employing the high precision for localizing biomolecules of ~15nm. Brightness and single molecule colocalization analysis allows us to study stable or transient molecular associations *in vivo* (1). In particular, I will present results on the interaction between antigen-loaded MHC and the T cell receptor directly in the interface region of a T cell with a mimicry of an antigen-presenting cell (2,3). We found considerably accelerated kinetics of the unbinding process compared to *in vitro* data on purified proteins; the increased off-rate was only observed with intact actin cytoskeleton within the T cell. We proposed a model, in which pulling forces applied to the TCR-pMHC bond improve the capacity of the T cell to discriminate between stimulatory and non-stimulatory ligands (4).

Moreover, we developed a method for *in vivo* micropatterning of plasma membrane proteins to measure molecular interactions (5). This technology brings together our interest in immune signaling, and the capability for ultra-sensitive readout of large biochip surfaces. Cells transfected with a fluorescent fusion protein (“prey”) are grown on micropatterned surfaces functionalized with specific antibodies to the extracellular domain of a membrane protein (“bait”); the fluorescence copatterning is used as readout for the bait-prey interaction. We applied this technology for the study of the interaction between CD4 – the major coreceptor for T cell activation – and Lck, an important tyrosine kinase in early T cell signaling. In addition to the well-known zinc-clasp structure, we found strong contributions of Lck membrane anchorage to the binding of the two proteins.

