December 9, 2003

California Institute of Technology
Applied Physics Department, MC 128-95
1200 E. California Blvd.
Pasadena, CA 91125

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

Dear Search Committee:

Enclosed please find my application materials for the Experimental Biophysics faculty position at the Biocomplexity Institute at the University of Indiana. I am very excited about the prospect of joining this innovative institute, and I am eager to initiate the research objectives I have outlined. In particular, I intend to use scanning probe techniques to study and control molecular-scale biological systems. Within, I have briefly described my research as a postdoc and Beckman Senior Research Fellow in the biophysics group of Prof. Stephen Quake at Caltech, where we developed a scanning-probe fluorescence microscope with spatial resolution below 10 nm. I believe this experience has been excellent preparation for my future endeavors at Indiana.

The enclosed materials include my CV and an outline for my planned research activities. In addition, I have asked those listed as references to send their letters to the above address. Please feel free to contact me if any questions arise. I can be reached by phone at (626) 395-4823 or by email at jgerton@caltech.edu .

Sincerely,

Jordan Gerton, Ph.D.
Beckman Senior Research Fellow

JORDAN MITCHELL GERTON

PROPOSED RESEARCH

The focus of my research plan is to develop scanning probe techniques to study and control nanoscale biological systems. In particular, I intend to use the sharp tip of an atomic force microscope (AFM) probe or the end of a single-wall carbon nanotube (SWNT) for simultaneous optical and topographical microscopy with molecular-scale spatial resolution. Further, I intend to functionalize these probes at their ends with single biomolecules, e.g. enzymes or ligands, to achieve single-molecule biochemical precision. The combination of molecular-scale microscopy with single-molecule biochemical precision will enable a new class of biophysical experiments where specific biochemical sites can be addressed, and further, triggered to induce a particular reaction. Development of scanning probe techniques to facilitate such experiments will build on my current research, which combines atomic force microscopy (AFM) and fluorescence microscopy to achieve spectroscopic imaging with spatial resolution below 10 nm.

Tip-enhanced microscopy

A conventional lightning rod works by concentrating an external electric field near a sharp metal protrusion due to the increased charge density there. In the case of an oscillating external field, the instantaneous charge density at the protrusion can still be very high, resulting in a strong local enhancement of the oscillating field strength. This effect can be used to amplify field-dependent processes such as Raman scattering and fluorescence. In my postdoctoral work, I helped develop a fluorescence microscope built on the lightning-rod principle. We used this instrument for simultaneous AFM and fluorescence microscopy of quantum dots with spatial resolution below 10 nm[1], limited only by the sharpness of the AFM probe. This was the first experimental demonstration of the feasibility of spectroscopic imaging at the length scale of the individual proteins that make up the complexes of various biological nanomachines.

Although this microscope provides unprecedented optical resolution, it can be refined further. I will improve the spectral differentiation capabilities to better distinguish between multiple biomolecule species. In addition, I will achieve liquid compatibility to study dynamic biological systems. I also have a strategy to improve both the microscope spatial resolution and sensitivity even further. To increase resolution, I will utilize recently developed techniques to attach single-wall carbon nanotubes (SWNT) to AFM probes[2]. The SWNT probes are mechanically robust, are suitable for use both in liquid and air, and have been used for AFM imaging at ~1 nm resolution[3]. Additionally, they provide a functional platform for attachment of single biomolecules, as described below. To increase sensitivity, I will improve our current analysis techniques to better suppress background fluorescence resulting from the relatively diffuse laser spot used to induce the lightning-rod effect. The laser spot also causes photobleaching which interferes with single-molecule imaging. To reduce the background illumination, I will investigate the possibility of attaching a SWNT to the end of an apertured optical fiber used in conventional near-field microscopes. This idea is based on a recently reported technique whereby a metal protrusion is grown on the aperture of such an optical fiber[4]. The spatial resolution of this technique is limited, because the probes are very fragile and must be held several nanometers off the surface. Using the aperture probe to excite the lightning-rod effect in the SWNT should nearly eliminate background illumination while maintaining molecular-scale resolution.

Single-molecule functionalization of AFM probes

In collaboration with Pat Collier's group at Caltech, we are currently developing techniques to tether single biomolecules to the ends of SWNT probes. The very end of a SWNT can be electrically etched by application of a 5-30 volt pulse of ~100 μs duration between the probe and a contacted substrate. When etched in an oxidizing environment (e.g. ambient O_2), the ends become functionalized with carboxyl groups. The tip can be chemically modified further by coupling organic amines to the carboxylate to form amide bonds. The use of reactive amino chemistry is a common biochemical conjugation technique, and can be exploited for attaching fluorophores, antibodies, ligands, proteins, or nucleic acids to the ends of

the nanotubes with well-defined orientations. This method for single-molecule functionalization of AFM probes is very promising because the chemistry is relatively straightforward, and the biomolecule is attached uniquely at the very end of the nanotube, where it can most easily interact with the substrate. A biochemically functionalized probe can be used for simultaneous biochemical, optical, and topographical interrogation of a sample, providing unparalleled specificity. A particular research project that I plan to initiate to exploit these probes is described briefly below.

