Attonewton Sensitivity Cantilevers for Single-Electron Spin Detection

CNF Project Number: 863-00
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Primary CNF Tools Used: JEOL 6300, SC4500 evaporator, Oxford 80

Abstract:
Magnetic resonance force microscopy (MRFM) is a scanning-probe technique that overcomes the sensitivity and resolution limitations of conventional magnetic resonance imaging (MRI) by detecting density of magnetic spins as a force or force-gradient on a high-compliance silicon micro-cantilever. One of the primary goals of this research project is to advance MRFM as a tool to image the tertiary structures of individual membrane proteins and biomolecular complexes. This report discusses developments in cantilever fabrication and sample preparation conducted at the Cornell NanoScale Facility (CNF) to enable advancements in nanoscale magnetic resonance imaging through single electron-spin detection of nitroxide spin labels.

Summary of Research:
In 2009, Moore et al. demonstrated a protocol to detect nitroxide electron radical spins, used as a label in electron spin resonance (ESR) studies of biological systems, in an MRFM experiment [1]. In this protocol, the magnetization of electron radicals was modulated, allowing the spin density to be measured as a shift in the resonance frequency of an oscillating magnet-tipped cantilever. This innovation suggests the possibility of attaching spin labels to a protein or complex at known residues, imaging the electrons, and using the locations of the spins to make inferences about the tertiary structure (Figure 1).

Detecting electron spins, rather than nuclear spins offer a distinct advantage; the large magnetic moment of electrons (660x 1H), offers increased sample polarization, and increased force, i.e. far fewer spins required to measure a signal. Detecting a single electron-spin would allow the locations of individual labels on the molecule to be observed.

The magnitude of the MRFM force-gradient signal from an individual spin is proportional to the field-gradient of the cantilever's magnetic tip. Smaller magnets have larger field-gradients, and can detect a smaller number of spins and with better resolution. For this reason, Longenecker et al. developed a batch-and-serial protocol at CNF to prepare cobalt nanomagnets on attonewton-sensitivity microcantilevers [2]. The 200 nm-diameter cobalt nanomagnets were shown to have record field gradients of ~ 5 mT/nm, significantly greater than the micrometer-scale nanomagnets used in previous MRFM ESR experiments. Nuclear magnetic resonance experiments
were able to detect a signal from just 500 proton magnetic moments, demonstrating the sensitivity required for single electron-spin detection.

Over the past year work has been done at the CNF to fabricate of attonewton-sensitivity magnet-tipped cantilevers, with magnet diameters as small as 100 nm using the Longenecker protocol (Figure 2). In this protocol, cobalt metal was evaporated onto a separate silicon-on-insulator wafer and this chip was later attached to the cantilever body. The wafer was patterned by a JEOL 6300 electron-beam lithography system and the magnetic material was deposited by electron beam evaporation in a SC4500 evaporator. A magnet overhang, ~ 200 nm, was achieved through plasma etching in an Oxford PlasmaLab 80+ RIE System with a sulfur hexafluoride and oxygen recipe. Magnet patterned chips were lifted off via ion beam milling and attached to pre-fabricated silicon cantilevers by platinum deposition using a dual beam FEI Strata 400 STEM FIB system available at the Cornell Center for Materials Research (CCMR).

The main limiting factor in the sensitivity of MRFM experiments to date has been surface noise. Varying electric and magnetic fields in the sample interact with the cantilever tip and are observed as fluctuations in the cantilever resonance frequency. These fluctuations — frequency noise — obscure the signal in force gradient MRFM measurements. Based on the frequency noise observed by Longenecker, et al. [2], we hypothesized that the surface noise was exacerbated by both charges accumulating on the sample surface and eddy currents originating in the metal microwire below the sample. To mitigate this surface frequency noise, we applied a 12 nm gold top contact over the sample using an electron beam evaporator and grounded the gold contact using wire bonds (Figure 3). We then applied a bias to the cantilever tip to minimize contact potential differences between the magnet tip and the metal surface — resulting in significantly reduced frequency noise (Figure 4). We have developed protocols to deposit gold over a narrow region of our sample using a shadow mask or over a larger surface area by taping off the sample surface using Kapton® tape.

With noise mitigation protocols in place, we are currently developing frequency radiation sources to more effectively polarize electron spins and an updated detection protocol for higher resolution electron spin imaging. Efforts are currently underway to prepare samples for a proof-of-concept single-electron spin detection experiment on a biological sample.

