

SPM Workshop

19 July 2010, SILC Building

Morning Session

Micheal Higgins

9.30 am *Probing Single Cell and Protein Interactions at Conducting Polymer Interfaces using Atomic Force Microscopy*

Raymond R. Dagastine

10.10 am *The Visualization of Dynamic Forces in Soft Matter using Atomic Force Microscopy*

10.50 am Morning tea (30 mins)

Christopher T. Gibson

11.10 am *Attaching Nanostructures to Atomic Force Microscope Probes*

Victoria A. Coleman

11.50 am *Probe-based nanoparticle metrology*

John E. Sader

12.30 pm *Dynamics of Cantilever Devices in Fluid Environments*

1.10 pm Lunch (1 hour)

Afternoon Session

Ruth Zoehrer

2.00 pm *AFM-based nanoindentation on human fragility fractured bone as a function of bone mineral density distribution*

Yanyan Liu

2.20 pm *Microscratch characterization using semi-contact AFM*

Daniel Tune

2.40 pm *Single Walled Carbon Nanotube Network Electrodes for Dye Solar Cells*

Cathal O'Connell

3.00 pm *Probing Dynamic Nanomechanical Properties of Single Living Cells at Biomaterial Interfaces using Atomic Force Microscopy*

3.20 pm Afternoon tea (20 mins)

Diana Pham

3.40 pm *Multi-scale characterisation of collagen type I tensile material properties within the intervertebral disc*

Brad Simons

4.00 pm *Modification of non-conducting substrates using diazonium cation chemistry*

Rhiannon Creasey

4.20 pm *Supramolecular assembly of arabinogalactan-like proteins*

Amy Gelmi

4.40 pm *Measuring the Adhesion of Proteins on Conducting Polymer Surfaces as a Function of Dopant and Electrical Stimulation*

5.30 pm Dinner

Probing Single Cell and Protein Interactions at Conducting Polymer Interfaces using Atomic Force Microscopy

Michael Huggins

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University of Wollongong*

The surfaces of traditional biomedical implants employ a single material, or surface chemistry, with the intent of presenting an effective wetting behaviour or topography to enhance biocompatibility. However, these coatings are static, have limited function and very few have the ability to enhance desired biological functions, like promoting adhesion of certain cell types, while also decreasing selective proteins and bacterial adhesion. The design of biocompatible surfaces has been a perpetual challenge (e.g. to prevent bacterial infection), but is ever more critical as the worldwide production of biomedical devices and related materials rapidly expands into a \$150 billion per year industry. Any new strategies to this problem must shift the focus from single materials to dynamic or tunable interfaces, capable of facilitating multiple biological processes. Such growing demands in industry have turned to nanotechnology to develop complex surface architectures and/or biodegradable polymers for the release of chemicals. Switchable polymer coatings that can flip back and forth their properties under the influence of external stimuli, such as temperature, pH, light and electricity, are also of great interest in the area. The challenge will be to exploit these sophisticated and switchable materials without unduly complicating large-scale production for clinical research. To successfully incorporate these switchable interfaces into a device, one must elucidate the different levels of cell-material interactions responsible for mediating biocompatibility and tissue growth. These interactions include non-specific cell membrane-material interactions, adsorption of blood serum and cell-secreted proteins to the material, and cellular interactions with adsorbed proteins and chemical factors that facilitate cell adhesion and growth. Furthermore, the ability to control these interactions and processes, for example by turning them 'on' or 'off', via switching of the interfacial properties is envisaged in the development of next generation biomaterials and coatings.

In this presentation, we highlight our current research aimed at designing in vitro and in vivo scaffolds for the regeneration of damaged nerve and muscle tissue. As a part of the design process, a variety of surface characterisation techniques such as Atomic Force Microscopy (AFM) and Electrochemical-AFM (EC-AFM) have been used to determine the interactions of proteins and living cells with conducting polymer substrates as they undergo electrical stimulation. In particular, AFM probing of single living cells will be presented to show how the nano-mechanical properties of single cells respond to the electrically stimulated conducting polymer substrates.

The Visualization of Dynamic Forces in Soft Matter using Atomic Force Microscopy

Raymond R. Dangastine

*Department of Chemical and Biomolecular Engineering and
the Particulate Fluids Processing Centre
The University of Melbourne*

Atomic Force Microscopy (AFM) has enabled an explosion in the amount of research focused on the visualization of materials at the nano-scale. Twenty years after its invention, many have expected these measurements to become as routine as using a toaster, yet in many applications the use of AFM is still more attune to playing a violin. This is certainly true in the visualization and study of the dynamic interaction forces on the nano-scale in soft matter materials. These dynamic interactions on the nano-scale between objects (particles, droplets, bubbles and other soft matter materials including living cellular systems) mediate or control behaviour on the macroscopic scale in complex fluids (e.g. emulsions, foams and particle suspensions). Our research has focused on the development of innovative methods to quantitatively study the dynamic forces between drops, bubbles, particles and cells on the nano-scale.