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# **Protein-Protein Interaction evaluated by confocal FRET microscopy**

Martin Muik

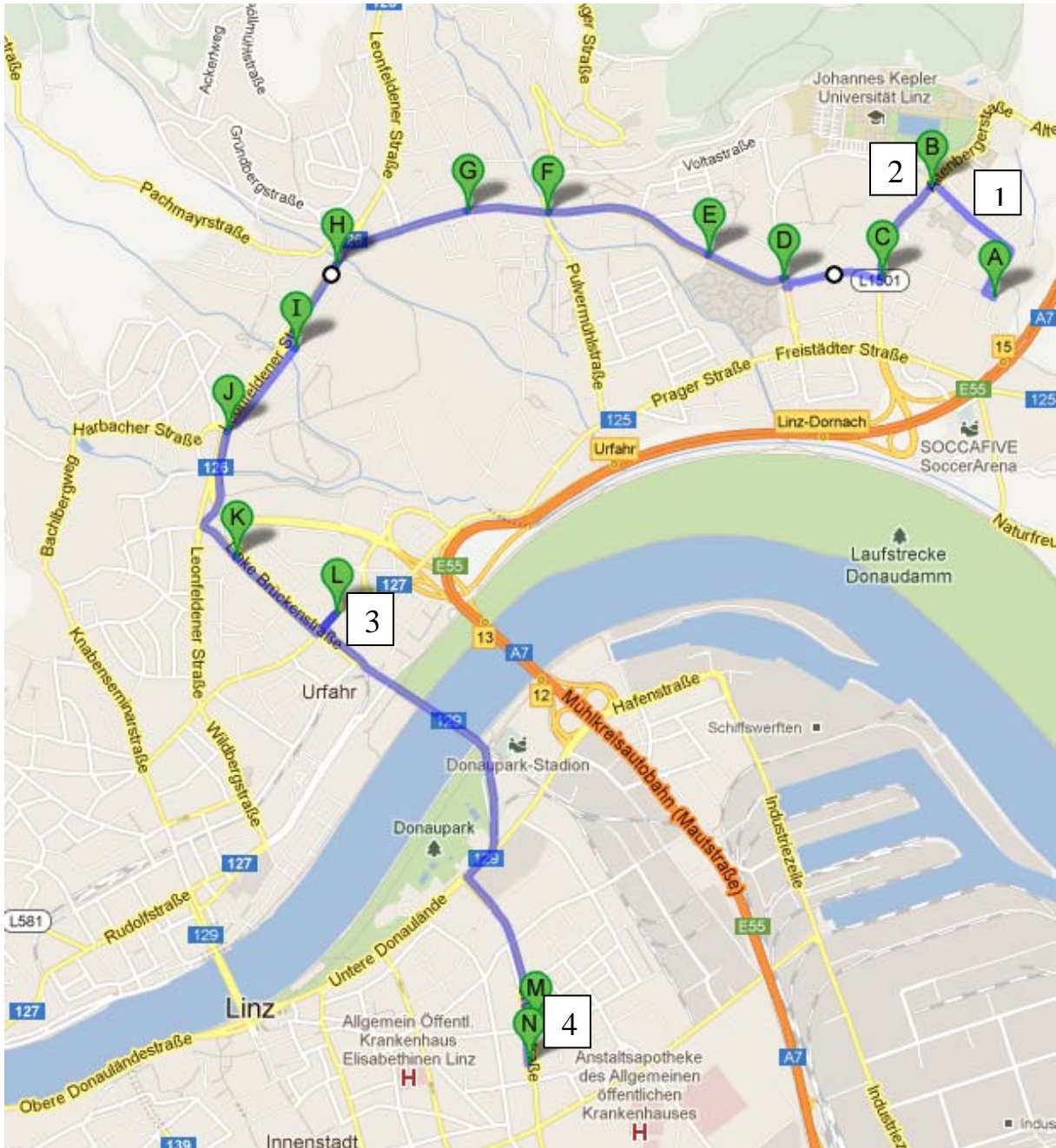
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FRET (Förster Resonance Energy Transfer) can be used to qualify and quantify interactions between biomolecules. The applications range from studying protein folding to mapping intracellular signal transduction pathways. In principle this transfer of energy from a donor fluorophore to an acceptor dye without the emission of a photon depends primarily on the distance between them and the overlap of the donor emission and the acceptor absorption spectrum. Here, FRET is determined by taking three images of the appropriately labeled specimen. Due to spectral overlap the FRET image (excitation: donor; emission: acceptor) has to be corrected. The other two images provide the basis for this correction. For the subtraction of the acceptor fluorescence directly induced by the FRET excitation the acceptor image (excitation: acceptor; emission: acceptor) is multiplied with a predetermined correction factor and subtracted from the FRET image. In analogy the donor fluorescence which bleeds through to the FRET image is eliminated by subtracting the donor image multiplied by a donor correction factor.

