

Optical Trapping & Ablation

“Putting Light to Work”

Neurophysics Laboratory Final Report

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Abstract. The feasibility of optically trapping and laser ablating synthetic vesicles was explored in these experiments. The combination of optical trapping and femtosecond laser ablation is a potentially useful method of safely delivering exogenous materials to a location with a large degree of spatial and temporal resolution. Vesicles can be optically manipulated into place and ablated to release their payload to a spatially confined area. Our experiments successfully demonstrated the two main tasks of a) optical trapping and b) laser ablation of vesicles independent of each other. The experiments have also highlighted the parameters in which these operations work best.

Introduction

There has been a veritable revolution in our understanding and ability to utilize optical phenomena as tools for scientific and technological ends. The ability to create and control lasers was only the beginning of this new age. In a sea of potential uses, two techniques, in particular, seem to hold a great deal of promise for use in biological applications. These are a) optical trapping or tweezing and b) laser ablation.

Optical trapping is a technique using laser light to trap and manipulate small (nm scale) spherical objects. Laser ablation is a technique using femtosecond high power laser pulses to cut through various materials. Both techniques are in common use today in scientific laboratories for a variety of purposes. However, few applications have utilized both techniques in tandem.

In our experiments, we propose that laser ablation and optical trapping can be combined to create a novel delivery system. There are currently few practical methods to precisely deliver small amounts of chemical substances with a high degree of spatial resolution.

Applications for such a delivery system would include micro and nano scale chemistry, cellular biology, and neuroscience, amongst other uses. For example, one envisioned application is for the study of controlled neurotransmitter release. A vesicle containing the neurotransmitter of choice is created, then optically trapped and moved to a specific location close to the point of release, presumably near the active site of a specific synapse. Then, a laser is used to ablate the vesicle to cause it to release its chemical payload. (see Fig. 1)

There would be a number of advantages of such a delivery system over present alternatives such as molecular uncaging. For one, the chemical would not need to be suspended in a homogenous bath. This would require a large amount of the material, which would not be practical for rare or expensive substances. Furthermore, certain classes of material are extremely difficult to chemically cage, whereas trapping into vesicles is a relatively straightforward process that is applicable to a wide range of materials.

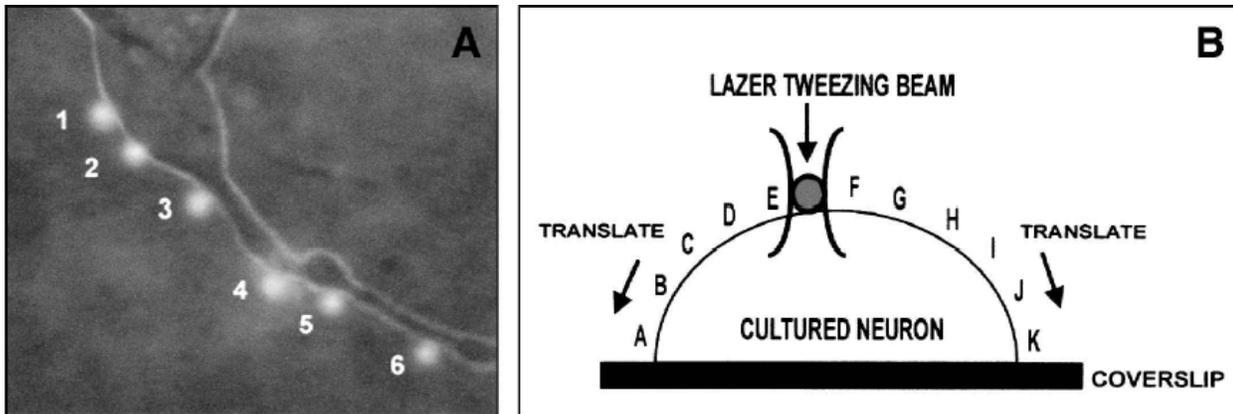


Figure 1 – (A) An overlay image showing the controlled positioning and translation (from positions 1–6) of a vesicle along the processes of an NG 108–15 cell using optical trapping. The vesicle was labeled with 2% DiO, a membrane stain. **(B)** Schematic illustrating the concept of using single vesicles, which can be positioned at different locations outside a neuronal cell, as high-spatial-resolution microsampling devices. (Chiu 2001)

In this report, a brief overview of optical trapping and laser ablation, and their respective histories and physical workings will be explored. Then a section on creation of the synthetic vesicles is followed by experimental results and discussion.