This talk will focus on the complexities involved in measuring the collision and coalescence of micro-bubbles. The dynamic forces between micro-bubbles are crucial for understanding applications as wide ranging as foams in mineral processing to ultrasound contract agents. We employ gas bubbles in water, the simplest and arguably the most shape sensitive soft matter systems, to quantitatively link the dynamic coupling of bubble shape with external forces that control their behavior. We have extended the experimental and theoretical methods developed to study the dynamic interactions between droplets using Atomic Force Microscopy (AFM) to study the collisions and coalescence behavior of two micro-bubbles in aqueous solution. These measurements show that bubble collisions, even at speeds comparable to Brownian motion, are dependent on a combination of equilibrium surface forces, hydrodynamic drainage forces and interfacial deformation. These results have implications in applications as wide ranging as froth floatation to micro-fluidics.

Attaching Nanostructures to Atomic Force Microscope Probes

Christopher T. Gibson

School of Chemical and Physical Sciences, Flinders University

The geometry and durability of atomic force microscope (AFM) probes is critical to the instruments' resolution and performance. In recent years a number of new methods have been developed to improve data acquired using AFM by attaching carbon nanotubes (CNT) to AFM tips. CNTs are ideal since they have a diameter typically less than standard silicon or silicon nitride AFM probes, high aspect ratio, high strength and virtually no wear. One technique that has been developed to attach CNTs to AFM tips is known as the pick-up method. The pick-up technique involves scanning a tapping mode probe in air across a CNT bearing surface until a nanotube attaches to the tip. When stable CNT attachment is achieved an abrupt improvement in image resolution can be observed and maintained. CNT attachment can then further be verified by performing dynamic force curves or by using scanning electron microscopy (SEM). In this work we will investigate methods to improve the stable attachment of CNTs to single and multiple AFM probes as well as attaching CNTs to contact mode probes which, until now, has yet to be achieved using this technique. We will also demonstrate, for the first time, that these improved pick-up methods can allow nanostructures other than CNTs, such as ZnO nanorods, to be attached to AFM probes.

Probe-based nanoparticle metrology

Victoria Coleman

Nanometrology, National Measurement Institute, Lindfield

Accurate and precise measurements are of critical importance in nanotechnology to support and control manufacturing, enable research and development, and understand and manage potential health, safety and environmental risks. The Nanometrology section of Australia's National Measurement Institute (NMI) is currently developing capabilities for the traceable measurement of nanoscale systems, in particular, of particles. Scanning probe microscopy forms the basis of traceable nanoscale length measurements, and probe-based techniques are also highly suited for particle measurement. As such, they form a significant portion of NMI's particle characterisation activities. In this talk I will present two techniques under development at NMI. The first is a metrological scanning probe microscope, which will enable traceable dimensional particle characterisation through the use of interferometers to monitor stage displacement in three dimensions. The second technique is a relatively new instrument based on a microfluidic microresonator which uses a cantilever to weigh individual nanoparticles. Preliminary results from this instrument with a range of different particles will be presented, and compared with other more traditional particle characterisation techniques such as dynamic light scattering.

Dynamics of Cantilever Devices in Fluid Environments

John Elie Sader

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The University of Melbourne*

The dynamic properties of microcantilevers underpin numerous applications in sensing, force measurements and imaging. Due to its relevance to biological and colloidal systems and sensing applications, there is growing interest in the application of dynamic AFM methods to the quantitative determination of forces in fluid environments. While operation in fluids presents no conceptual difficulty, additional complexity arises since the dynamic properties of microcantilevers are strongly dependent on the surrounding fluid.

In this talk, I will give an overview of recent work in my group dealing with the behaviour of cantilever devices in the presence of fluid. This will cover three topics. The first will discuss recent developments focusing on cantilever sensors with embedded microfluidic channels, which display dramatic reduction in energy dissipation, i.e., enhanced quality factor. The underlying physical mechanisms behind this phenomenon will be presented. Second, I will discuss theoretical work aimed at exploring the behaviour of cantilever devices immersed in fluid that are operating in their higher order modes. This inherently accounts for the three-dimensional flow generated by such devices. Finally, I will present work dealing with the effect of surface stress on the stiffness of cantilever sensors. These exhibit fundamentally different behaviour to the commonly assumed axial force model, as I will discuss. These works are aimed at developing a theoretical framework for quantitative measurements using cantilever sensors in fluid environments

AFM-based nanoindentation on human fragility fractured bone as a function of bone mineral density distribution