In this presentation the background of FRET and its implementation in our lab are demonstrated by means of the interaction between stromal interaction molecule 1 (STIM1) and ORAI1, two proteins involved in the Calcium Release Activated Calcium current (CRAC). In some cases non-excitabile cells regulate their internal calcium concentration by activating calcium influx from the extra-cellular space after releasing calcium from the internal stores (endoplasmatic reticulum; ER). STIM1 acts as calcium sensor in the ER and ORAI1 forms the ion permeating pore in the plasma membrane. ORAI1 is activated by forming multimeric complexes with STIM1 after store depletion.



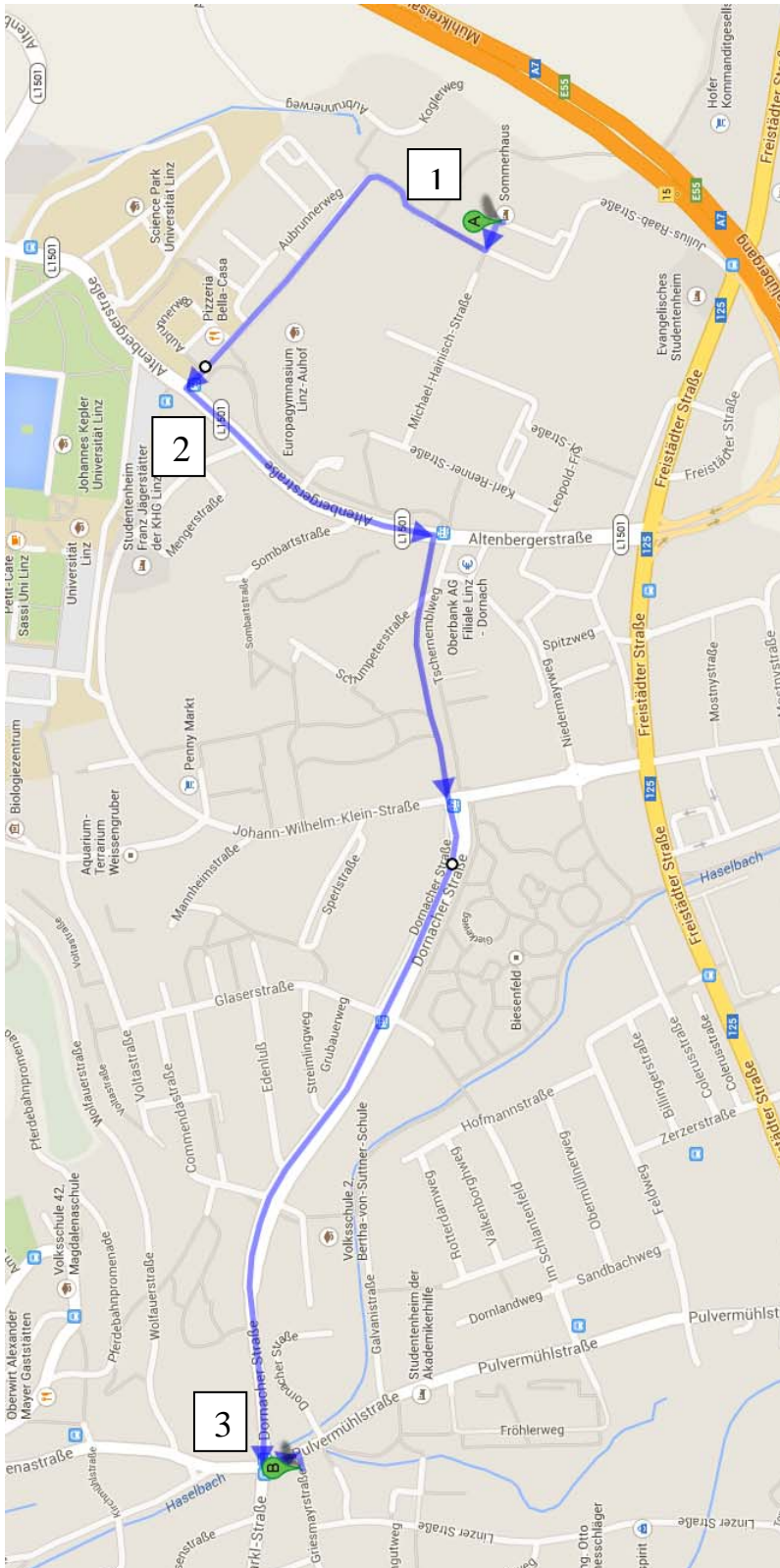
**Suggested path from Julius Raab Heim (Linz, Julius-Raab-Straße 10) to the Institute for Biophysics and CBL, Gruberstraße 40, 4020 Linz.**



- 1: Walk from Raab-Heim (A) to Tram-Station “Universität” (B)
- 2: Take Tram 1 or 2 to Tram-Station “Linke Brückenstraße” (L)
- 3: Take the Bus Nr. 12 or Nr. 25 to Bus-Station “Kaplanhofstraße” (M)
- 4: Walk about 160 m south to Gruberstraße 40, JKU Building, Institute for Biophysics (N)

## “Weinstadt”

Tram station: St. Magdalena (tram number 1 or 2)  
Griesmayrstraße 18, 4040 Linz



1. Walk from Raab-Heim to Tram-Station “Universität”
2. Take Tram 1 or 2 to Tram-Station “St. Magdalena”
3. Walk about 70 m south to Pulvermühlstraße/ Griesmayrstraße