Overview of Optical Trapping

Optical trapping began as the brainchild of Art Ashkin and Steven Chu, physicists at Berkeley in the 1978. The Nobel Prize was later awarded to Steven Chu in 1997 for his application of the technique to the manipulation of neutral particles. However, the applications of optical trapping are manifest in a wide range of applications in all branches of science and technology.

For example, in Physics and Nanotechnology, optical traps are used for measuring fluid and flow forces, and Constructing and deconstructing microstructures. In micro and nano scale chemistry, individual droplets of chemical compounds can be manipulated and combined. In biology and Medicine, DNA and organelles can be manipulated to study their properties. Also, cellular manipulation and cell sorting can be performed. Furthermore, coupled with laser ablation, microsurgery can be performed on the cellular level. Perhaps the most common use in biology today, however, is the use of optical traps in measuring the forces exerted by biological structures, such as kinesin or other motor molecules.

The advantages to laser tweezer systems are many. For one thing, they are easily integrated into microscope imaging systems as the laser can simply be routed through the optics of the microscope. They are flexible & versatile, being easily scaled from one trap to many through multiplexing of the laser to trap many particles simultaneously. And they are thus altogether inexpensive.

They have excellent spatial resolution (10nm - 100um) and thus confer a large degree of dexterity and accuracy in the manipulation of micron scale objects. For biological applications, the fact that the lasers are noninvasive, sterile, and operate in the near-IR ($\lambda = 800 - 1064 \text{ nm}$) range means that there are little to no biological effect on cells.

The main drawback of optical trap systems for use in manipulation is that they produce limited amounts of force (typically pN) in comparison with other techniques using mechanical mechanisms.

Physics of Optical Trapping

Optical traps make use of radiation pressure forces on its form of scattering and gradient forces, to trap dielectric particles. The study of these interactions can be divided into two regimes, depending on the size of the particles related to the wavelength of light.

Because optical traps are usually made for biological purposes, we will focus our discussion into the Mie regime, where the diameter of the particle is much larger than the wavelength ($d \gg \lambda$). The model assumes dielectric particles transparent to the incident wavelength and with a higher index of refraction than the medium. Also, spherical shape is assumed for simplicity.

Scattering and Gradient Forces

When light is focused inside a roughly spherical object, there are two main forces that act upon it, the scattering force and the gradient force. (see Fig. 2) The scattering force results from the deflection of the photons reflecting away from the object, causing a force moving away from the light source. The gradient force results from the refraction of the light through the sphere. For a spherical object, these gradient forces are homeostatic, or they cause the sphere to center towards the center of focus of the laser beam.

Within the Mie regime, optical tweezers action can be understood, using ray tracing, as the total transmission of momentum to the particle due to light being reflected by (scattering force, F_s) and refracted through (gradient force, F_g) the particle. Under conservation of momentum, the model results in a force F_s , in the direction of incident light, and a force F_g whose direction is that of the momentum change due to the refraction. Fig. 2A shows the effect of the scattering force.