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The mechanical competence of bone is characterised by a complex interplay of structural and material variables from the nano-scale up to the macro-scale level, where the latter one has been investigated extensively in the recent years. The purpose of this study was to describe the nano-level material properties of trabecular bone in fragility fractured (FF) patients and age-matched control (CTL) specimens. AFM-based nanoindentation was used to compare nano-mechanical properties to the bone mineral density distribution (BMDD) obtained via backscattered scanning electron microscopy (BSEM). The AFM-based nanoindentation was performed on anatomically equivalent areas, showing explicit primary mineralization (forming) or no newly formed bone (resorbing). The BSEM results showed that the FF individuals are significantly under-mineralised relative to the control cohort ($P < 0.01$). The BMDD curve of the FF-group exhibited a wider curve, characterising a more homogenous mineralisation, indicated in the BSEM images by lower density packets along the trabecular surface. The AFM-based nanoindentation showed no differences in hardness (H) as well as elastic modulus (E) in the FF-group in resorption sites when compared to controls. In contrast, significant differences could be observed in areas undergoing new bone formation in H as well as in E ($P < 0.001$). Interestingly the FF-group exhibited increased H but decreased E compared to the CTL-group, which may be due to a change in the properties (i.e. remodelling) in response to the increasing number of micro-fractures in the tissue. Further investigations are needed to be able to identify material and structural factors at the nano-scale level influencing bone strength.

Microscratch characterization using semi-contact AFM

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Minute micrometer-scale wear particles can be problematic in many technological applications of polymers such as ultra high molecular weight polyethylene. Wear particle production mechanisms have been studied on samples of this material using microscratching. Using AFM the polymer surfaces and the morphology of scratches have been imaged. Furthermore cross-sections of scratches have been quantitatively depth-profiled. A NT-MDT instrument and semi-contact mode was used. The three-dimensional spatial sensitivity of AFM allows for detailed volumetric measurements of scratch grooves and of debris particles not readily achievable with electron microscopy. Cantilevers (NSG 01) with cone angles of about 22 degrees, a curvature radius of 10 nm, a force constant of the order of 2.5-10 N/m, and a resonance frequency between 115-190 kHz were used. Scratch depths of the order of 5 micrometers can reliably be measured. The AFM characterization is crucial in identifying wear mechanisms such as 'wall' formation in intersectional scratching which is a precursor of debris particle detachment.

Single Walled Carbon Nanotube Network Electrodes for Dye Solar Cells

*Daniel D. Tune, Benjamin S. Flavel, Jamie S. Quinton, Amanda V. Ellis and Joseph G. Shapter
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The photovoltaic properties of a new working electrode for dye sensitised solar cells, consisting of networks of covalently bound single walled carbon nanotubes on indium tin oxide, have been investigated. Following covalent sensitisation of the carbon nanotube networks with a ruthenium dye an appreciable cathodic photocurrent is measured upon illumination with simulated sunlight. By building up sequential layers of carbon nanotubes cross-linked with ethylenediamine to form a three dimensional dye sensitised single walled carbon nanotubes network significant increases in photocurrent density are observed. Such electrodes are promising for the future fabrication of low cost, minimal material use solar cells.

Probing Dynamic Nanomechanical Properties of Single Living Cells at Biomaterial Interfaces using Atomic Force Microscopy

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Conventional techniques assay cellular response to a biomaterial at the microscopic, population level. However, as bionics enters the nano-domain, so its tools of characterization must become nano-sensitive. Optimising the nanobionic interface requires unprecedented focus on the level of individual cellular response to mechanical, chemical and electrical cues. Biological-Atomic Force Microscopy represents a powerful, multifaceted technique for the acute observation of individual biological events.

In the present study, living C2C12 skeletal muscle cells have been imaged using Bio-AFM in physiological fluids at 37°C. The AFM tip has been utilised as a mechanical probe to make indentation measurements of mechanobiological properties with piconewton sensitivities. These properties are significant as many cellular processes, including differentiation[1] and responses to chemical stimulus[2] and mechanical environment[3], are often accompanied by changes in cell mechanical properties. The elasticity of nominated living cells has been probed throughout adhesion. As an investigation into the usage of cytomechanics as a novel biomarker to assay cellular response to controlled drug release, cell elasticity was probed as a function of growth factor introduction to the cell medium. Results have been correlated with cell height, cell spread-area and rate of cell migration. Future work will probe cellular mechanics on soft nanopatterned biomaterial substrates of varying stiffness.

References

- [1] A.M Collinsworth, S. Zhang, W. E. Kraus, G. A. Truskey, *Am J Physiol Cell Physiol* 283: C1219–C1227, 2002.
- [2] C. Rotsch, M. Radmacher, *Biophysical Journal* 78, S20-S35 (2000).
- [3] T. Yeung et al., *Cell Motility and the Cytoskeleton* 60:24–34 (2005).