Probing intracellular signal networks

A complex electronic circuit is composed of multiple inputs, specific gain elements such as amplifiers and filters, and multiple outputs. The circuit elements are interconnected in such a way that a signal applied at an input may result in a non-linear response at one or more of the outputs. One way to determine how to illicit a particular response is to analyze the circuit diagram, determine the various gains and coupling constants, and then apply the appropriate combination of signals to the inputs. A better way is to design a circuit for the desired response. When the circuit diagram is unknown, however, we are stuck with a top-down approach of exploring the vast parameter space of input signals while measuring the response.

Analogously, a biological cell is a complicated signal network complete with inputs, outputs, and gain elements. Very generally, the inputs are composed primarily of receptor protein complexes that transduce biochemical signals across the cell membrane. The outputs can be either cellular products such as enzymes, or actions such as cell growth, death, division, and chemotaxis. The gain elements are a variety of biochemical reactions that allow the input signals to interact in a nonlinear fashion. Natural selection has led to a vast variety of cell types, each with its own circuit diagram that is basically unknown to us. I intend to develop a general top-down approach to mapping the signal network of a cell. Armed with this circuit diagram, it should be possible to optimize the input parameters in order to invoke a desired response. In particular, as in electronic circuits, there may be resonances which can be exploited to amplify a particular behavior or product. Alternatively, it may be desirable to suppress such resonances in the case that they lead to unwanted behaviors or products.

At first, this appears to be an overwhelmingly difficult task. Fortunately, molecular and computational biology techniques can provide rough sketches of intracellular signal networks. These sketches can be used to identify the particular inputs (receptors) associated with specific pathways, but do not really address nonlinearities in the cellular response. The strategy then is to stimulate a particular receptor with a locally applied ligand at a particular time and follow the subsequent response. The localized stimulation can be done in the presence of a background ligand concentration of either the same, or different, species to probe for nonlinearities. Additionally, a given receptor can be stimulated multiple times with a variable period in order to probe the frequency response.

The local probes can be used to locate specific receptors either optically via spectroscopic signatures in the fluorescence emission, or biochemically via the specificity of the functionalized probe for the target receptor. Most likely, some combination of optical, biochemical, and topographical signatures will work best. In any case, it should be possible to differentiate between various receptors with molecular-scale spatial precision.

Once a specific receptor is located, there are several possible ways to trigger it. The most straightforward method may be to simply tether a ligand to the probe and then use the fine controls of the AFM to hold the ligand in the immediate vicinity of the receptor. By controlling the background biochemical environment, it may then be possible to control the ligand-receptor binding constant and thereby induce binding or anti-binding at specific times. Another possibility is to functionalize the probe with an enzyme whose product is the receptor's ligand. Then, by controlling the background concentration of the enzyme substrate, the local concentration of the ligand can be adjusted in time.

These two methods may require development of novel fluid-exchange techniques to facilitate fast switching of the background biochemical environment. Alternatively, it may be possible to control the activity of the enzyme directly via an electrical contact between it and the probe (nanowiring). This has been demonstrated recently with the enzyme glucose oxidase using gold nanoparticles attached to macroscopic electrodes[5]. Finally, if a ligand can be nanowired directly to the probe, it may be possible to alter the receptor-binding affinity via electrical control of the ligand structure.

After the receptor is triggered, the response of the cell must be measured. Many signal pathways culminate in expression of a gene or combination of them, so molecular biology techniques will be heavily utilized. In particular, single-cell gene expression assays will be needed to monitor either the RNA or protein content of a cell. It may be possible to use fluorescently labeled oligomer probes to screen for RNA sequences that correspond to particular genes within an intact cell. Alternatively, it may be possible to use quantitative reverse-transcription polymerase chain reaction (qRT-PCR) to quantify the target RNA sequence of a lysed cell. To quantify protein expression, it may be possible to use gene-splicing techniques at the single-cell level to express the protein of interest and a fluorescent protein (e.g. GFP or variant) simultaneously. Alternatively, injection of fluorescently labeled antibodies might be used to screen for protein content. In the case that the expressed proteins make their way to the cell membrane, they can be detected with molecular-scale spatial precision. Otherwise, the bulk-fluorescence measurement capabilities of the microscope will be utilized.