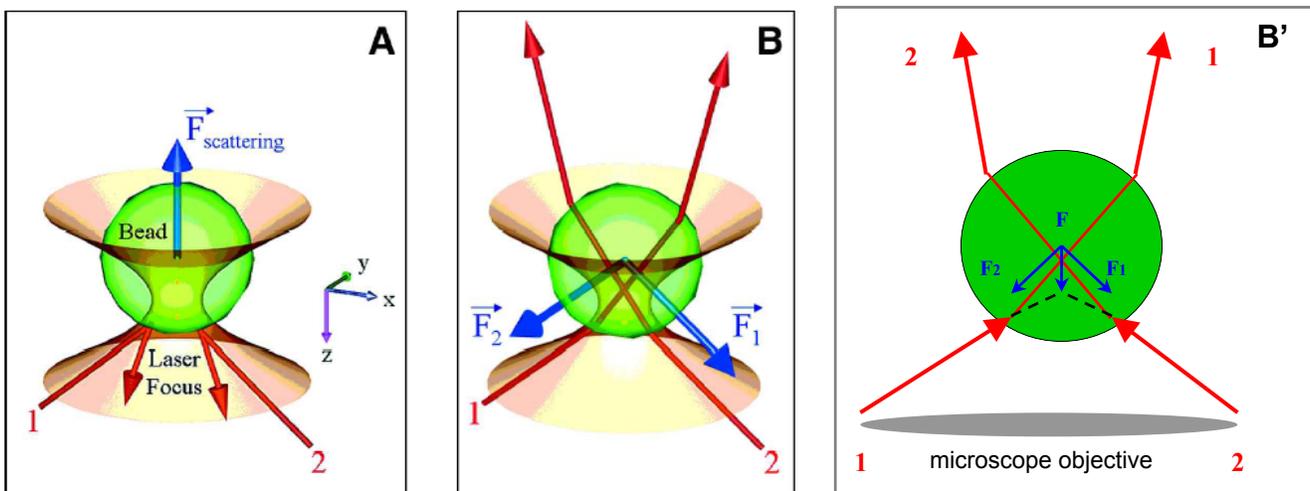


Figure 2 – (A) Effect of the scattering force on a bead. Force is in opposite direction of the z component of incident light. **(B)** Effect of gradient force on a bead when two beams of same intensity are focused below the center of particle. **(B')** Resultant force in the direction of focus.

The best way to visualize the gradient force effect is to trace two parallel rays of different intensities that are brought to focus in different places inside the particle (Fig.3)

Once the principle is understood, the number of rays traced and their intensities can be set to simulate a gaussian beam profile and get the tridimensional effect.

As the incident light is transmitted through the particle, the last interface encountered is the particle-medium one. Application of Snell's Law to this interface will result in an exiting direction of the beam with different components.

Figures 2B and 2B' shows the case where light is brought to focus in a point below the center of the particle. Both beams exit the particle at with a larger z component and a smaller x component. All the x components cancel each other, resulting in a force F directed toward the focus.

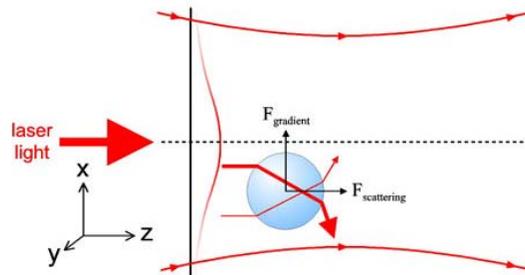


Figure 3 Visualization of gradient and scattering forces

Important Parameters

From the above analysis it can be seen that as for the scattering force concerns, the important parameter for trapping is the intensity of the laser beam.

The expression for this force is:

$$\bar{F}_{scatt} \sim \frac{1}{\lambda^4} I_o r^6 \left(\frac{m^2 - 1}{m^2 - 2} \right)^2 n_m$$

Where m is the relative index of refraction, and n_m is the index of refraction of the medium.

For the gradient force, the contrast of the index of refraction between the particles and the medium, the high numerical aperture of the microscope objective and the intensity of the laser, are the fundamental parameters. In the Mie approximation, the trapping forces do not depend on the radius of the particles.

Optical Trap Setup

Stable trapping is achieved when the gradient force in the region beyond the focus overcomes the scattering force (Svoboda). In practice, this conditions are met focusing the light into the sample plane with the highest possible numerical aperture. This will provide the steepest possible light gradients.

The optical trap setup used is shown in Fig 4.

An anamorphic prism was used to correct the elliptic beam profile due to the exit aperture of the diode laser. A telescope with a couple of $f=20\text{cm}$ and $f=30\text{cm}$ were used to increase the beam spot by a factor of 1.5, and a pinhole was placed in the inside focus of the

telescope, to clean the beam profile. The telescope is setup such that the spot size completely fills the back aperture diameter of the objective.