Multi-scale characterisation of collagen type I tensile material properties within the intervertebral disc

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The intervertebral disc is located between the vertebrae of the spinal column, and serves as a strong, flexible junction that cushions and redistributes the complex, multi-directional forces transmitted through the spine.

Presently, a finite element model of the intervertebral disc that can adequately describe this loading response is lacking. Such a model could be used to predict the behaviour of the disc without requiring invasive monitoring or in vitro testing. Current models are based upon the macro structure of the disc; it is hypothesised that the key to a more accurate model could be the inclusion of the disc's characteristics at nanoscopic and/or microscopic levels.

Collagen type I is a major component of the annulus fibrosus, the fibrous, cross-ply, layered region that lies on the periphery of the disc, and is a strong contributor to the annulus' overall mechanical behaviour. This research aims to investigate the mechanical behaviours of collagen type I at different hierarchical levels in the annulus fibrosus (i.e. fibril and fibre), and through comparisons between healthy and diseased specimens, possibly shed some light on the aetiology of disc degeneration.

Using atomic force microscopy, collagen type I fibrils from the human annulus will be tensile tested with the aid of anti-collagen-functionalised tips. The preliminary results of this research will be presented, namely topographical images of collagen type I from sheep annulus at different structural levels, and force volume images comparing unaltered and functionalised tips.

Modification of non-conducting substrates using diazonium cation chemistry

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Surface modification is an effective way of tailoring the surface properties of a substrate while maintaining the bulk properties of the material.

Aryl diazonium salts are widely used for grafting thin (usually less than 10 nm) organic films to a variety of surfaces. These films are generated by forming a reactive aryl radical in solution by reduction of the diazonium cation. This reactive species forms a covalent bond with the substrate surface.

An advantage of this modification strategy arises from the stability of the grafted film due to the strong covalent bonding to the surface. Grafting can be carried out using electrochemical, chemical or spontaneous reactions on a variety of substrates including carbon, industrial metals, noble metals, silicon, plastics and glass.

This research is concerned with investigating chemical grafting of diazonium cations onto a variety of non-conducting substrates including glass, PTFE and other polymeric materials. This is carried out by adding a chemical reducing agent to a solution of the diazonium cation, promoting the formation of the aryl radical. The grafted layers are characterised using techniques including immobilisation of gold nanoparticles, measurement of water contact angles, and AFM depth-profiling. The latter technique gives information about the thickness of the organic layer by removing a section of the film by scratching with the tip of an AFM cantilever. The trench can then be profiled by conventional tapping mode.

Supramolecular assembly of arabinogalactan-like proteins

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Protein aggregation is of significant interest to various disciplines, from clinical understanding of Alzheimer's disease to the formation of novel functional biomaterials. The symbioses of arbuscular mycorrhizal (AM) fungi in root apoplasts are thought to be carried out by the formation of protein structures via aggregation. Identification and modelling of three arabinogalactan-like (AGL) proteins expressed at the cell-surface interface of AM fungi, *Glomus intraradices* (1), suggests protein aggregation formation designed to assist AM fungi colonise plant roots to form this wide-spread symbiosis.

Atomic force microscopy (AFM) is a surface-sensitive technique developed in the 1980s (2), and is one of the foremost tools for the study of surfaces at a nanoscale. Here, we use AFM to investigate the supramolecular assembly of GiAGL1 and GiAGL3 in a variety of environments.

The structural properties of these proteins are vital to the understanding of this symbiosis, but also represent an opportunity for the development of a novel, controllable self-assembling biomaterials.

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Measuring the Adhesion of Proteins on Conducting Polymer Surfaces as a Function of Dopant and Electrical Stimulation

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Conducting polymer films are currently being studied for a wide range of biomaterial applications, hence the biocompatibility of these materials is very important. During biocompatibility studies protein coatings can be put down onto the surface which will enhance cell adherence, and subsequent proliferation and differentiation.

However, is this a clear indicator of a biocompatible material?

By functionalizing AFM probes with specific proteins we can directly measure the protein adhesion force to the naked conducting polymer surface, which will provide a more definitive indication of cell adherence. Proteins such as fibronectin (FN) and vitronectin (VN) are produced by cells to form an extracellular matrix which enable the cells to bind to a surface in culture and so are excellent proteins to work with in this study.

The conducting polymers used in this study are a set of polypyrroles doped with different biochemicals, such as hyaluronic acid and chondroitin sulphate. The adhesion force between the protein functionalized probes and polymer surface was then analysed as a function of dopant and also as a function of electrical stimulation.

This study hopes to reveal more about the interaction between cells and biomaterial surface and the influence from the chemical advantage of the dopants used. As electrically stimulating biomaterials is also an area undergoing intense research, as a way to enhance the success of cell growth, by measuring changes in the surface-protein adhesion during stimulation will help us understand the effect of electrical stimulation on cell adhesion.