For Z-movement we have now used a faster piezo assembly with a resonant frequency of ~18 kHz, similar to the ones we used for hair bundle deflection. Despite having a less sensitive strain gauge sensor, the vertical resolution of the system remained the same (~5nm). After adjusting the proportional-integral-derivative controller of the Z-scanner (~50µs delay) and increasing the speed of approach, we were able to obtain high-resolution images of live hair cell bundles at a frame rate of 12 min/bundle or less.
We tested the performance of the improved HPSICM system in live rat inner hair cells (IHC) and showed, for the first time in live cells, the presence of characteristic stereocilia features at an X-Y resolution of ~11nm. We also imaged IHC bundles from the *Shaker2* and *Whirler* mice due to their short stereocilia with abundant stereocilia links (typically ~5nm in diameter and ~100-300nm in length). We confirmed the reproducibility of links in continuous time-lapse scanning and also their absence after chemical disruption with BAPTA-buffered Ca^{2+}-free medium.
Our results demonstrate that the improved HPSICM technique successfully visualizes the extremely convoluted surface of stereocilia in live auditory hair cells at a high resolution and a faster speed.
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4025-Pos Board B753
Compressed Sensing Based Atomic Force Microscopy
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The Atomic Force Microscope (AFM) is a powerful tool that has had a tremendous impact on the understanding of systems with nanometer-scale features. Efforts in improving the temporal resolution of the instrument have recently yielded high-speed AFMs with frame rates of approximately 10 frames per second. These instruments, however, achieve such speeds through various trade offs and as a result have limited imaging modes and scan sizes. In addition, despite these advances, typical commercial instruments continue to have frame rates well below one frame per second.
This work develops and implements a novel sensing matrix for the application of compressed sensing (CS) to image acquisition in AFM, with the goal of improving the temporal resolution of the instrument by reducing the amount of data that needs to be acquired to create a high-quality image. In traditional CS, each measurement is, by design, a linear combination of the elements of the signal under study. In AFM however, the physics of the sensing process require that each measurement contains information about only a single point. The CS measurement matrix used here takes this into account and allows the user to balance image acquisition time against image quality. The proposed method is demonstrated through simulation. These simulations show faithful recovery with a reduction in imaging on the order of a factor of ten. By accepting a reduction in image reconstruction quality, additional gains in imaging time, up to a factor of twenty, were achieved.

4026-Pos Board B754
A Novel Platform for Simultanoues Mechanical Stimulation and Characterization of Single Cells Based on Dielectric Elastomers and Atomic Force Microscopy
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We have developed a novel set-up to simultaneously 1) apply static and dynamic deformations to adherent cells in culture 2) optically image cells under fluorescence microscopy and 3) assay the near-membrane mechanical properties with atomic force microscopy. In this system, the cell culture substrate is formed by a film of dielectric elastomer which can be eletro-actuated. The geometry and position of the actuating electrodes and the applied potential can be manipulated to obtain specific strain fields over the cell culture chamber. We have modeled the electro-mechanical behavior of the actuated elastomer film and using optical markers we have established an experimental procedure to optimize and quantify the strain at the adherent cells. This cell culture device has been integrated together with a commercial atomic force microscope coupled with an inverted optical microscope equipped for fluorescence. This novel set-up allows us to temporally assess, with sub-micron spatial resolution, single cell topography and elasticity, as well as ion fluxes, all during static or cyclically applied deformations. Preliminary results on fibrobalsts (3T3 NIH) show reproducible and reversible increase in cell elastic modulus as a response to 4% applied uni-axial stretch; additionally high resolution elasticity maps of an area of 40x40 µm on a single fibroblast could be obtained while stretching a single cell. When measuring cardiomyocites from mouse embryo, profiles of Ca^{2+} intracellular concentration could be also monitored while applying static and dynamic stretches. This study provides proof-of-concept for this set-up as a flexible experimental platform to investigate mechano-transduction mechanisms at the single cell level.

4027-Pos Board B755
High Resolution Mass Spectrometric Imaging for Single Cell Metabolic Analysis
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Mass spectrometry imaging makes possible simultaneous measurement of a large number of biomolecules of lipids, peptides, proteins, etc. from the same sample. Of special interest are ambient ionization techniques that can be carried out at room temperature in air. We report the development of a new type of ambient mass spectrometry named laser desorption ionization droplet delivery mass spectrometry (LDIDD-MS). It utilizes a pulsed laser for desorption of molecules from cells or tissue substrates. The desorbed ions are picked up and delivered with directed sprayed liquid droplets on the laser-irradiated region to a mass spectrometer. By translating desorption region on XY moving stage, two-dimensional images of the desorbed/ionized ions can be formed. As the region of desorption/ionization of LDIDD-MS is spatially limited to the laser beam spot size, the spatial resolution can be ideally reduced to several microns.
We obtained spatial resolution as low as 2.4 µm in microcontacted standard samples and ~7 µm for a pancreas tissue sample. We employed the LDIDD-MS imaging for single cell analysis and observed a significant heterogeneity in cellular apoptosis of HEK cells. LDIDD-MS also enables real-time measurement/imaging of exocytosed biomolecules in live cells. Exocytosis of neuropeptides and enzymes in PC12 upon biochemical or biophysical stimulation has been acquired and we believe that this will make it possible for use to obtain spatiotemporally resolved maps of neurosecretions at single-cell resolution.

4028-Pos Board B756
Atomic Force Microscopy Reveals the Structure and Dynamics of the Cell Cortex
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Recent studies have shown that processes such as eukaryotic cell-cell interactions, differentiation and tissue development are controlled by mechanical signals. These mechanical stimuli come from the outside of the cells and induce remodeling of the cytoskeleton in the cell interior. Unfortunately, no dynamic data at high spatial resolution could be acquired so far on the cytoskeleton of live cells, and the mechanical heterogeneity at the subcellular level remains unknown. In particular, the cell cortex is a major determinant of the cell mechanics but its spatial arrangement is poorly understood, and the dynamic behavior of its elements could only be inferred through indirect methods. Here we demonstrate that simultaneous topography imaging and mechanical mapping of live cells under physiological conditions at high resolution and low forces is possible using atomic force microscopy. We applied our methods to perform direct imaging of the cell membrane actin cortex, reaching a resolution inferior to 100nm and a maximal 10s image acquisition rate. The cell cortex is structurally, mechanically and dynamically heterogeneous at the subcellular level, and its fastest rearrangement time was in the 10s range. Our resolution enabled direct sizing the sub-membrane actin meshwork, confirming estimates from electron microscopy and molecular diffusion studies. Furthermore, we can attribute dynamic parameters to actin meshworks of various architecture and estimate the forces that they can exert on neighboring cells.

4029-Pos Board B757
Nanopipet Based Nanoprobes for Single-Cell Analysis
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Molecular Biology has advanced our knowledge of the individual molecular components that make up living cells. The challenge, however, is to fully

understand the functional integration of these components. Our group have pioneered the development of an array of new and powerful biophysical tools based on Scanning Ion Conductance Microscopy (SICM) that allow quantitative measurements and non-invasive functional imaging of the surface of living cells down to the level of single protein molecules. Scanning ion conductance microscopy and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial and temporal resolution of living cells, but also in the rich combination of imaging with other functional interrogation methods [1-3]. Recently we develop a novel class of nanoprobes based on double-barrel nanopipettes. One barrel is selectively functionalized with carbon and act as a nanoelectrode while the other one is used for ion conductance microscopy. These nanoprobes allowed simultaneous electrochemical and ion conductance imaging [4] and functional mapping of the electrochemical activity of the surface of living cells [5]. Current work in our group is directed to the use of these nanoprobes for intracellular sensing. We will present recent data demonstrating their ability to measure intracellular molecules in real time both in adherent cells and in tissue.
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4030-Pos Board B758
RNA Isolation from Single Living Cells using AFM
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Many protein-RNA complexes exist within cells and have various functions, including shuttling mRNA from the site of transcription to specific cellular regions. Often, these protein-RNA complexes consist of multiple mRNAs of which some are known and others remain unknown. The traditional method used to isolate protein-mRNA complexes is immunoprecipitation (IP). IP involves lysing a population of cells and isolating the protein-mRNA complexes using antibodies specific to the protein to "pulldown" the corresponding protein-mRNA complex of interest. We demonstrate the use of AFM as a complementary analysis tool for studying protein-RNA complexes in single living cells. While IP homogenizes the composition of protein-mRNA complexes from the cellular population under investigation and represents an "average", the AFM can interrogate not only a single cell but also subcellular regions of a single live cell, allowing stratification of protein-mRNA complexes from different regions of the cell. The result is the ability to spatially discriminate different populations of mRNAs that may be carried by the shuttling protein. To demonstrate extraction of a protein-mRNA complex, we selected the shuttling protein, Zipcode Binding Protein 1 (ZBP1) and its known associated mRNA, beta-actin, as a model system. Rat fibroblasts expressing a fusion protein, ZBP1-mCherry, were visualized using the fluorescent microscope of the AFM and then the cells of interest were punctured with an AFM tip conjugated with antibodies to ZBP1. Subsequently, the AFM tip was collected and analyzed for beta-actin mRNA using RT-PCR. BioAnalyzer results confirmed the extraction of beta-actin mRNA. This work shows that the AFM can be used as a tool to extract protein-RNA complexes from different regions of single living cells, potentially expanding the cell biologist's toolset.

