University of Central Florida

Biocomplexity Faculty Search Committee,
c/o Prof. Rob de Ruyter van Steveninck,
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School of Optics/CREOL
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Re: Faculty Search

November 17, 2003.

Dear Chair of the Physics Search Committee:

I am interested in a faculty position, which I saw advertised on the web page of American Institute of Physics. My research interests are in the area of Experimental Biophysics, specifically single molecule microscopy and spectroscopy of molecular interactions *in vitro* and in living cells.

Please find enclosed the documents, which are required in the position announcement. Also, I have requested my professional references to send their recommendation letters directly to the Search Committee.

Thank you for considering my application.

Sincerely,

Svitlana Berezhna.

Encl.

Svitlana Berezhna

Single Molecule and Single Particle Imaging and Tracking for Biomolecular Dynamics.

(Summary of Proposed Research)

The research effort proposed here is focused primarily on establishing and development of single molecule and single particle optical microscopy methodologies for studies of biomolecular dynamics and interactions *in vitro* and in living cells.

A new era of biomolecular and cellular studies has been introduced since recent advance in optical microscopy, allowing for making measurements on single biological molecules. Whereas past experiments required model-dependent inferences from ensemble measurements, these new techniques allow a direct observation of the parameters that are relevant to answering the following questions: *How does a biological molecule move within the cell? How does it manage to target the right place inside the cell and get it in the right time? Does molecule mobility vary with the position in the cell and is it modulated? How does it interact with other molecules? How does it respond to applied signals?* Besides the scientific relevance for single molecule analysis in physics, chemistry and biology, these techniques are also of increasing importance in pharmacologic drug screening.

A range of scientific problems, which can be addressed by single molecule microscopy, is exploding. The remarkable demonstrated applications include, for example, studies of enzymatic reactions with identification of transient intermediates, receptor oligomerization, dynamics of lipids rafts, ion channel functions, the vizualization of the nucleo-cytoplasmic transport inside living cells, the pathways of the single adeno-associated viruses during their infection entry into living cells, the determination of step sizes of individual myosin V heads.

A long-term aspiration of the applicant is to establish a laboratory that builds and applies light microscopy imaging and analysis tools to the discovery of basic aspects of cellular organization and dynamics. The major initial effort is to build a two-color single molecule tracking microscopy setup configurated in epi- detection and total internal reflection geometries and further develop software for tracking that will provide observation with a precision in the *nanometer* range *within* cells, which would in the ideal case offer a full view of intracellular molecular dynamics.

It is also aimed to apply this single molecule imaging methodology to attempt and solve real scientific problems in cell biology. As an initial endeavor, it is planned to focus on the dynamics and interactions of mitochondria proteins (cytochrome c) and phospholipids (cardiolipin) and of proteins in cytosol (binding of Apaf-1 to cytochrome c) under a variety of conditions during apoptosis. Understanding of the molecular mechanisms of apoptosis is significant for the development of strategies designed to selectively induce apoptosis in cancer cells. The experiments will involve an in parallel *in vitro* and *in vivo* approach such as using artificial membrane systems of giant unilamellar vesicles that will mimic mitochondria membrane. Although apoptosis is a cellular phenomenon, some aspects of the specific molecular interactions during this process can be better understood outside the complex cellular milieu, using well controllable albeit simplifies systems.

Another planned activity includes further understanding mechanisms of lipofection (lipoplex-mediated transfection of eukaryotic cells) at the level of interaction between single DNA and

cationic lipids molecules. The efforts will be aimed at defining a structure – function relationship of the lipoplex particles (LPs) to suggest methods for rational design of effectual lipofection compounds, which improve nucleic acid delivery leading to gene expression. It is also intended to use lipoplex nanostructures for potential delivery of quantum dots, entrapped in lipids coating, into the cells.

