RESEARCH PROPOSAL
Dr. Joseph E. Reiner

INTRODUCTION

Fluctuations have long played an important role in the study of large systems in biology, chemistry and physics. Temporal autocorrelation functions inform the experimenter about the underlying dynamics of a noisy system. The single molecule community has been using this fact for the last decade to observe intermediary pathways that are lost in bulk sample studies[1,2]. Devices capable of exerting piconewtons of force such as optical tweezers[3], magnetic tweezers[4] and AFM tips[5] have been used to study molecular motors. Optical tweezers have also been used to observe spontaneous binding and disassociation of antibody-antigen coated microspheres[6]. In all of these examples it is the fluctuations that carry information about the system dynamics.

Studying fluctuating signals from a single molecule improve upon bulk studies only if the signal is fluctuating around more than two events. Fluorescence correlation spectroscopy (FCS) applied to an on-off telegraph signal will only inform the experimenter about the rate and width of the on portion of the signal. Interesting things happen when a signal jumps between many discrete or even continuous states. FCS applied to this data shows several decay rates corresponding to jumps between different conformations. Interpreting this data can be difficult and usually a model dependent fit is used to extract results. An alternative approach that has begun to gain theoretical notice[7, 8] considers three-time correlation functions of a fluctuating signal. Three-time correlations extract information about multiple events triggered by some initial event. I believe an exciting and fruitful research path would study fluctuations beyond two-time autocorrelation techniques to pursue three-time correlation functions that could be used to better understand complicated biodynamics at the single molecule level.

RESEARCH BACKGROUND

I obtained my Ph.D. in the quantum optics group at SUNY Stony Brook under the supervision of Luis Orozco. My research focused on three different projects that spanned the fields of optical cavity quantum electrodynamics (CQED), quantum feedback applied to the CQED system, and atomic laser cooling. Our CQED system consisted of a collection of two-level atoms strongly coupled to a single mode of a high finesse Fabry-Perot cavity. Strong coupling, in this context, means that a single photon saturates the atom-cavity system. The emission of a single photon initiates a large fluctuation in the intracavity field. In the theory of quantum measurement it is the emission of a single quanta (photon) that represents a measurement. This measurement initiates a large fluctuation that can only be described by quantizing the atom-cavity degrees of freedom. We used conditioned field and intensity measurements to study these quantum fluctuations.

The first part of my thesis studied how spontaneous emission from the two-level atoms degrades the size of the quantum fluctuations. I used numerical simulations to model the individual quantum jumps that give rise to the ensemble-averaged results. Studying the fluctuations induced by the spontaneous emission from the atoms led to a

better understanding of the overall quantum dynamics of the system. This work appeared as part of a larger review on conditional homodyne detection in the *46th Volume of Progress in Optics* edited by Emil Wolf.

Each time a photon is emitted from the cavity in the steady state, the fluctuation is well defined by the system parameters. We collaborated with Howard Wiseman, from Griffiths University in Australia, in proposing and implementing a quantum feedback protocol that used our knowledge of the quantum fluctuations to apply feedback to alter the dynamics of the intracavity field following a photon emission. The quantum feedback was implemented by applying a step in the intensity of the driving field conditioned on a single photon detection. This step forced the quantum fluctuation into a different steady state. This was the first time that anyone had applied quantum feedback in a system at the level of individual quantum jumps. These feedback results were also featured as part of a year-end review issue of *Optics and Photonics News*.

In the last year of my thesis I designed and constructed a 2D magneto-optical trap (2DMOT) to provide a continuous source of slow atoms free of any laser beams or magnetic fields. This arrangement will serve as a next generation source for future CQED experiments. One of these experiments will test an analytical expression I developed that describes the dependence of the size of the quantum fluctuations on the random distribution of atoms within the cavity mode volume.

After completing my degree I decided to apply my knowledge of fluctuations to biomolecular systems. I was awarded a National Research Council Fellowship to carry out postdoctoral research studying applications of optical tweezers. I have been working on three different projects in the Laser Cooling and Trapping group at the NIST campus in Gaithersburg, MD.