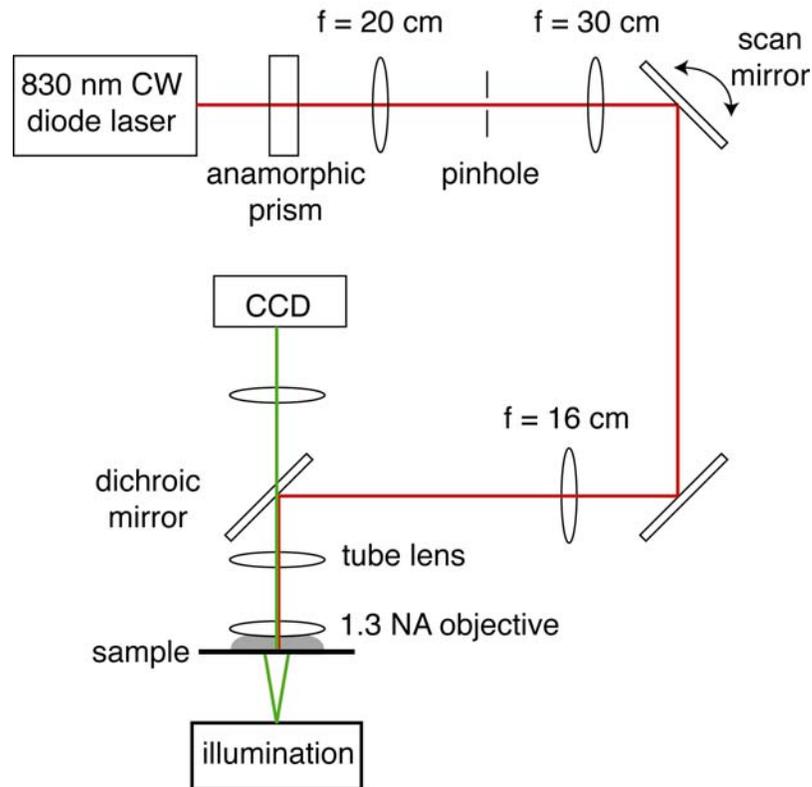


Figure 3 – Optical trap setup. Laser beam diameter fills back aperture of objective and is brought to tight focus at the image (sample) plane. Beam steering obtained by imaging scan mirror into the back aperture of the objective.

A scan mirror allows beam steering. The beam is directed toward a modified microscope, where a dichroic mirror directs laser beam towards the sample, and allow the telescope’s illumination system to image the sample into a CCD camera placed in the vertical port. Light inside the microscope tube is brought to focus by the microscope objective. A lens ($f=16\text{cm}$) was used to accomplish the adequate trapping.

The main challenge of a successful optical trap setup is achieve both of the next conditions:

1) Laser in tight focus at image plane.

This conditions is met using a high NA objective (oil immersion). A 160 mm microscope objective is made such that images nearly collimated light that is at 16 cm from its back aperture. The microscope also has a tube lens ($f=160\text{mm}$). The approximate combined focal length of this lenses is 9.4cm, as the lenses are 4.8cm apart.

To correctly focus the laser in the image plane, we should have a nearly collimated beam at a distance of approximately 14.2 cm from the objective’s back aperture.

2) Steering of the laser beam in the image plane.

Adequate steering of the beam into the image plane without vigneting is accomplished by imaging the scan mirror into the objective’s back aperture. This was done introducing a lens L1 ($f=16\text{cm}$) at a distance 42.2cm from the microscope’s back aperture. The scan mirror should be placed at 15.5cm from L1, as obtained by the lens’ maker formula.

Optical Ablation

Optical ablation is a technique that uses femtosecond pulses of laser light to cut through materials. In the experiment, the high-energy short pulses increase the probability of non-linear phenomena to occur. This way, laser causes ionization by non-linear mechanisms that damage the cell walls and cause the release of the vesicle payload into the surrounding solution. A layout of the ablation setup is shown in Fig. 6.