References:
Design and Characterization of a Microreactor for Thin Film Deposition and in situ Surface Analysis

CNF Project Number: 1239-04
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Primary CNF Tools Used: Arradiance ALD Gemstar-6, Oxford ALD FlexAL

Abstract:

Atomic layer deposition (ALD) is a technique capable of precise control of film thickness and conformal film growth due to the self-limiting nature of the precursors involved. The Engstrom research group has built a microreactor that delivers and confines reactants of ALD in a small region for deposition. This microreactor is coupled to an ultra high vacuum (UHV) chamber for surface characterization such that the deposited film is transferred in vacuo, without an air break, to the analysis chamber. The most well-studied precursor of in the ALD community, trimethylaluminum (TMA) is used to characterize the microreactor to verify whether it is able to deposit $\text{Al}_2\text{O}_3$ film in ALD fashion with water as the co-reactant.

Summary of Research:

ALD has emerged as a potential approach capable of matching the rapid rate of downscaling of semiconductor devices. The self-limiting nature of ALD precursors brings about two major advantages unique to the technique: precise control of thickness of deposited film and conformal growth [1]. As described in Figure 1, ALD is sequential binary reaction separated by purge steps to prevent any unwanted parasitic reactions between the precursor and co-reactant in gas phase. TMA, the most-studied precursor in the ALD community, is introduced to a surface in alternating sequence with water as the co-reactant. The deposited alumina film is transferred in vacuo to and characterized in a UHV chamber coupled with the microreactor using x-ray photoelectron spectroscopy (XPS).

The thickness of deposited film (~ a few nm) is often in the range that is most effectively probed with surface-sensitive characterization techniques that require UHV, $p < 10^{-9}$ Torr. Conventional ALD is typically conducted at low to medium vacuum conditions ($p \sim 10^{-3}-10^{-2}$ Torr), thus in most cases UHV based analysis of the deposited thin films occurs in a separate chamber, requiring an air break that may significantly alter the surface composition, oxidation state, structure of the deposited film, and/or underlying substrate. Avoiding this air break is critical for fundamental studies of the growth of ultrathin films, particularly in the early stages. Figure 2 describes how a sample is exposed to precursors at the upper stage and transferred down to the lower stage of the chamber without exposure to air for post-deposition characterization.

In this report, we assess the performance of the microreactor probe with various experiments. In a 10-cycle ALD experiment with $\text{Al}((\text{CH}_3)_3$ and $\text{H}_2\text{O},
we deposited an ultrathin film of alumina (Al₂O₃) on a hydroxyl-group terminated SiO₂ at substrate temperature of 180°C. From the integrated intensity of a XP spectrum of Al(2p) peak (refer to Figure 3), photoionization cross section, inelastic mean free path, and kinetic energy, thickness of alumina is calculated to be 14.2 Å [2]. Previous studies have reported growth rates ranging from 1.1 to 1.2 Å per cycle, indicating that thickness of the alumina film from ten ALD cycles using the microreactor lies close within the range of reported values [3]. In addition, elemental ratio of oxygen to aluminum is estimated to be 1.52, which corresponds to the stoichiometric ratio of elements in Al₂O₃ film.

A half-cycle ALD experiment is performed with the same precursor, Al(CH₃)₃, substrate, and temperature to verify linear correlation between thickness and number of cycles. The thickness of the ultrathin film from this experiment is 1.36 Å, approximately 9.6% of that from the 10-cycle one. This linear relationship between the thickness vs. number of cycles is a unique characteristic of ALD and confirms that the microreactor probe is capable of depositing films via ALD. The absolute atomic density of Al atom is calculated using calibration of semi-infinite Au film and methods described elsewhere [4]. Approximately 1.03 × 10¹⁵ Al atoms·cm⁻² are detected from the half-cycle experiment whereas ~ 1.02 × 10¹⁵ Al atoms·cm⁻² are present in the two top-most layers of α-Al₂O₃ <0001> surface [5].

References:
Chemical Bonding Across the Periodic Table at High and Ambient Pressures

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Primary CNF Tools Used: CNF Computer Cluster

Abstract:
We address problems of bonding, structure, and emergent properties such as superconductivity in a wide range of materials – from discrete molecules through polymers to extended solids. The behavior of matter under high pressure is of special interest, as it forms a fruitful collaboration with the group of Neil Ashcroft in Physics. The specific project addressed in 2016-2017 is the design of gold hydrides, as well as ternary gold-hydrogen-alkali metal or earth compounds with potential superconductivity.

