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Applications of Optical Trapping to Single Molecule DNA

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Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). The project focused on the methodologies required to integrate optical trapping with single molecule detection (SMD) so as to demonstrate high speed sequencing through optical micromanipulation of host substrates, nucleotide cleavage, and single molecule detection. As part of this effort, the new technology of optical tweezers was applied to the confinement and manipulation of microsphere handles containing attached DNA fragments. We demonstrated substrate optical trapping in rapid flow streams, the fluorescence excitation and detection of fluorescently labeled nucleotides in an optical trapping system, and the epifluorescent imaging of DNA fragments in flow streams. We successfully demonstrated optical trapping in laminar flow streams and completely characterized the trapping process as functions of fluid flow velocity, chamber dimension, trapping depth, incident laser power, and fluorescence measurement geometry.

Background and Research Objectives

This project is a collaborative effort between Los Alamos National Laboratory (LANL) and the Beckman Laser Institute (BLI) at the University of California-Irvine (UCI) to study the applications of optical laser trapping (optical tweezer) techniques to DNA sequencing and single molecule detection (SMD). In particular, the efficacy of optical tweezers to capture DNA molecules attached to latex microsphere "handles" in a flow stream, and subsequently acquire biophysical data via fluorescence and force microscopies, was investigated. The basis for this work is the fact that single DNA fragments can be sequenced through a process of (a) synthesis with tagged nucleotides, (b) attachment and suspension of the molecules to microspheres, and (c) cleavage and detection, by laser-induced fluorescence of the microsphere-attached DNA, in a flow stream. Optical tweezers are extremely effective in the confinement and manipulation of microspheres in stationary and rapidly flowing streams. Hence, optical tweezers, when used in

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conjunction with nucleotide fluorescence labeling and DNA fragment cleavage procedures, offers the potential for the rapid sequencing (100 - 1000 bases/sec) of large fragments that are attached to a "host substrate," yet which are free to flow in a laminar stream.

This research complements existing LANL efforts in laser-based DNA sequencing [1-9] and the BLI efforts to develop and apply the new technology of non-invasive laser trapping [10] to biological sample confinement and manipulation [11-15]. The key issues for investigation include: (1) development of an optimum chamber design for microsphere/fragment trapping and flow; (2) study of the attachment process and fragment binding strength to a microsphere handle; (3) measurement of fragment fluorescence as a function of trapping and dye excitation conditions; (4) sequencing of the largest DNA fragments possible; and (5) preliminary study into the use of the optical trap as a scanning force DNA probe.

Importance to LANL's Science and Technology Base and National R&D Needs

By combining laser trapping with fluorescence and other flow cytometric techniques, it may be possible to achieve, for the first time, the rapid sequencing of large DNA fragments and the detection and study of individual nucleotides. Beyond the immediate study of DNA molecules, the integrated system proposed herein, which can combine optical confinement with fluorescence and scattering detection, could also find widespread use and applications in the physiological study and identification of other biological cells and organelles (e.g. chromosomes, sperm cells) which serve as the transport vehicles for DNA genetic material. Applications include sample sizing, determination of optical properties, study of antigen and body reactions, and the study of micro-irradiation effects on biological systems at the molecular and cellular levels, for uses in genetics, immunology, and cancer biology. This work seeks to establish the limits of trapping and fluorescence detection at the molecular level, and define the parameters necessary for rapid DNA sequencing, which is essential to the mapping, identification, and manipulation of the human genome.

Scientific Approach and Accomplishments

For the past five years, Berns and Sonek at BLI have collaborated on the fundamental and applied study of optical trapping [11,15], specifically with respect to the confinement and movement of dielectric and biological samples in fluid solutions. In addition to performing many of the first optical trapping experiments on chromosomes and

sperm cells, they have also developed an extensive electromagnetic model for predicting the forces produced by highly focused laser beams. LANL has exceptional expertise in flow cytometric and fluorescence detection systems. By combining trapping and laser-based detection in a common flow system, the LANL-BLI collaboration could ultimately lead to the development and commercialization of a new laser-based human genome mapping instrument that greatly outperforms existing systems. This project has focused on the methodologies required to integrate optical trapping with SMD with the ultimate objective of demonstrating high-speed sequencing through optical micromanipulation of host substrates, nucleotide cleavage, and single molecule detection.

We had success in two key areas, including the characterization of the performance of optical traps in a microchamber flow system geometry, and the implementation of laser-induced fluorescence measurements on optically trapped latex microspheres in a flow stream. We developed a flow system, integrated with a microscope, to facilitate the simultaneous optical trapping and fluorescence excitation/detection of micron-sized samples that have been tagged with fluorescent probes. Using a Nd:yttrium aluminum garnet (YAG) laser (1064 nm) as the trapping beam, one- and two-micron sized latex microspheres were stably trapped in a flow stream, where flow velocities as high as 1-12 mm/s were achieved. Such flow velocities are commensurate with those required to stretch and measure fluorescence from DNA strands in high-speed sequencing applications.

High spatial resolution ($\sim 1 \mu\text{m}$) and high signal-to-noise ratios ($>10^3$) were achieved using a high-numeric-aperture objective lens for trapping and fluorescence detection within a confocal microscope geometry. In this system, samples were displaced with the scanning laser trap by up to $\pm 20 \mu\text{m}$ off the trapping beam axis in the sample plane while maintaining, at the same time, a large fluorescence detection efficiency. At a trapping depth of $20 \mu\text{m}$ below the chamber surface, a laser power of 100 mW was sufficient to trap a $2\text{-}\mu\text{m}$ diameter microsphere and confine it for a period in excess of 60 sec in a flow stream having a velocity of 0.5–5 mm/s and a particle concentration of $\sim 10^6/\text{ml}$ while its fluorescence was measured. A systematic study investigating the effects of trapping efficiency, trapping depth, flow velocity, and tweezers holding time in a $500 \mu\text{m} \times 500 \mu\text{m}$ flow microchamber system was performed. In particular, we demonstrated the noninvasive positioning of samples, upstream and downstream from the immediate detection probe volume, and characterized the spatial dependence of optical collection efficiency in a flow cytometric fluorescence detection system.

During the last year of the project, we also studied the optical trapping of $1 \mu\text{m}$ latex beads and $2.8\text{-}\mu\text{m}$ paramagnetic beads containing attached λ -DNA fragments. The DNA

was primed and tagged with the YOYO fluorescent dye (λ excitation = 488 nm) and epifluorescence imaging performed on the optically trapped beads in a stationary fluid. We also developed an improved bead delivery system, so that we can introduce fewer beads into the sample chamber during our trapping studies. This work indicated that paramagnetic beads could not be easily trapped in the existing laser trap, the fluorescence imaging of the DNA strands could be problematic, and that new laser trapping parameters would need to be established to accommodate the conventional DNA-coated substrates used in the SMD experiments.

In summary, this work has established the system parameters for a trapping-fluorescence system that can potentially facilitate nucleotide detection and the direct integration of the UCI/BLI optical laser trap into the LANL DNA sequencer for high-resolution, high-throughput measurements on DNA fragments.

Publications

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