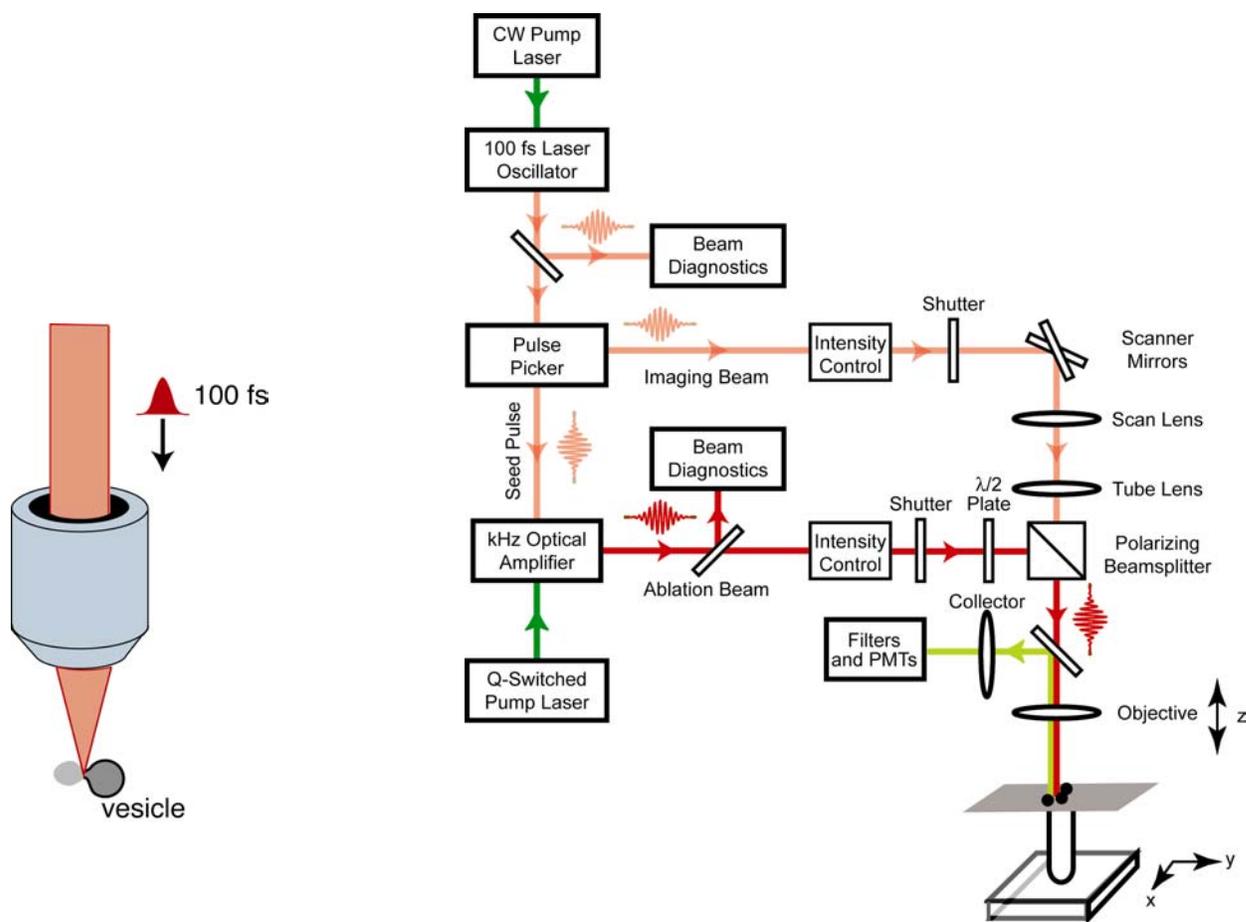


Figure 5 (left) – Optical ablation technique focuses high energy femtosecond laser pulses on a vesicle to damage walls. **Figure 6 (right)**- Optical ablation setup. Two-photon microscopy is used to image fluorescein labeled vesicles. High-energy femtosecond pulses ablate vesicles membrane.

Methods

Synthetic Vesicles

Vesicles are lipid bilayer “bubbles” containing fluid. They can be created via a mixture of phospholipids suspended in an organic phase and aqueous phase (containing the vesicle

payload). The mixture is then evaporated at high temperature in vacuum to spontaneously form vesicles.

These vesicles, or liposomes, are excellent model systems for studying the dynamics and structural features of many cellular processes, including viral infection, endocytosis, exocytosis, cell fusion, and transport phenomena. In addition to having importance for basic research in biological disciplines, liposomes are used as vehicles for drug application, for gene transfer in medical therapy and genetic engineering, and as microcapsules for proteolytic enzymes in the food industry. Vesicles also open many exciting possibilities for chemical reactions in small confined volumes.

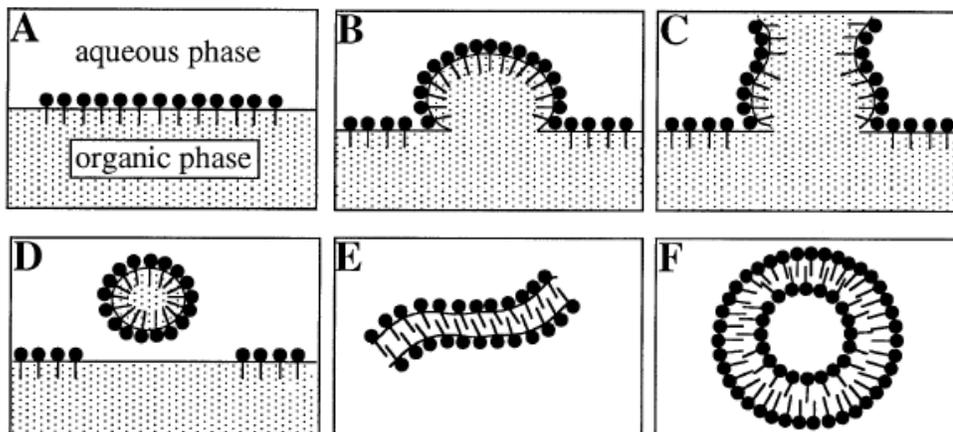


Figure 7 - Diagram of the suggested formation mechanism for GUVs. Initially there is an ordered monolayer of phospholipids at the interface between an aqueous and an organic phase (A). During evaporation, bubbles form (B) that rupture the phospholipid film into fragments (C). The resulting phospholipid monolayer fragments fuse to bilayers (E), which spontaneously vesiculate (F). An alternative way for the formation of bilayered phospholipid fragments (D) involves micellar structures having entrapped liquid organic solvent. (Moscho)