Summary of Research:
Gold is one of few elements that does not have a known hydride in the condensed phase, and the making of one presents an extraordinary experimental challenge. A few years ago, we gained some expertise with gold in a study (also done with CNF resources) metastable AuO [1]. In the present work, our structure searches (using CNF computing resources, in last year’s report) began by showing that there are no stable binary phases of gold hydride below 300GPa.

More recently, we have concluded a study that comes to a more positive outcome, predicting specific new alkaline/alkali-gold hydride ternaries [2]. One of these, KAuH₂, which calculates as thermodynamically favorable with respect to the elements and other decomposition products are shown in Figure 1. We have also used CNF resources to validate our approach by confirming the experimentally known ground states of related A₂PdH₂ (A=alkali metal) ternaries [3].

![Figure 1: Calculated 3-component phase diagram of K, Au and H as T → 0K. [Red] squares denote unstable compositions (above the convex hull). Black lines between [green] circles connect stable phases. KAuH₂ is predicted to be stable with respect to decomposition into all binaries considered here. In addition to running structure prediction calculations, we are also using the CNF cluster to study the behavior of these phases over a range of pressures, to modulate their conductivity, band gap, and relative heat of formation. The mix of light hydrogen and heavier gold gives rise to both high and low frequency phonons in the lattice, which is interesting since electron-phonon coupling is a key ingredient in BCS superconductivity, which we are trying to understand. Three superconducting compounds have thus far been identified following CNF-supported structure searching: KAuH₂\(_x\) (\(T_c = 0.3 K \at \120GPa\)), Ba(AuH)\(_x\)\(_y\) (\(T_c = 30K \at 1atm\)), and Ba(AuH)\(_x\)\(_y\) (\(T_c = 10K \at 1atm\)) [2]. The predicted structure of one of these, Ba(AuH)\(_x\)\(_y\), is shown in three views in Figure 2.](image)
Figure 2: Three views of predicted I4 structure of metastable Ba\(\text{AuH}_2\), at 1atm. Large balls are Ba, medium ones Au, smallest ones H. In the view at right, the Ba-H sublattice is highlighted as prisms centered with Ba ions.

References:
Substrate Preparation for Ultrafast Vibrational Spectroscopy Experiments

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Abstract:
Water is ubiquitous and an active component in many natural and technological processes. Vibrational spectroscopy can be used to probe the structure and dynamics of water in a variety of environments. We use sum-frequency generation, a surface specific vibrational spectroscopy, to probe the structure and dynamics of interfacial water at chemically tunable surfaces. Interfaces with tunable surface character are created with self-assembled monolayers. In order to create substrates compatible for both silane self-assembly and sum-frequency generation, infrared and visible transparent CaF$_2$ windows are coated with SiO$_2$. Then after surface functionalization with silane monolayers, the water structure and dynamics at the interfaces can be explored with sum-frequency generation.

Summary of Research:
Water is an active component in many natural and technological processes [1]. Interfaces terminates the H-bonded network of water. We aim to study the structure and dynamics of water at self-assembled monolayers (SAMs) with varying surface character using sum-frequency generation (SFG) spectroscopy. In SFG, an infrared photon interacts with a dipole transition of the molecule and a visible photon excites the molecule to a virtual electronic state where it can undergo an anti-Stokes Raman transition resulting in a photon at the sum of the two incident frequencies being emitted [2,3]. In order to collect SFG spectra of solid-aqueous interfaces, we must probe through the window so the infrared photons are not absorbed by water. However, silica, a common SAMs substrate, also absorbs in the infrared.

To create an infrared and visible transparent substrate compatible with SAMs syntheses, we start with a CaF$_2$ window, which is transparent through the visible and infrared. Then approximately 10 nm of SiO$_2$ is deposited on the CaF$_2$ window via atomic layer deposition (ALD) with the Oxford ALD FlexAL. The SiO$_2$ layer is thin enough to not absorb all the IR photons and prevent SFG spectra of the sample from being collected, but thick enough to form a surface compatible with the self-assembly of silanes.

Once the SiO$_2$ is deposited, hydrophobic, hydrophilic, or mixed monolayer are synthesized with self-assembly of silanes on the surface. Figure 1 shows a schematic of the surface in contact with water. Then, the surfaces and water at the surfaces are analyzed with SFG [2,3].