One more planned research project is related to probing the viscoelastic properties within cells by use of single particle tracking methodology. Viscoelasticity plays an important role in the behavior of the cells. For example, it is a key factor in the regulation of the cell shape of resting and moving cells. The cell viscoelasticity is determined in a complex way by the multipart plasma membrane composed of the lipid-protein bilayer with the associated actin cortex and by the internal cytoskeleton. Single particle tracking allows determining local mechanical properties in the cytoplasm in a dynamic and rate-dependent manner, which offers tools to probe cell response to different mechanical stimuli.

This proposed research activity will not only prospectively lead to the development of new generations of a single molecule sensitive microscope for biomedical imaging, but will aid in resolving imperative demanding problems of molecular and cell biology. The development of instrumentation and the associated software to effectively track single molecules in living cells will enhance the infrastructure for doing intracellular real-time protein dynamics research. It is planned to develop this experimental technique into a relatively simple optical microscopy system design that can be routinely accessible to researchers in biology. In addition, the broadly based multidisciplinary environment, which would pottentially be established to perform this research will provide excellent training and education opportunities for the next generation of optical and molecular biology scientists.

It seems fitting to conclude this brief summary of the proposed research with a quote from S. Brenner, who wittily defined developmental restrictions of modern biological research: "...In looking back, one realizes that in molecular biology there are technical advances, discoveries and ideas – usually in that order...". The research program proposed here follows a similar philosophy offering to develop potentially important techniques (the imaging methodology based on single particle tracking) and to apply them to fundamental problems in cell biology in a way that will lead to discovery and new ideas.

Svitlana Berezhna

Description of teaching experience

My teaching experiences so far were mostly interactions with undergraduate and graduate students, with interests in different research area (optics, physics, biophysics, experimental mechanics), either in small groups or on a person-to-person basis.

As a PhD student I worked as a teaching assistant for the graduate laboratory classes "Nonlinear Optics" and "Optical Spectroscopy" at Lviv I.Franko University, Ukraine. This work included organization of laboratory courses and providing technical assistance during laboratory works in a small group (10-13 students), giving seminars on theoretical background for experiments, being around during the class to help, solve problems and answer questions, and assisting in oral examinations at the end of the semester.

During my work as a JSPS Postdoctoral Fellow at Aoyama Gakuin University, Tokyo, Japan I was assigned to supervise two graduate students and two undergraduate students, who closely worked with me for two years. The topics of the Master degree theses of those students were related to stress tensor field optical tomography, which I worked on at this time. My responsibilities included introducing students to new experimental techniques and providing theoretical background. Also I assisted them in digital image processing and writing programs. I organized the work so that they solved small projects independently and we together analyzed the results. If they were not able to get good experimental data or explain the facts they encountered, I encouraged them to find the solution on their own through reasoning. Also I helped them to organize their work in teams and pass their knowledge to each other either at laboratory meetings or through one-to-one discussions. In addition, I assisted the students in their preparing presentations for several international conferences.

My postdoctoral work at Department of Physics, Northeastern University, USA as well as my current activity as a Research Associate at School of Optics/CREOL, University of Central Florida have given me an unique opportunity to closely interact with many international students and gain new teaching experiences in the American academic environment. Currently I closely work with and supervise one graduate optical student in the laboratory. Also I have assisted in preparing written tests for midterm and final exams for "Introductory Physics", and was involved in grading procedures. In addition, during my multiple research visits to Max-Plank-Institute for Biophysical Chemistry, Goettingen, Germany I work together and supervise two biophysical students.

With my background I consider myself qualified to teach general physics courses at the undergraduate level and biophysics, general optics and nonlinear optics at the graduate level. My teaching philosophy is based on organizing students to work in teams, ***actively*** involving them in real research projects and stimulating them to find solutions of problems independently through reasoning and verifying the possible explanations. I consider active participation of students in work on ongoing research projects in the laboratory as one of the most efficient way to stimulate their reasoning and motivation, to grow their analytical abilities and to extend their scope. It will help students to learn how to cope with minor and major problems, which constantly occur in any work on daily basis so that in future these minor obstacles do not prevent them from achieving their final goals. The important part of my teaching philosophy is to help students to gain abilities of thinking broadly, independently and differently and to be ready for challenging nontrivial problems and finding nontrivial solutions.