The first project was to develop a technique for creating polymer nanotubes by pulling on the edge of a polymersome vesicle with optical tweezers. The polymersomes are formed with a surfactant that promotes stretching. The polymer is a covalently linked polyethylene oxide-polybutadienne (PEO-PB) diblock copolymer, which allows cross-linking along the PB portion of the molecule. We have created the first chemically cross-linked polymer nanotubes. We are currently working to characterize the properties of these tubes with SEM and TEM imaging. In future experiments we will inject DNA molecules into the polymersome vesicles to study transport through these tubes in the cross-linked state. The ultimate goal is to reach the nanofluidic regime where the channel dimensions affect the conformation of single DNA molecules.

The second project involves single molecule detection with an optical tweezer setup. This work is being carried out in collaboration with Lori Goldner in the Optical Technology Division at NIST. The majority of work on single molecule detection involves attaching molecules to a surface or observing the molecules diffuse through an interaction region defined by the focus of a laser. One of the major disadvantages of attaching molecules to surfaces is that the surface changes the dynamics of the biomolecule in an unknown manner. Laurie Locasio's group, a member of the Chemical Science and Technology Laboratory at NIST, has developed a new method for encapsulating single Cy3 dye molecules inside liposomes with microfluidic flow channels. We are working to optically trap a single liposome and study the photophysical properties of the dye molecule inside the vesicle. The long-term goal for this project is to

provide a novel method for single molecule detection without the need for attaching the molecule to a surface.

The third project is also related to single molecule detection. It involves the trapping and detection of single molecules contained within a reverse phase water-in-oil emulsion or hydrosome. Using a fluorinert liquid, FC-77, that has a lower index of refraction than water, we can optically trap micron sized water droplets. Two major advantages of hydrosomes over other vesicles are that it is trivial to incorporate molecules inside the droplets and we have found that the fusion between two droplets is spontaneous once the droplets are brought into contact with each other. We have so far used sonication to create hydrosomes. We are currently developing techniques for creating micron-sized droplets on demand with the goal of performing single molecule studies.

RESEARCH PLAN

I am interested in combining my knowledge of fluctuations with single molecule detection to move beyond the standard methods established in FCS. To do this I would set up an experimental biophysics laboratory that specializes in studying two- and three-time correlation functions on single molecule dynamics. I will accomplish this by setting up an optical tweezer arrangement for manipulation, trapping, and force measurement, setting up a confocal detection system for observing single molecules and finally developing a method for measuring three-time correlation functions that can be used to study the dynamics of single molecules. With the apparatus in place, I would like to study the fluctuations of two different biophysical systems.

Helmerson's group has initiated studies of spontaneous antibody-antigen adhesion when the molecules are attached to micron-sized spheres held in two optical tweezer traps[6]. I will use this work as a starting point to perform adhesion studies on free ligand-receptor molecules. A single ligand molecule will be held in a trapped microdroplet while another trapped microdroplet will contain a single receptor molecule. Both molecules will be tagged with FRET pair dye molecules. The two droplets will be fused and standard FCS methods will be used to study the spontaneous adhesion and disassociation of the ligand-receptor pair. Three-time correlation surfaces can be extracted from the photon counting data and they will be used to show any intermediate dynamics of the ligand-receptor system.

A second experiment studies the hybridization of DNA strands at the single molecule level[9]. The experiment could be set up in the following way: one single stranded DNA molecule has Cy3 attached at both ends and a complementary strand has Cy5 attached at both ends. Observing the fluctuations in the fluorescent signal from the Cy5 in a FRET type experiment with FCS and three-time correlation studies will show what pathway the strands follow when they hybridize. Does the DNA zip up from one end to the other? Will it combine in the middle and merge in both directions? Do the DNA strands remain attached once they are hybridized? These are some of the important questions that I hope to answer with this experiment.

OBJECTIVES AND GOALS:

Year 1:

- Recruit personnel.
- Design and build an optical tweezer setup.
- Build a confocal single molecule detection system.
- Design and implement photon counting apparatus and time correlator.
- Trap and detect single fluorescent dye molecules inside hydrosome droplets.
- Begin writing proposals to: NSF, NIH, ACS/PRF, …

Year 2:

- Begin hybridization experiment by observing FRET signal of mixed DNA strands.
- Perform initial adhesion studies of ligand-receptor system inside trapped hydrosomes. Utilize FCS and three-time correlation functions to study dynamics of adhesion.

Lab Requirements:

I require approximately 500-1000 square ft. of research space with appropriate electrical (110V and 220V) outlets, benchtop, chemical hood, water, temperature stability to within 2-3 degrees C, and relative humidity stable to within 15%.

Personnel:

I would initially recruit two graduate students to work on setting up the laser and detection systems. One student could work on the adhesion studies and the other will work on the three-time correlator system by studying single dye molecules in hydrosomes. I will also seek to hire a postdoctoral associate (2 years) or research assistant with a strong background in biochemistry.

Budget:

A detailed budget for laboratory start-up is available upon request.

FINAL STATEMENT

The success of any academic researcher depends strongly on the ability to attract the best graduate students. This can be accomplished by establishing a vigorous research program in an area that I feel to be one of the most interesting in the field of biophysics, single molecule detection. In my doctoral research I learned about the importance of understanding the system fluctuations in describing the underlying quantum nature of the system. The strength of studying fluctuations has been shown recently in single molecule detection. I feel that an exciting avenue of research is to continue further exploration of fluctuations in biomolecular systems.

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TEACHING STATEMENT
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The growth of scientific knowledge has divided scientific disciplines into different subfields. This division has been extended to include the categorization of scientists as researchers or teachers. I believe that teaching is a universally important endeavor. My motivation to teach is driven by the universal goals of creating a scientifically and technologically literate society and educating future scientists.

Scientists with a strong background in research can provide a valuable resource in the classroom. A research scientist has direct knowledge of recent developments and can use that information to pique the curiosity of students. It can also be beneficial to the researcher to try and explain these modern results at the introductory level.

My experience teaching introductory physics courses comes from years of tutoring at the undergraduate level and working as a TA for two sections of an introductory mechanics lab along with two sections of a calculus recitation for life science majors. I have found myself, when trying to make sure the beginning students understand the material, in a situation familiar to most professors. How does one convey the concepts, profit from the knowledge and tools that are already present in the student, and at the same time develop the skills in problem solving? The balance of these three questions is fundamental in the success of any teaching. It requires good planning, carefully chosen conceptual questions, lecture and laboratory demonstrations, homework, and some humor.

My experience teaching upper level physics courses includes working as a TA for a junior level optics lab and delivering a handful of lectures to my adviser's junior level electromagnetism course. I found that students at this level are usually proficient at the mathematics, but they lack the insight required to make progress in the derivation of a physics formula. A successful approach includes providing the physical motivation for solving a particular problem, deriving the appropriate formulas, and assigning challenging homework problems on a weekly basis. As these students become more proficient, some more complicated ideas from recent research should be presented.

I briefly mentioned in my cover letter that I would be interested in teaching an advanced topics course in fluctuations. Advanced topics courses should introduce advanced undergraduate and graduate level students to recent ideas and results in the field at a sophisticated technical level. I believe an interesting approach is to stress a generalist approach to physics. Fluctuations and noise are an obvious choice for such a course. One could start out from statistical mechanics and cover topics from quantum optics, atom optics, stochastic resonance, biophysics, condensed matter, and many other areas. My hope is that teaching this course would help attract prospective graduate students to work in my lab and the interaction with students at this level may help me to think differently about problems in my research.

My professional development has relied upon guidance from great teachers in the classroom and the laboratory. I would like to contribute back to the physics community by teaching students how to look at problems from a scientific point of view. I feel that teaching is an important part of what it means to be a professor and I intend to teach well and provide a strong learning environment for my students.