References:
2016–2017 Research Accomplishments
Biomechanics of Bacteria

CNF Project Number: 1970-10
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Primary CNF Tools Used: ASML, Oxford 100, VersaLaser, MOS clean anneal

Abstract:
The mechanical properties of the bacterial cell envelope influence cell growth, cell division and subcellular localization of membrane proteins. Here we demonstrate the ability to apply mechanical loads to live bacteria, the first step toward determination of mechanical properties of bacterial components in vivo. Additionally, we show that devices based on the same concept have the ability to separate bacterial species/strains from one another based on the cell mechanical phenotype.

Summary of Research:
In bacteria, the ability to resist mechanical forces is necessary for survival and growth, allowing cells to withstand osmotic pressures while maintaining cell shape, cell growth and division. Hence, the mechanical properties of bacteria and bacterial structural components influence species competition and resistance to toxins and antibiotics.

Our work involves the use of micro/nano fabricated devices as tools for mechanical testing of live bacteria. Within our devices individual bacteria are flowed into tapered channels and trapped at points within the channels based on whole cell stiffness in which less stiff cells are able to travel further in to the channels (Figure 1). Key advantages of this microfluidic platform for profiling the biomechanical properties of bacteria include: minimal sample preparation, no chemical immobilization or labeling, and the ability to analyze hundreds of cells at once.

In our first series of experiments we manufactured devices on silica glass wafers using deep UV photo-

Figure 1: Bacteria under fluid pressure (p) are forced into tapered channels. The distance a cell travels into a tapered channel depends on cell stiffness with more compliant cells traveling further into the channels. The distance traveled by a cell into the tapered channel (d₁) is therefore an indicator of cell stiffness. Viewing the deformation of a cell under two different applied pressures can be used to determine the mechanical properties of the cell envelope.

Figure 2: (A) The position of bacteria occupying trap channels at twelve different pressure levels (where level 1 is lowest and level 12 is greatest) in a single experiment are shown. Horizontal lines indicate averages at each pressure level. E. coli travel further into the traps than B. subtilis overall (p < 0.0001, ANCOVA) as well as at each individual pressure level (p < 0.0001, t tests). (B) Differences in bacteria stiffness between species can be detected in a mixed culture. E. coli expressing GFP ([green], indicated by horizontal arrows) traveled further into the trap channels than B. subtilis (indicated by tilted arrows), demonstrating the possibility of separating bacteria based on mechanical phenotype.
lithography to achieve nano-scale features (250 nm smallest dimension). These glass on glass devices were manufactured using the ASML, Oxford 100, VersaLaser and MOS clean anneal tools at the Cornell NanoScale Science & Technology Center.

In the first device design, cells from a population are submitted to up to 12 different applied pressures was used to establish the biomechanical profile of two model organisms, *E. coli* and *B. subtilis*.

Our results demonstrated differences in stiffness between *E. coli* and *B. subtilis* (Figure 2) and suggest that a device with a shorter channel length would allow transport of *E. coli* but not *B. subtilis*, potentially allowing for separation of bacteria based on the biomechanical properties [1]. When combined with theoretical mechanics models, it allows us to determine the stress distribution within individual bacteria and study their response to mechanical stimulation [2]. In our recent work, we have explored the effects of mechanical loads on the assembly/disassembly of multicomponent efflux pumps. Multicomponent efflux pumps are three part channels that cross the inner membrane, periplasm and outer membrane of bacteria and are used to remove toxins (excessive metal ions, antibiotics, etc.) [3].

Our preliminary data suggests that the assembly and function of multicomponent efflux pumps is sensitive to mechanical stress and strain, in particular shear stresses across the cell envelope appear to impair the function of efflux pumps while hydrostatic stresses tend to promote the function of efflux pumps.