4031-Pos Board B759
The Effects of Marine Bacteria on Barite Growth and Morphology
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Several studies have shown that bacteria can influence calcite ($CaCO_3$) crystal growth, although little is known about the effects of bacteria on barite ($BaSO_4$). Calcite and barite are found in comparable environments- namely under oceanic or sedimentary conditions. The purpose of this investigation is to determine the effects of a marine gram-negative bacterial strain on the growth and morphology of barite ($BaSO_4$). Using an atomic and lateral force microscopy, we established the typical crystal growth pattern and step velocity in supersaturated $BaSO_{4(aq)}$ solution. We then introduced the bacteria to the barite system and monitored changes in barite topography, friction, and step velocity. Preliminary data suggest a determinable alteration in the growth and morphology of the crystal when in the presence of the bacteria, leading us to infer that bacteria and sedimentary crystals discernibly affect one another. Future studies will connect these results to current discrepancies in oceanic mineral concentrations.

4032-Pos Board B760
Study Sub-Membrane Structure and Corresponding Functions of Conductive Bacteria Cable by SPMs
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As a kind of surface characterization tool, traditional scanning probe microscopy (SPM), especially atomic force microscopy (AFM), is hard to explore the structures underneath sample surface and correlate them with the corresponding functions. With the development of advanced AFMs, this obstacle has been overcome gradually, for example, with the application of quantitative dynamic AFM mapping, the hollow helical amyloid self-assembly fibrils have been identified;[1] and the protein structure flexibility on inner/outer sides of membrane is also possible to determine[2]. However, distinguishing the sub-surface features and corresponding function of macro-size biological samples, such as bacteria and cells, is still challenging to SPM. In this presentation, we combined quantitative dynamic AFM, AFM based manipulation, electrostatics force microscopy and scanning ion conductance microscopy to illustrate the inside membrane feature of recently identified conductive bacteria cable[3]. Basing on the SPM results, we proposed the model to explain the reason of the bacterial function to transport electrons over centimeter distance along cable direction. On one hand, this work will help biologists to understand the bacterial cable and promote the future application in nano-conductive cable field; on the other hand, it will inspire the further applications of SPM to beyond surface limitation on macro-size bio-systems.
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4033-Pos Board B761
Structural Dynamics of Proteasome: AFM Perspective
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Conformational transitions and allosteric signaling are the major contributors to catalytic activity of enzymes. The proteasome is a giant, multisubunit enzyme constituting a prime example of highly dynamic and allosterically regulated structure. This essential protease of the ubiquitin-proteasome pathway is a target of successful anti-cancer drugs. To explore alternative means of proteasome regulation, a better understanding of its mechanism is highly desired. However, its size and complexity puts it beyond the reach of most of the methods probing the global structural dynamics. Here we report results of testing dynamics of a catalytic core of proteasome with atomic force microscopy (AFM). We focused on the mechanism of elements comprising the gate controlling access to a channel that leads to a catalytic chamber internalized within a tube shaped particle. We identified conformational diversity within a single molecule of the proteasome using the oscillation mode AFM in liquid and followed the allosteric routing between the gate and active centers. We established that a gate opening and closing cycle represent activity readout of the catalytic centers. Moreover, we found that reciprocally the gate elements affect kinetics of catalysis. Now, we extended these studies by applying the high speed AFM to investigate the mechanism underpinning the very process of gate movements and position shifts of surrounding subunits. For the first time, it was possible to directly follow a complete cycle of the working gate in the live 20S proteasome with a high temporal resolution. The highest speeds of a tip reached 163 nm/ms enabled to examine changes in the gate structure occurring within 10 microseconds. Partition of the gate conformers and analysis of single molecule gate cycles detected with diverse tip speeds brings a promise to reveal details of proteasome structural dynamics not available with any other structural approach.