It may also be useful to probe the cellular signal network further upstream, before gene expression occurs. This could reveal the signal propagation rate and timing between individual chemical steps, or the minimum biochemical threshold for initiation. This can be accomplished by screening for intermediate biochemical products. For example, signaling pathways which involve the generation of inositol triphosphate (IP3), intermediately release intracellular calcium ions that can be detected with a calcium-dependent dye, such as Fluo-3.

Potential collaborations

I have other projects in mind, in addition to the one described above. These include studying the conformational changes in membrane-bound protein complexes during signal transduction, and studying the interactions between proteins and chromosomes during transcription. I believe the optics and nanoscience aspects of my research plan will compliment the current focus areas in the Physics Department at Indiana, and I fully appreciate the value of soliciting the expertise of biologists and chemists to bring these studies to fruition. I have already developed collaborative relationships with scientists here at Caltech, including Scott Fraser in Biology and Pat Collier in Chemistry, and I expect these relationships to grow. More importantly, I intend to vigorously pursue collaborations among the faculty in the Biocomplexity Institute at Indiana, and to recruit students from various departments and backgrounds.

1. Gerton, J. M., Wade, L. A., Ma, Z., Lessard, G. A. & Quake, S. R. Tip-Enhanced Fluorescence Microscopy at 10 nm Resolution. *submitted to Phys. Rev. Lett.* (2003).
2. Hafner, J. H., Cheung, C. L., Oosterkamp, T. H. & Lieber, C. M. High-Yield Assembly of Individual Single-Walled Carbon Nanotube Tips for Scanning Probe Microscopies. *J. Chem. Phys. B* **105**, 743-746 (2001).
3. Wade, L. A., Shapiro, I. R., Ma, Z., Quake, S. R. & Collier, C. P. High Yield Production of Carbon Nanotube Scanning Probe Tips. *in preparation* (2003).
4. Frey, H. G., Keilmann, F., Kriele, A. & Guckenberger, R. Enhancing the Resolution of Scanning Near-Field Optical Microscopy by a Metal Tip Grown on an Aperture Probe. *Appl. Phys. Lett.* **81**, 5030-5032 (2002).
5. Xiao, Y., Patolsky, F., Katz, E., Hainfield, J. F. & Willner, I. Plugging into Enzymes: Nanowiring of Redox Enzymes by a Gold Nanoparticle. *Science* **299**, 1877-1881 (2003).

JORDAN MITCHELL GERTON

TEACHING STATEMENT

My teaching experience extends back into high school and college with various tutoring stints. In graduate school at Rice University, I was a physics laboratory teaching assistant for five semesters under the supervision of Prof. Stanley Dodds. My duties included preparing short lectures that tied the classroom material together with the lab experiments, supervising the labs themselves, grading reports, and occasionally conducting recitation sessions. In addition, while at Rice, I gave several guest lectures for a quantum mechanics course taught by Prof. Frank Tittel in the Electrical Engineering Department. More recently, while a postdoc at Caltech, I searched out a visiting instructor position in the Physics Department at Pomona College in Claremont, CA. This position was not a part of my postdoctoral responsibilities, but I wanted to gain valuable teaching experience, particularly at a top-tier liberal arts college like Pomona. My duties there included teaching one section of a laboratory course, and supervising two undergraduate teaching assistants. The Physics faculty at Pomona is a group of extremely talented teachers, and I learned much in my semester there.

I received very positive feedback on student evaluations throughout my graduate teaching assistantship and during my stint at Pomona. Recently I saw one of my former Pomona students give an oral presentation at the Optical Society of America annual conference in October, 2003. As a bright first-semester freshman in my lab section, he was wavering on whether to major in physics or to take a pre-law track. After his presentation, he credited me as the biggest influence in his decision to go into physics. Score one for science!

I will enjoy teaching most standard physics courses, including introductory physics (both for majors and non-majors), modern physics, quantum mechanics, statistical mechanics/thermodynamics, electricity and magnetism, optics, and any laboratory. In addition, my graduate work in the laser cooling and Bose-Einstein condensation group of Randy Hulet at Rice has prepared me to teach atomic physics, laser physics, quantum optics, or laser spectroscopy. My postdoctoral work in the group of Stephen Quake at Caltech has prepared me to teach biophysics and single-molecule imaging techniques. I would also like to develop courses that survey a variety of more specialized subjects, such as near-field and nano-optics, microscopy techniques in biology and medicine, and single-molecule biosensors. If possible, I would enjoy building a course on experimental methods in physics, which might include topics such as electronic circuit design for physicists, metal machining (in combination with a machine shop), laser techniques (tuning, frequency stabilization, etc.), CADD design, and possibly others.