References:


Figure 3: (Top) A single mechanical testing device is shown with arrows indicating direction of flow. Bacteria trapped within the tapered channels are visible in bright field images. PALM microscopy is used to visualize a photo switchable membrane protein, making it possible to view the cell morphology and measure changes in cell shape in response to changes in flow rate/pressure.
Micrometer-Scale Coplanar Waveguides for Nanoscale Magnetic Resonance Imaging

CNF Project Numbers: 2125-12, 863-00
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Primary CNF Tools Used: Gamma automatic coat-develop, ASML 300C DUV stepper

Abstract:
We report on the design, fabrication, and characterization of broadband micrometer-scale coplanar waveguides capable of delivering millitesla strength magnetic fields on a 200 milliwatt power budget with minimal sample heating. These waveguides, operating from DC up to 40 GHz, have been integrated into a cryogenic scanning probe microscope to enable the detection of electron spin resonance and nuclear magnetic resonance in a single force detected magnetic resonance experiment for the first time. Additionally, the ability to irradiate both nuclear and electron spins with a single device allowed for the first mechanical detection of hyperpolarized proton magnetization achieved via cross-effect dynamic nuclear polarization [1]. These enabling advances are the first steps toward achieving three-dimensional, nano-scale magnetic resonance imaging (nano-MRI) using magnetic resonance force microscopy.

Summary of Research:
Having a universal platform for imaging individual biomolecules or biomolecular complexes with isotopic specificity and nanometer or sub-nanometer resolution would be an enabling advance for a variety of scientific disciplines. By detecting magnetic resonance as a force on an attonewton-sensitivity microcantilever (Figure 1), magnetic resonance force microscopy (MRFM) offers the sensitivity and depth-of-view required for three-dimensional, nano-MRI. While recent advances in MRFM have demonstrated the ability to perform 3D imaging of a single virus particle with < 10 nanometer resolution [2] and the sensitivity required to detect a few hundred proton magnetic moments with a record-high gradient magnet tipped cantilever [3], these experiments required hours or days of signal averaging as a result of detecting statistical polarization or random spin fluctuations commonly referred to as ‘spin-noise’. To achieve greater imaging resolution, while decreasing signal averaging time, our approach was two-fold: (1) increase nuclear spin polarization to generate a well-defined polarized spin signal and (2) develop a universal sample platform in which biological samples can be deposited for single electron spin imaging. The enabling advance for both experiments was a broadband coplanar waveguide (CPW) to deliver radiofrequency waves for nuclear magnetic resonance (NMR), microwave frequency irradiation for electron spin resonance (ESR) and combining these techniques to transfer polarization from nuclear spins to electron spins via dynamic nuclear polarization (DNP). The challenge in the development of these CPWs was to achieve a high (millitesla) strength oscillating magnetic field, from a few megahertz to tens of gigahertz while operating on the < 200 milliwatt power budget of our microscope to avoid sample heating under operation at 4.2 kelvin. The CPWs were designed and simulated (Sonnet) to have a 50-Ω characteristic impedance.
throughout the device. With this design constraint, the waveguides could be coupled via multiple wire bonds to a ceramic coplanar waveguide board equipped with SMA connectors for use with commercial radiofrequency (rf) and microwave (MW) signal generators. To achieve the millitesla rf magnetic fields necessary to invert nuclear spin polarization and microtesla MW magnetic fields required to saturate electron spin resonance, the coplanar waveguides were designed to maintain a 50-Ω impedance while the centerline tapered from a 480 µm wide wire with a 230 µm wide gap to a 5 or 10 µm wide wire with a 3 µm or 6 µm wide gap, respectively. This constriction generated a high current density in the microwire capable of producing magnetic fields up to 5 millitesla for rf waves and a few microtesla for MW frequency irradiation with just 200 mW of input power. Additionally, this coplanar waveguide has demonstrated losses of just a few milliwatt across the device meaning sample, heating at cryogenic temperatures is negligible.

Furthermore, patterned silicon gridlines, seen above and below the CPW microwire in Figure 2, have been implemented into the ground plane of the coplanar waveguide to assist in the alignment of the cantilever and microwire under vacuum at temperatures down to 4.2 kelvin. Additional optical features may be added in future biological imaging experiments to rapidly locate specific molecules of interest.

In addition to using CPWs coupled to commercial frequency generators as a universal sample platform, we have further developed coplanar waveguides to be coupled to cryogenic chip scale microwave sources developed by Prof. Ehsan Afshari and coworkers (Figure 3). Coupled to our coplanar waveguides, these 36 GHz CMOS oscillators generate > 300 µW of output power at temperatures down to ~ 12 kelvin. Simulations show that this should be sufficient to saturate electron spin resonance in our magnetic resonance force microscope. This unique combination of CMOS oscillator and coplanar waveguide would be the first of its kind integrated into any cryogenic scanned probe microscope experiment.

References: