

Final Report for Benedictine University

The Modification of an Optical Tweezers in Order to Study the Motility Strength of E. coli under Different Environmental conditions

Student Researchers

Swetha Sabu
Tricia Avanzado
Alexander Seymour

Alonso Valerio
Michael Fralaide
Beatriz Ibarra

Faculty Sponsor: Dr. Andrew Wig

Project Overview:

Our chapter proposed using an optical tweezers to measure the strength of E. coli's motility within different environments. To achieve this goal the optical tweezers system needed to be upgraded and improved. This involved completely rebuilding and testing our system as well as developing new protocols for sample preparation. Over the course of the year we have had 6 student researchers participate in the project from three different majors; Physics, Biology, and Biochemistry and Molecular Biology (BMB).

The laser and optical systems have been improved through the purchase of a more powerful laser to replace the He-Ne laser we were using previously. This resulted in the development of a new laser safety barrier system and other safety protocols. The optical system was completely rebuilt and new components were added that improved the magnification and imaging capabilities of the system. Experiments were conducted to calibrate and test the imaging system.

Along with the improvements to the optical system a fluid flow cell system was installed and tested. Sample preparation and handling procedures for the E. Coli samples were also developed.

At this time we have successfully trapped and moved microspheres as well as E. Coli but we have not reached our goal of determining the motility strength. The project will continue into next year.

Preliminary results from the project have been presented at the 43rd Associated Colleges of the Chicago Area (ACCA) in April of 2010 at Lewis University, the Benedictine University Summer Research Symposium in August of 2010, and our local SPS meeting.

Optical Tweezers in Biophysical Application

Introduction

Optical tweezers are an instrument that traps particles using a focused laser beam. Incoming photons from the laser impose forces on the particle towards the center of the laser trap through photon refraction or scattering according to Newton's Third Law. There are 3 main requirements for optical trapping: the gradient force must be stronger than the scattering force, the particle's refractive index must be larger than that of the medium, and the particle's kinetic energy must be smaller than the potential energy depth of the optical trap. Pioneered by Arthur Ashkin in 1970, optical tweezers are quickly becoming a popular topical field of study in physics.

Apparatus

The setup contains the basic essentials for optical tweezers: a laser, a beam expander, a simple microscope, and an imaging system. The diode laser outputs 660 nm wavelength light with a power output of upwards of 60mW. This is a power increase from the previous helium-neon laser. The laser light passes through a beam expander, which includes a focusing lens, a pinhole, and a converging lens. The beam expander serves two purposes: it cleans up the intensity distribution of the beam as well as allowing adjustment of the width of the collimated beam waist. The clean Gaussian curve of the intensity distribution is crucial because the strength of the trap should be the greatest at the center of beam. The lenses are adjusted such that the aperture of the 100x oil immersion objective lens is filled by the laser beam. The μ -Slide I^{0.2} Luer flow cell is mounted on the XYZ translator stage. The height of the flow cell channel is .2 mm, which is ideal for flow assays with small amounts of shear stress. Using a syringe as a gravity feed, as well as a simple clamp, the sample can be pushed through the flow cell at an adjustable rate. The condenser and light source located behind the stage help to produce a bright image on the CCD camera, which is connected to a computer with appropriate video software.

Experiment

The first sample examined consists of a diluted solution of polystyrene microspheres approximately 2 μ m. The sample is placed onto microscope slide and imaged by adjusting the oil immersion objective lenses and focusing lenses in front of the

CCD camera. Once a relatively clear image is attained, the laser and beam expander are meticulously aligned and integrated into the setup until the polystyrene microspheres are optically trapped. Then, *E. coli* must be prepared to be imaged and trapped. A colony of *E. coli* strain RBB1050 is placed in a test tube with a liquid medium. After being left undisturbed overnight, an aliquot of the solution is diluted with the fresh growth medium. Approximately 800 μ L of this diluted solution is then placed in a petri dish with 200 μ L of 1% hydroxypropylmethylcellulose of about 7pH. After the mixture has been swirled by hand, approximately a sample of 17 μ L is placed onto a microscope slide using micropipettes. The *E. coli* use their peritrichous arrangement of flagella to generate torque and thus be motile. Using the optical tweezers, the *E. coli* can be trapped and their motility strength can be calculated after finding the strength of the optical tweezers.

Results

The initial task of aligning the optical trap begins with the trapping of the polystyrene microspheres on a standard microscope slide. The stochastic movement of the microspheres is observed, which is caused by Brownian motion, the collisions of the suspended particle with the molecules of the fluid. Brownian motion is the simplest form of diffusion and is the main force trying to pull the particles out of the optical trap when the sample is on the standard microscope slide. One key factor in the success of the optical tweezers is having the focus of the laser being at the same depth as the focus of the CCD camera. With the laser properly aligned with the optical components, the optical tweezers can trap the moving microspheres while being in focus on the computer screen. Next, a sample of *E. coli* is placed on the microscope slide and imaged. The *E. coli* sample seems to have a different index of refraction than the polystyrene microspheres as the bacteria tend to be more translucent in appearance. Staining the bacteria with a dye was considered, however their ability to flourish and be motile would likely be affected by the staining process. The optical tweezers trap the *E. coli*, which attempt to swim out of the optical potential well created by the laser. The slide is then replaced by the flow cell with a gravity feed from a syringe containing a polystyrene microsphere sample. By clamping a pinch into the tubing below the syringe, the velocity of the sample can be greatly decreased in order to observe the movement of the individual microspheres. Using fluid dynamic equations to approximate the speed of the sample, the strength of the

optical trap, which is typically in the range of piconewtons, can be experimentally approximated by using Hooke's law, $f = -kx$. The force constant can be approximated using a visual analyzing software to find the position change in the microspheres. To increase the precision of the position detection, a quadrant photodiode and a fast Fourier transformation spectrum analyzer will be incorporated into the setup in the future. Once this force calibration is found, E. coli can be placed into the flow cell and their motile strength can be approximated using the optical trap. An E. coli solution will then be prepared with 210 μ L of 1% hydroxypropylmethylcellulose and 10 μ L of 0.5M sodium fumarate. The sodium fumarate will serve as a chemoattractant for the E. coli specimen. The E. coli will again be trapped using the optical tweezer setup to compare the motility strength while in the presence of the chemoattractant. The sample can be altered again by changing its pH from slightly basic to slightly acidic using aspartate. The optical tweezers should prove to be a crucial instrument in studying the motility strength of the E. coli under different environmental conditions.

| Budget | |
|--|--------|
| Expenses | |
| Laser – 60mW Laser Diode Module and Power Supply | \$1199 |
| Flow Cells | \$123 |
| Optics and Optical Mounts | \$627 |
| Material for E. coli growth and preparation | \$56 |
| Total : | \$2005 |
| Income | |
| SPS Research Grant Funds | \$1796 |
| Benedictine University Funding | \$209 |
| Total : | \$2005 |