Vesicle Preparation

There are two solutions, the organic and the aqueous phase that should be prepared ahead of time. For the organic phase, lipids were dissolved in chloroform (0.1 M), and 20 ml of this solution was added to a 50-ml round-bottom flask containing 980 ml of chloroform and 100–200 ml of methanol. The aqueous phase consists of Hepes buffer (10 mM, pH adjusted to 7.4 with NaOH), and the desired chemicals that will go inside the vesicles, in this case, fluorescein (10 mM). This aqueous phase (7 mL) was then carefully added to the organic by pouring along the flask walls.

This mixture was then heated to 40 degrees Celsius, a vacuum tube was attached and the whole assembly (flask and vacuum tube attachment) set upon a rotary shaker. There should be two boiling points observed within minutes of applying the vacuum. After about 3 minutes, the liposomes should be formed.

To separate the liposomes, pour 1 mL of the supernatant into an Eppendorf and centrifuge at 10,000 rpm for 5 minutes. Pour off supernatant and add Hepes buffer (1mM) until 1mL. Centrifuge and pour off supernatant. Repeat as many times as necessary until the solution becomes clear.

Experimental Results & Discussion

Optical Trapping

Only successful 2-D trapping was observed. Although the required condition $F_g > F_s$ was met, stable z trapping was not achieved. A possibility of trapping was only found when the beam was focused near the bottom place of the slide, allowing trapping against the surface of the slide. Different beads and vesicle samples were used in order to increase contrast of the refraction indexes, and the beam was cleaned at different points of the setup, but with no positive results.

Energy losses from poor beam profile seems to be the answer (see Fig. 7)

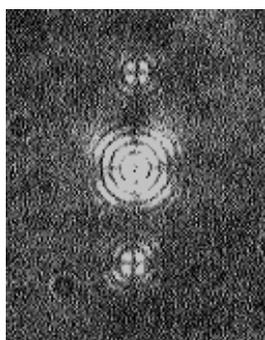


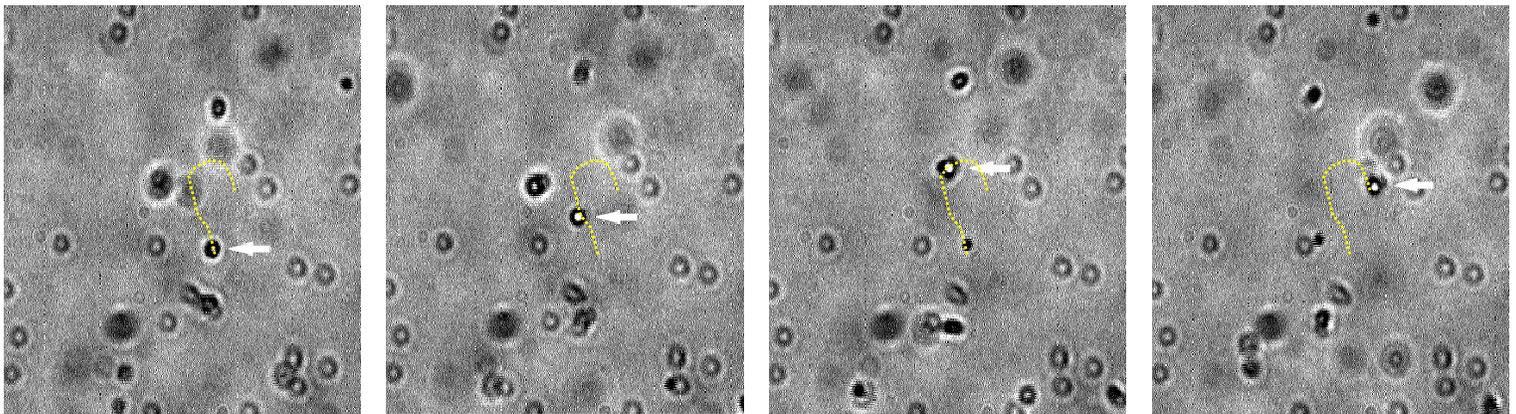
Figure 7 – Beam profile at the image plane. Two second-order diffraction spots coming from laser aperture. Circular interference pattern due to thin film of scan mirror.

A clean tight focus is fundamental for effectiveness of the gradient force. Although the microscope objective's back aperture was completely filled, and a high NA was used, intensity looses and a poor beam profile decreased our gradient force. Two second-order diffraction spots due to the rectangular cavity of the laser create a triple trap, and an interference pattern generated at the thin film coat of the scan mirror, avoided a clean focus. The later problem can be addressed by simply using a different mirror. The earlier, will only be solved by used a different laser with a better mode.

The reader will find extremely useful reference [7] to follow and understand the optical setup. Although theoretical calculations using geometric optics can be done to find the appropriate parameters for the optical setup, it would be kept in mind that further adjustments will be made if the calculations are not done for the wavelength of the laser being used.

A further step in the experiment would be to characterize the trap by measuring its trapping efficiency and stiffness (N.Malagnio, et.al.)

Sucrose Vesicles Trapping



t = 0 s

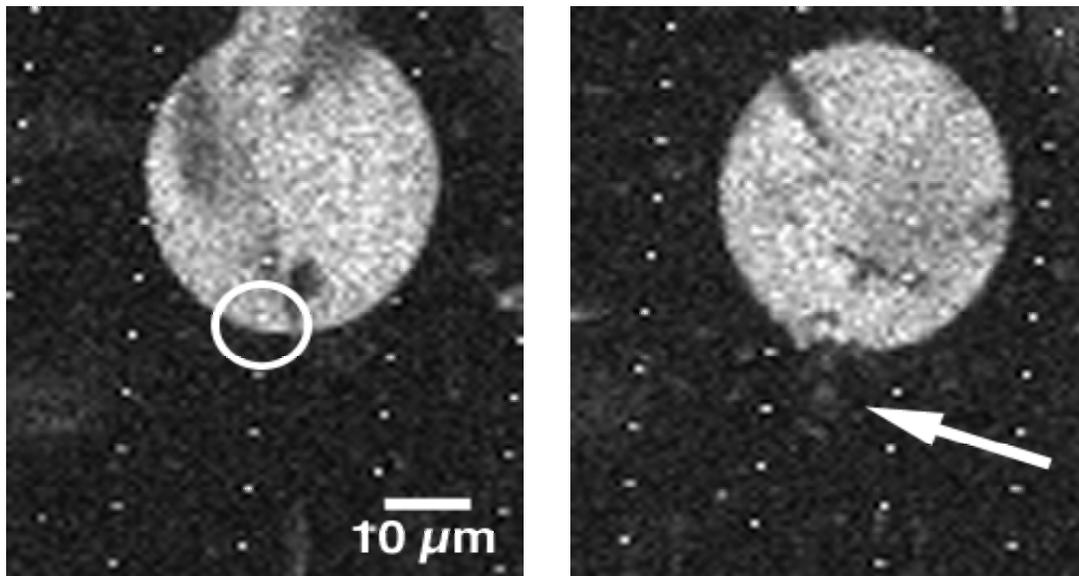
t = 1 s

t = 2 s

t = 3 s

Figure 8 – Successful x-y trapping was achieved. Sequence show a sucrose vesicle (1 μ m) moved along a curved path. Vesicles other that the one marked with the arrow, moved from frame to frame due to Brownian motion.

Fluorescein-Dextran Vesicle Ablation



t = 0 s

t = 250 ms

Figure 9 – Fluorescein-Dextran vesicle ablation. The circle indicates the laser target. The arrow points to the hypothesized diffusion emanating from the hole.

Ablation

Two major challenges presented themselves during the ablation process. A) It was found that the vesicles were much more resilient than originally thought. Quickly after ablation, the vesicles seemed to reseal very quickly. B) It was unclear whether there was the temporal resolution to see the diffusion occur.

To address the second question, we used the large molecule Fluorescein-Dextran in order to slow diffusion times. Some calculations were performed to see what the time scale of diffusion is. Diffusion time traces were calculated using Fick's Diffusion Equation. A [.01 M] source was step-pulsed and diffusion across space was calculated.

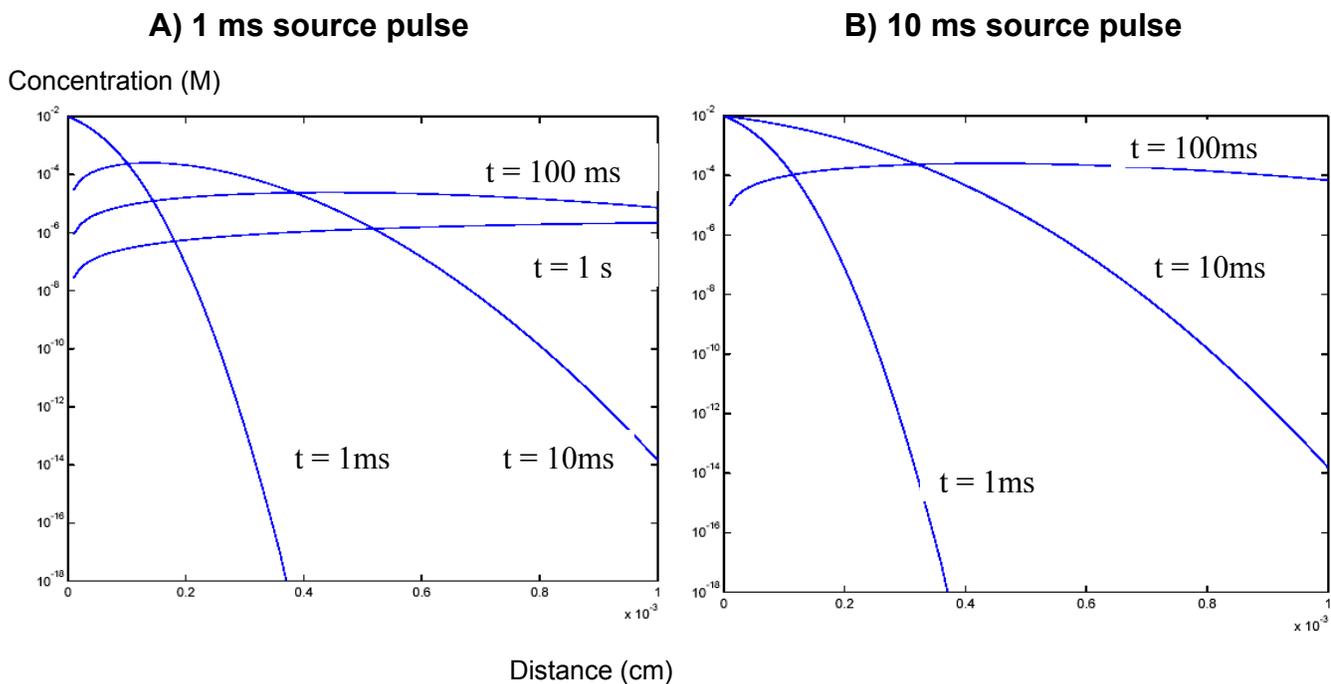


Figure 10 – Calculated Diffusion curves of various starting concentrations of Fluorescein-Dextran diffusing across space. A) 1 ms source pulse. B) 10ms source pulse

From the graphs above, it becomes clear that diffusion becomes quickly difficult to see within 250ms of the pulse. Unfortunately, we were not able to achieve such temporal resolution with our photographic equipment, despite increasing the scan rate. This might be one explanation for our inability to clearly define when diffusion was occurring.

To address the first question of creating a long lasting “hole” in the vesicle walls that would allow significant payload diffusion we did the following: A) Using Bengal-Rose oxidizer to disrupt the membrane so that membrane reforming would be hindered. B) Changing the laser scan geometry to excise larger holes near the apex of the vesicles. C) Increasing inner payload and fluorescence concentrations. However, none of these changes seemed to make visualizing diffusion any less elusive.

Conclusions & Future Directions

We demonstrated that the 2 main parts of the overall system - optical trapping of vesicles and vesicle ablation - work well independently. Both systems seemed to have aspects that could be improved upon. The optical trapping can be improved with better optics and a better laser, for example, in order to overcome the problem that we faced in trapping in the z direction. The ablation system seems to work well, but our imaging systems do not yet have the ability to scan at a speed fast enough to detect the diffusion. This could also be countered with faster scanning equipment. All in all, the problems that surfaced are not insurmountable, and can largely be solved with equipment upgrades, if not improved techniques.

Several methods of overcoming limitations in our equipment had to be derived, including creating vesicles with optimized properties for imaging, trapping, and ablation. However, these are either limitations in equipment that can easily be overcome, or they were experiment specific parameters (such as being able to visualize diffusion) that were imposed by us. For example, other experiments may not need to visualize the diffusion, and thus may not have the same stringent requirements placed upon the project.

In conclusion, both laser ablation and optical trapping subsystems are on track for full integration into a combined system. We anticipate no significant hurdles in combining the two subsystems to form a complete manipulation and ablation system. This would be the next step and would complete the goal of simultaneous trapping and ablating of vesicles. Afterwards, endless possibilities would exist in the application of such a completed system.

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