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I am submitting herewith a thesis written by William Neil Robinson entitled “Simulation of Single Molecule Trapping in a Nanochannel.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of requirements for the degree of Master of Science, with a major in Physics.

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Simulation of Single Molecule Trapping in a Nanochannel

A Thesis
Presented for
the Master of Science
Degree
The University of Tennessee, Knoxville

William Neil Robinson
August 2008
Dedication

This thesis is dedicated to the doctors and nurses who worked at the Blanchfield Army Community Hospital in 1983. I’m grateful for their hard work back then and glad to prove them wrong.
Acknowledgements

The writing of this thesis could not have been completed without the patience and guidance of my advisor, Lloyd Davis. Also, many thanks go to Bruce Whitehead and Horace Crater for agreeing to be on my committee at short notice. I would also like to thank my parents for their support and for always pushing me. I owe Zbigniew ‘Peter’ Sikorski a large debt for letting me share his office my first months here and for helping me with my simulation. I’d also like to thank Dr. Parigger for being so supportive. He always had time to answer my questions even though he wasn’t on my committee. Finally, I would like to thank the friends I’ve made at UTSI. My sanity would have run out a long time ago without you guys.
Abstract

Trapping of single fluorescent molecules in solution is numerically simulated. Optical trapping provides insufficient force for trapping molecules much smaller than the optical wavelength. Instead, a means for trapping by sensing the molecule position and applying real-time feedback of flow to compensate diffusional displacement is used. The solution is contained in a nanochannel, reducing the problem to one spatial dimension. The position of the molecule is estimated from the fluorescence signals generated by two focused laser beams, which originate from a single laser source that is split and temporally alternated between the two focal spots. Photon collection is time gated, and photons collected in the two detection channels are used to find the maximum-likelihood estimate of the molecule position and adjust the electrokinetic motion to reposition the particle. Adjustment of the simulation parameters leads to a multi-variable analysis of the trapping effectiveness. For the range of parameters considered in this thesis, trapping is found to be robust and stable. However, the maximum speed of electrokinetic motion that would be possible in an experimental implementation limits the capabilities of the trap. Accordingly, the maximum likelihood position estimate provides little or no advantage for trapping over simpler algorithms. A simpler feedback algorithm is proposed and demonstrated to provide effective trapping. Also, in consideration of when molecular photobleaching becomes significant, an algorithm for quickly reloading the trap with a new molecule is developed and tested in a second simulation.
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1 Introduction

A single molecule may be readily detected in a confocal microscope but diffusion limits the molecule’s residence time within the confocal volume and hence the maximum observation time. This thesis deals with the spectroscopy and trapping of a biomolecule in a nanochannel and presents an approach for countering diffusion.

In work by Enderlein [1], feedback is used to cancel particle diffusion, enhancing observation capabilities. He proposes that confocal microscopy can be used to track a fluorescent molecule across a spatial range in a two dimensional membrane. This could be done with wide-field microscopy, but this approach has a worse signal-to-noise than confocal microscopy. Also, confocal microscopy allows for observation of sub-nanosecond timing of fluorescence events with single-photon avalanche diode (SPAD) detectors. Time-correlated single-photon counting has the desirable property of being able to measure the fluorescence decay lifetime, but the detectors used, photomultiplier (PM) tubes and SPAD detectors, provide no spatial information in of themselves.

A focused laser spot scanning in a circular pattern can be used to determine spatial information and thereby perform tracking of a particle. The signal given by the detector will modulate according to the position of the fluorescent particle, being more constant when the particle is near the center of the scanning circle and more intensely modulated as the particle is displaced from this position. If polar coordinates are used, the intensity of the modulation would therefore give $r$ the radial coordinate and the phase of the modulation would provide $\theta$ the angular position [1]. Feedback can then be used to control a piezoelectric translator. Berglund
and Mabuchi have tracked individual fluorescent particles by use of a scanning laser focus with single-photon excitation [2]. Two-photon experiments with the same scanning pattern have been performed by the group of Gratton [3].

Cohen and Moerner [4-9] have developed the anti-Brownian electrophoretic (ABEL) trap, which uses feedback to trap a particle. They initially used a CCD camera to image the particle. To decrease the time delay before feedback in later experiments, they used the circular scanning focused laser spot technique. Electrodes provided for electrokinetic transport of the particle in two dimensions, the third dimension being confined by the walls of the fluidic device. This method was employed instead of laser tweezers, which generate insufficient forces for trapping particles smaller than ~100 nm [10].

Another technique for controlling particles is magnetic tweezers [11]. A DNA molecule or another particle can be attached to a magnetic bead a few microns in diameter and manipulated with electromagnets.

One group has performed tracking on quantum dots in three dimensions [12]. Using a confocal microscope, fluorescence was imaged onto four optical fibers each connected to a separate SPAD detector. The fibers collected light from points arranged in a tetrahedron to provide position information in all spatial dimensions. A translating sample stage, controlled by feedback, provided a means for recentering the quantum dot. The dots had a diffusion constant of ~0.7 µm²/s, which is far slower than the particles covered in this thesis. Quantum dots also have the benefit of not photobleaching, although the fluorescence is usually intermittent [12].
There is clear interest in tracking single molecules, particularly proteins, in living cells. Levi and Gratton have done work on this with various probes including colloidal gold and quantum dots [13].

This thesis discusses the simulation of the trapping of small particles in a nanochannel [14]. There has been increasing interest in single-molecule trapping in solution [15]. We desire to trap a particle at photon count rates of less than $10^5 \text{ s}^{-1}$ using maximum likelihood position estimation and electrokinetic control. Higher photon count rates would yield positional information more quickly but would be difficult to achieve experimentally and would decrease the time before photobleaching.
2 Principles of the Trapping Method

2.1 Irradiance and Fluorescence

The particle is confined to a narrow channel, which simplifies the problem to one dimension. In order to trap in this dimension, the position of the particle along the nanochannel must be determined. This information then provides the feedback to adjust the electrokinetic motion of the particle and thereby keep it in the region of observation. A CCD camera is not used for position determination because it is desired to use single photon detection with a SPAD detector for faster feedback.

In order to determined the position along the nanochannel, time-gated photon detection and a distinct laser irradiance pattern are used. This pattern is formed by splitting a single laser beam with a beam splitter into two beams, which are focused into the nanochannel at closely spaced points separated by an adjustable distance.

The laser is mode-locked and puts out picosecond pulses separated by $T=13.2$ ns. One of the two beams is delayed by 6.6 ns so that the excitation pulses at each focal spot alternate in time, with 6.6 ns between the excitations.

Photon collection is time-gated into two channels. Each channel counts photons that fall within the 6.6 ns intervals that follow each set of excitation pulses at each of the two laser foci. Fluorescence photons generated by each laser focus generally fall into the time channel corresponding to that focus. However, if the fluorescence decay takes longer than 6.6 ns, the released photon will be counted after the next laser pulse, causing the photon to be registered in
the incorrect time channel. Such events are called cross-talk and lead to decreased precision in
the prediction of the molecule position.

If the fluorescence lifetime is $\tau$, the probability that a photon will fall into the incorrect
time channel is $\alpha$, given by [14]

$$
\alpha = \sum_{n=0}^{\infty} \int_{(2n+1)\tau/2}^{(2n+2)\tau/2} \left(\frac{1}{\tau}\right) \exp\left(-t/\tau\right) dt
$$

$$
= \left(1 + \exp\left(T/2\tau\right)\right)^{-1}
$$

(1)

For $\tau = 3$ ns and $T = 13.2$ ns, $\alpha = 10\%$. The actual cross-talk experienced in the simulations is
discussed in Section 4.1.

The irradiance profiles for each laser spot are assumed to be Gaussian along the
nanochannel and virtually constant across the axial width of the channel [16]. The adjustable
distance between the laser foci is set to be two times the full width at half maximum (FWHM) of
each spot as shown in Figure 1, which is located in the Appendix along with all other figures and
tables. A separation distance of twice the standard deviation of the Gaussian profiles provides
greatest slope of the irradiance from each laser spot at the center of the trap and hence greatest
sensitivity for position determination. However, the narrower spacing would decrease the size of
the trap and hence require more stringent trapping conditions.

**2.2 Maximum Likelihood Position Estimation**

The detected photons are processed by maximum likelihood methods to estimate the
position of the particle and thereby provide feedback for the electrokinetic motion, which
displaces the molecule back towards the center of the trap. The distance of displacement is limited by the maximum electrokinetic velocity achievable in an experimental setup.

In the maximum likelihood method, the probability to obtain the observed numbers of photons in each of the two time channels if the molecule is at a given location $x$ is calculated; the location $x$ that yields the maximum probability is then the best estimate.

The probability to collect $n_1$ and $n_2$ photons in the time channels 1 and 2 if the molecule is at position $x$ is given by the binomial distribution

$$P(n_1, n_2 | x) = \frac{(n_1 + n_2)!}{n_1! n_2!} p_1(x)^{n_1} (1 - p_1(x))^{n_2},$$

where $p_1(x)$ is the probability that a photon is collected in the time channel 1 if the molecule is at $x$.

If the fluorescence signal were linearly proportional to the irradiance and if there were no cross-talk, $p_1(x)$ would be the ratio of the irradiance from beam 1 to the total irradiance:

$$p_1(x) = \frac{I_1(x)}{I_1(x) + I_2(x)}.$$  

With cross-talk, there is a probability $\alpha$ that a photon generated by one beam will be detected in the time channel corresponding to the other beam. In this case

$$p_1(x) = \frac{(1 - \alpha)I_1(x) + \alpha I_2(x)}{I_1(x) + I_2(x)}.$$  

With no cross-talk the position $x$ that yields the maximum of equation (2) can be found analytically [14]. In general, equation (2) can be evaluated at a grid of discrete locations $x$ and
the value of $x$ that yields the maximum of equation (2) can be found numerically. In practice, the combinatorial factor $(n_1+n_2)!/(n_1!n_2!)$ can be dropped as it is independent of position.

In response to the position estimate, voltages are to be altered to adjust the electrokinetic motion of the molecule. In practice, this would occur after a time delay or latency. Accordingly, an adjustable time delay, typically 0–6 μs, is included in the model before implementing the change in the position of the particle. Further details are given in section 3.3.
3 Simulation Components

3.1 Diffusion

The numerical simulation considers a single particle diffusing on a one dimensional grid. The grid must be fine compared to the size of the laser waist. The waist for most of the simulations was set to 0.5 µm, and the grid spacing was set at \( \Delta x = 0.01 \) µm. The long axis of the channel is taken to be \( x \), and the other dimensions are small by comparison. The channel has a region of interest with length of 40 µm, which corresponds to 400 grid points. If the particle leaves this region, it is no longer simulated.

Diffusion is modeled by Fick’s second law of diffusion,

\[
\frac{\partial \rho}{\partial t} = D \nabla^2 \rho, \tag{5}
\]

which is derived in Bunfield’s thesis [17]. Here, \( \rho(x)dx \) is the probability to find a molecule within \( dx \) of \( x \), and \( D \) is the diffusion coefficient. With the initial condition,

\[
\rho(x, t = 0) = \delta(x), \tag{6}
\]

where \( \delta(x) \) is the Dirac delta function, the solution of equation (5) may be shown to be

\[
\rho(x, t) dx = \frac{1}{\sqrt{2\pi\sigma(t)}} \exp\left[\frac{-x^2}{2\sigma^2(t)}\right] dx, \tag{7}
\]

which is a normalized Gaussian distribution with standard deviation \( \sigma(t) = \sqrt{2Dt} \).
The time step $\Delta t$ for the simulation of diffusion is chosen so $\sigma(\Delta t)$ is equal to the grid spacing $\Delta x$. For $\Delta x = 0.01 \mu m$ and a diffusion coefficient of $D = 4.14 \times 10^{-7} \text{cm}^2 \text{s}^{-1}$ [18], $\Delta t = 1.2 \mu s$. In each cycle $\Delta t$ of the simulation the particle will move $i$ grid spaces, where $i$ is a random number.

The diffusion algorithm is detailed in a flow chart in Figures 2a and 2b. Table 1 provides the probabilities $P_i$ mentioned in Figures 2a and 2b that the particle will diffuse the given number of grid spaces $i$ with each time step $\Delta t$. These were generated by the following integral over $\rho(x,t)$ with $\sigma = 1$,

$$P_i = 2 \int_{a_i}^{b_i} \rho(x,t)dx.$$  

The limits of the integral, $a_i$ and $b_i$, change for each value in the table. For a diffusion of zero grid spaces, limits of $(0, \sigma/2)$ are employed. The rest are listed in the table.

In theory, the particle could diffuse any amount within each time step $\Delta t$, but the probability to diffuse by more than $6 \sigma$ is less than one part in $2^{32}$. For the 32-bit random numbers used here, the probability of diffusing more than six intervals is beneath the precision available, hence the simulation provides for 0-6 grid spaces of diffusion per time step.

In simulation, a random number is generated between zero and one. If this value falls between two values in the Cumulative column of Table 1, then the molecule will move the number of grid positions equal to the corresponding $i$ value of the greater Cumulative value. For example, if the random number was 0.4, then the molecule would move one grid space. A second random number then determines whether this movement will be to the left (-1) or the right (+1) with equal probability for each case. If the first random number was 0.5, then the molecule would move zero grid spaces, and the second random number is ignored.
3.2 Verification of Discrete Diffusion Model

The simulations presented have assumed that a discrete model of diffusion correctly approximates smooth diffusion without bias. To verify this assumption, the diffusion algorithm was extracted from the simulations and placed inside test code adapted from Landau [19]. Landau supports this assumption and cites that in properly modeled discrete diffusion the magnitude of position increases linearly against the square root of the number of discrete steps.

Landau’s method measures diffusion over many trials each with a set number of steps \( N \). As each trial is being run, the magnitude of position for each step from one to \( N \) is recorded. These magnitudes are averaged into an array of data points which is plotted against \( \sqrt{N} \).

Landau’s algorithm had movement in two dimensions. A random number was thrown for movement in two dimensions, and the resulting translation vector was normalized. For the method used here, the motion was only in one dimension. The normalization is discussed in the following paragraph, and the details of the diffusion model are detailed in section 3.1.

The step size must be normalized to one in order to yield a slope of one. In Landau’s case, every step was the same size, so normalization involved dividing the actual step size by its own magnitude. In this case, the step size is between zero and six in magnitude. Thus, to normalize the step size, every step was divided by \( \sqrt{2\pi / 2} \), the integral of a Gaussian function with \( \sigma = 1 \) from zero to infinity, which serves as the average step size.

Figure 3 shows the results for ten thousand trials of one hundred thousand (\( N \)) steps. These values have yielded a highly linear relationship as theory predicts. The slope of the data is 1.029.
3.3 Photophysics

As shown in Figure 4, when irradiated by a laser, the molecule can become excited from the ground state $S_0$ to the $S_1$ manifold. Fluorescence is handled, like diffusion, by Monte Carlo methods. The photophysics algorithms used in the simulation discussed in this paper owe heavily to the simulations done by Bunfield [17] and Davis and Li [20].

The molecule has a rate of excitation $k_e$ given by

$$k_e = \sigma_a I(x)/E(\lambda).$$

(9)

Here, $\sigma_a$ is the absorption cross section, $E(\lambda)$ is the photon energy, and $I(x) = I_1(x) + I_2(x)$ is the total irradiance of the two focused lasers at the location of the molecule.

$N_0(t)$ is the probability of the molecule being in the ground state. The rate of transition from the ground state to the excited state $dN_0(t) / dt$ is given by

$$\frac{dN_0(t)}{dt} = -k_e N_0.$$ 

(10)

With the initial condition $N_0(0) = 1$, this yields a solution for $N_0$,

$$N_0(t) = \exp(-k_e t).$$

(11)

Therefore, the time of excitation is determined by an exponentially distributed random number with mean $1/k_e$.

Once excited, the molecule may decay to the ground state, with or without emission of a photon, cross to the triplet state, or photobleach as shown in Figure 4. The path followed is determined randomly. One random number is compared with the probability of decay without photon detection, the most probable event. If the random number is greater than this, then it is
compared to the cumulative probabilities of less probable events in order such that a minimum number of comparisons are made. For example, if a random number of .9886 were chosen, it would be greater than the probability of emission without detection (.94899) but less than that for emission without detection plus emission with detection (.94899 + .05), and hence the outcome would be emission with detection.

For the molecule, there is a rate of inter-system crossing ($k_{isc}$), a rate of fluorescence decay ($k_f$), a rate of decay without emission ($k_Q$), and a rate of photobleaching ($k_b$). In terms of collection efficiency $\Phi_c$ (which includes both collection and detection) and the aforementioned rates, the probability of decay without detection is the most probably event and is given by

$$\frac{k_f(1 - \Phi_c) + k_Q}{(k_f + k_Q + k_{isc} + k_b)} \sim .95.$$ \hspace{1cm} (12)

Detectable photon emission occurs in 5% of cases and is the next most probable event compared to the previous case. The probability of photon detection is

$$\frac{k_f \Phi_c}{(k_f + k_Q + k_{isc} + k_b)} \sim .05.$$ \hspace{1cm} (13)

It could also decay through a triplet state which decays to the ground state with an exponentially distributed mean time of 1 µs [17]. The probability of inter-system crossing occurring and subsequently the arrival of the particle at the triplet state is

$$\frac{k_{isc}}{(k_f + k_Q + k_{isc} + k_b)} \sim 10^{-3}.$$ \hspace{1cm} (14)

Finally, the probability of photobleaching is

$$\frac{k_b}{(k_f + k_Q + k_{isc} + k_b)} \sim 10^{-5},$$ \hspace{1cm} (15)
although in the initial simulation, this value is set to zero in order to avoid photobleaching so that the mechanism of the trap may be observed for longer times.

The desired outcome of excitation is detectable photon emission. At each time step, the number of photons detected from each laser since the last step is passed to the maximum likelihood algorithm to predict the molecule’s location.

A flowchart of the program is found in Figure 5. At the beginning of the flowchart, the program decides when the next excitation of the molecule would occur as explained in the beginning of this section. This time is dependent on the irradiance and thus on the position of the molecule. Hence, if this time of excitation would occur after the next diffusion event, then diffusion occurs first, and the determination of the next excitation event is recalculated using the new position of the molecule.

Once excitation occurs, the simulation resets the particle’s excitation/decay status and generates a random number to determine the decay outcome as detailed earlier in this section. If detection occurs, then the photon is counted and the cycle of excitation and/or diffusion restarts.

3.4 Trapping

Before the particle emits fluorescence photons, it cannot be seen. To simulate an experimental setup, the particle is transported towards the origin until it emits six fluorescence photons. Then it is assumed to be detected and trapping begins.

Once the position is estimated by the maximum likelihood algorithm, its estimate is passed to the translation subroutine for simulation of electrokinetic flow in the channel. The interfacing of this into the diffusion algorithm can be seen in Figure 2b. The molecule is shifted
back towards the center by an amount equal to its displacement but limited by the maximum achievable electrokinetic flow. This velocity was picked as 1.67 μm/ms. This value is a low-end estimate interpolated from the mobilities used by Cohen and Moerner [7] for an 80 kDa target molecule. This value was picked because the capabilities of adjustment for the experimental component of this project were not known, and it was desired to simulate a hardest-case scenario. The operating parameters for this case are listed in Table 2. For these, the amount of electrokinetic motion possible is only one grid spacing per five time steps. The block “Is it time for translation?” in Figure 2b is thus true one in five times the diffusion algorithm is run.

The strict limit of electrokinetic flow limits the usefulness of the maximum likelihood calculation. Replacement of this algorithm with a simpler one is discussed in Chapter 5.

Also, for the data presented in Figures 9-16, photobleaching is disabled. The capture times greatly exceed the actual lifetime of the particle, so to better determine the capabilities of the trap, it is necessary to allow it to perform for greater amounts of time than photobleaching allows. For 100 μW of power in each beam, a 0.5 μm beam waist, and a fluorescence lifetime of 3ns, the survival time of the particle, i.e., the length of time from entry into the simulation until photobleaching is thus an approximately exponential distribution with a mean of 8.2 ms. Figure 6 provides a graph of the lifetimes.

3.5 Second Simulation for Reloading the Trap

In other work conducted in parallel with this thesis, initial experiments in our lab with nanochannels fabricated in fused silica have indicated that diffusion is slowed by approximately a factor of 50 compared with that in bulk solution.
Lower diffusion rates make the requirements of trapping less stringent, but trapped molecules can be expected to photobleach before they escape the trap. In such a case, it will be important to actively transport a new particle into the trap as soon as the trapped molecule photobleaches.

A field programmable gate array (FPGA) would be used to control the experiment. Part of the goal of this next simulation is to test an algorithm that is simple to implement for use in the FPGA.

In an actual experiment, there will be a background count rate that the first simulation has thus far ignored. In the first simulation, on the order $10^5$ photons were received per second. Background generates only hundreds of photons per second. This is less than 1% of the photons received and was not modeled.

For this second simulation, the background was modeled. Though there is the same amount of background, it is necessary to model it in order to distinguish between an empty and occupied trap.

An algorithm with consideration of background has been developed to handle the entry and trapping of molecules. Figure 7 contains a flow chart of this algorithm. Upon detection of the $N$-th photon, the simulation checks the arrival time of photon $N$ against photon $N-2$. If the difference in their arrival times is less than a threshold time, then it can be assumed that the photons are from fluorescence and not from background.

If the timing between photon $N$ and photon $N-2$ is greater than the threshold time, then it can be assumed that the molecule has bleached and the emission is from background.
Also, there will be at least six photons collected before position correction begins. The initial velocity of the system is set to maximum to bring in a new particle as quickly as possible. After six photons are collected without their arrival times exceeding the timing threshold, the velocity is adjusted.

Each photon may be detected in the first or second time channel as before. If the photons detected in the first channel outnumber those from the second, the particle is assumed to be on one side of the center, the left for example. The velocity is adjusted to the right at maximum to move the particle towards the trap center. If second channel photons outnumber first channel photons, then the velocity is set to left. In the case that the number of first channel photons equals the number of second channel photons, the velocity will be turned off but will continue to be adjusted in subsequent time intervals.

See Figure 8 for a flowchart of the second simulation in total. This flowchart shows the principle difference between this simulation and the previous one very easily. In summary, the different features are the consideration of background, the measurement of the time between photons $N$ and $N-2$, and the mandatory use of the last six photons for velocity adjustment.
4 Data Analysis

4.1 Results of First Simulation

Results of the first simulation are displayed as graphs of the particle’s trajectory versus time. There is no clear way to judge whether the particle has escaped the trap unless the particle exits the simulation, so it is necessary before analyzing results to present examples of free diffusion and of what will be called good and loose trapping.

For an example of loose trapping, see the graph of the particle’s trajectory under the trapping force in Figure 9. The parameters utilized are a beam waist of 1 µm and 100 µW of laser power. The trapping is weak for these settings, with the molecule departing from the center and subsequently returning.

Examine Figure 10 for a graph of regular (non-trapping) diffusion for comparison. It is convenient to consider anything over 1 µm too weakly controlled to be considered trapped.

Compared with the 1 µm case and the case of non-trapping, Figure 11 displays the case of good trapping with 0.5 µm beam waist laser foci. The performance is improved visibly. The standard deviation from the center for the 1 µm beam waist case was 0.3 µm and for this case .09 µm. The laser foci have to be adjusted to remain at each other’s FWHM, so they are at the following positions (to the left and right of the center of the trap) dependent on the beam waist: 0.589 µm for the one micron beam waist and 0.294 µm for the 0.5 µm beam waist.

To measure trapping effectiveness, the root mean square (RMS) deviation about the origin is calculated. This is the same as the standard deviation of position with the origin taken
as the mean. The standard deviation is the square root of the variance. The variance is calculated in the simulation starting when the particle first passes through the origin to when the simulation ends. The following formula was used for variance,

\[ s^2 = \frac{1}{n-1} \sum x^2. \] (16)

Here \( x \) represents the molecule position at each time step of the simulation and \( n \) represents the number of time steps. The origin is between the laser foci at the center of the trap.

For the case of good trapping, the RMS position is .09 µm. Weak trapping has an RMS position of .33 µm, and free diffusion in the example above had an RMS position of 1.68 µm. For free diffusion, this value is highly variable, but in general, anything greater than 1.0 µm shall be considered unhindered diffusion.

See Figure 12 for a graph of RMS position versus beam waist, with the laser power held fixed at 100 µW. The performance decreases rapidly as the beam waist increases, but then the curve flattens out for a beam waist of about 1.5 µm. At this point, it is in the range of deviation for free diffusion. The trap is not effective for those values.

The other consideration for trapping performance is the number of photons per feedback interval. The size of the beam waist influences this significantly, with Figure 13 showing the number of photons per beam waist size. The performance from the previous graph is easily cross-referenced against the number of photons used per position adjustment. More photons create more accurate adjustments and tighter trapping.

Laser power also affects trap performance. The performance becomes very poor for a laser power less than 0.05 mW, and becomes steadily but slowly better for power greater than
that as seen in Figure 14. Power greater than about 0.3 mW is not feasible in a realistic experiment.

Another factor in the performance of the simulation is the fluorescence lifetime. An increased lifetime leads to increased cross-talk, and thus decreases the performance of the trap as seen in Figure 15. In general, an increase in the lifetime was found to decrease performance, as might be expected due to increased cross-talk. However, there is an increasingly large band of error in the RMS position. In Figures 12-15, the error bars were obtained from the standard deviation of the data that was averaged for each point.

The maximum likelihood method for position determination predicts the most probable position based on received photons. Figure 16 shows a graph of estimated versus actual molecule position. For examination of this technique, a set of parameters has been produced that generates a working number of photons per position estimation (~10^5 s⁻¹). This graph shows the estimated position versus the actual position for ~3.5 photons per estimation. The graph also reveals a weak correlation because of the effects of shot noise, i.e., statistical error due to the small number of photons. Cross-talk, occurring for 11% of collected photons (1% higher than predicted), contributes only slightly to the weak correlation of the estimation. For these parameters, maximum likelihood calculation of the particle’s position has not been shown to be better than simple photon counting whereby the laser beam with the higher photon count per interval receives the full electrokinetic push. The estimation here guesses the correct polarity of the position at a ratio of ~ 2:1, and the use of the simple photon counting algorithm developed for the second simulation provides very similar results.
4.2 Results of Second Simulation

Figure 17 presents the particle trajectories during the second simulation for rapid reloading of the trap. The time between particle exit and entry of the next molecule is not recorded on this graph to prevent the graph from being primarily white space.

A key factor that affects performance is the timing threshold. If the threshold is too large, then the simulation won’t be able to distinguish fluorescence from background. If it’s too short, then the simulation will eject active particles when photon emission fluctuates.

There is an expected background count of 500 s⁻¹, which corresponds to 1 photon per 2 ms on average. The threshold is set to 1 ms in simulation. With these settings, performance was such that approximately half the time, the simulation ejected the particle early. The ratio of proper exits to early exits was 5:6 when the particle was started in simulation at a distance of 1 µm away from the origin. The distance of 1 µm is within the irradiance profiles. When the particle is started at 5 µm, the ratio of early exits was over 100:1.

Adjustments of the threshold value did not significantly change the performance, and setting the threshold near or above 2 ms would prevent the simulation from properly registering photobleaching.
5 Conclusions

In conclusion, these results show that trapping in a nanochannel should be feasible in an experimental setup. The maximum likelihood calculation provides enough information to control the particle’s position. However, the calculation determines particle position with limited reliability because of statistical error due to the small number of photons available for each determination. Thus, given the slow rate of electrokinetic adjustment, it cannot be shown to be better than simple photon counting. Regardless, it is sufficient for trapping.

The beam waist is a critical part of the setup, and a beam waist of 0.5 µm coupled with the other settings in Table 2 will produce good trapping between the laser foci. Within a broad range of achievable parameters, it will be possible to have the particle trapped until photobleaching occurs. The fluorescence lifetime also has an effect on trapping performance, but being unique to the chosen particle, cannot be adjusted like laser power or beam waist.

The trap performance would improve significantly if the laser power could be increased past 0.1 mW to produce a higher photon count rate. Even doubling it would result in a significant performance increase. In an experiment, the effect of increasing laser power will have to be weighed against the increased rate of photobleaching.

The simulation has demonstrated trapping for times spanning the time of several photobleaching events. Indeed, it’s actually difficult for the particle to diffuse far from an area of interest before photobleaching even when trapping is not active. The trap serves more to decrease the area of confinement than to actually prevent the particle from leaving the area.
The algorithm for handling reloading of the trap is effective despite its frequent pre-emptive ejections. It should be possible to refine the algorithm to less frequently abort fluorescently responsive particles. Lower background counts would allow for longer periods of study with the existing algorithm if the threshold could be lowered.

Other possibilities would depend on the requirements of the experiment. If the amount of time between bleaching and the study of a new particle must be minimized, then the current algorithm is acceptable. If it becomes important to maximize the time that each particle is studied, then without altering the time threshold for background, it would be possible to implement repetitive testing. A ‘multiple strike’ rule could be implemented, whereby the particle had to exceed threshold several times before being declared photobleached. Also, the problem might simply be solved by adding a delay after (possibly early) detection of photobleaching and before particle ejection.

Future simulations will involve three-dimensional trapping regions. Tracking in three dimensions has been done [21], and it is believed that trapping in three dimensions using similar methods employed in the current setup should be possible.
References


Appendix
Figure 1. Intensity versus position. This shows the irradiance profiles along the nanochannel of beam one, $I_1(x)$ and beam two, $I_2(x)$, and the total irradiance (dashed line).
Figure 2a. Flowchart of diffusion algorithm part 1.
Figure 2b. Flowchart of diffusion algorithm part 2.
Table 1. Diffusion Probabilities. These numbers are the probabilities of diffusion on the grid.

<table>
<thead>
<tr>
<th>i</th>
<th>$a_i$</th>
<th>$b_i$</th>
<th>$P_i$</th>
<th>Cumulative</th>
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<tr>
<td>1</td>
<td>$\sigma/2$</td>
<td>$3\sigma/2$</td>
<td>0.4834606750</td>
<td>0.4834606750</td>
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<td>0</td>
<td>0</td>
<td>$\sigma/2$</td>
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<td>0.8663855976</td>
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<tr>
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<td>$3\sigma/2$</td>
<td>$5\sigma/2$</td>
<td>0.1211950719</td>
<td>0.9875806695</td>
</tr>
<tr>
<td>3</td>
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<td>$7\sigma/2$</td>
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<td>0.9995347420</td>
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<tr>
<td>4</td>
<td>$7\sigma/2$</td>
<td>$9\sigma/2$</td>
<td>0.0004584628118</td>
<td>0.9999932048</td>
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<tr>
<td>5</td>
<td>$9\sigma/2$</td>
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<td>0.00006757367124</td>
<td>0.9999999622</td>
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<tr>
<td>6</td>
<td>$11\sigma/2$</td>
<td>$13\sigma/2$</td>
<td>0.0000003789880492</td>
<td>1.0</td>
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</table>
Figure 3. Position magnitude vs $\sqrt{N}$, where $N$ = number of steps. The green line is from simulation and the red from theory.
Figure 4. Photon decay possibilities. This is a Jablonski diagram for the decay possibilities of the molecule. \( S_0 \) is the singlet ground state. \( S_1 \) is the singlet excited state, and \( T_1 \) is the triplet state.

- **a** \( \rightarrow \) Decay with no detected photon emission
- **b** \( \rightarrow \) Decay with detected photon emission
- **c** \( \rightarrow \) Cross to triplet state
- **d** \( \rightarrow \) Photobleach

\[ k_e \quad k_Q \quad k_f \quad k_{isc} \quad k_T \quad d \quad k_b \]
### Table 2. Simulation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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<tr>
<td>Fluorescent Decay</td>
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<tr>
<td>Triplet Decay</td>
<td>$10^{-6}$ [s]</td>
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<tr>
<td>Absorption Cross-Section</td>
<td>$2 \times 10^{-16}$ [cm$^2$]</td>
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<tr>
<td>Laser Power</td>
<td>$10^{-4}$ [W]</td>
</tr>
<tr>
<td>Beam Waist (weak trapping)</td>
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<tr>
<td>Beam Waist (strong trapping)</td>
<td>0.5 [μm]</td>
</tr>
<tr>
<td>Photon Energy</td>
<td>$3.6811 \times 10^{-19}$ [J]</td>
</tr>
<tr>
<td>Laser Foci Distance from Center (weak trapping)</td>
<td>$5.89 \times 10^{-5}$ [cm] or</td>
</tr>
<tr>
<td>Laser Foci Distance from Center (strong trapping)</td>
<td>$2.94 \times 10^{-5}$ [cm]</td>
</tr>
<tr>
<td>Laser Spacing (Temporal)</td>
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<tr>
<td>Photon Detection Probability</td>
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<tr>
<td>Triplet Probability</td>
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<tr>
<td>Bleach Probability</td>
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<td>Width of Simulation</td>
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<tr>
<td>Grid Resolution</td>
<td>$1 \times 10^{-2}$ [μm]</td>
</tr>
<tr>
<td>Diffusion Constant</td>
<td>$4.14 \times 10^{-7}$ [cm$^2$s$^{-1}$]</td>
</tr>
</tbody>
</table>
Figure 5. Flowchart of program cycle.
Figure 6. Approximately exponential distribution of molecule lifetime until photobleaching.
Figure 7. Flowchart of FPGA algorithm.
Figure 8. Flowchart of simulation for particle reloading.
Figure 9. Diffusion with weak trapping. Settings in use: 1 µm beam waist, 0.1 mW laser power, 3 ns fluorescence lifetime. Compare this with Figures 10 and 11. Laser foci positions shown as dashed red lines.
Figure 10. Free Diffusion. The trapping was disabled here and the particle was free to diffuse in each case. Each color represents a separate simulated instance. The particle started at the origin in each case. The dashed red lines represent the laser beam spacings for a beam waist of 0.5 µm.
Figure 11. Diffusion with strong trapping. The beam waist is set to 0.5 µm and the laser foci are spaced at 0.294 µm as the FWHM distance is dependent with the beam waist. The particle is visibly more tightly confined in this graph than in the case of weak trapping or free diffusion.
Figure 12. Root mean square position of particle versus beam waist. Settings in use: 100 µW laser power, 3ns fluorescence lifetime.
Figure 13. Photons per electrokinetic adjustment versus beam waist. A set number of photons are used to adjust the electrokinetic flow at intervals. That number is related to the beam waist.
Figure 14. Root mean square position versus laser power in each of the two beams. Below 0.05 mW, there is a sharp decay in trapping performance. Above it, there is a broad area of acceptable performance.
Figure 15. Root mean square position versus fluorescence lifetime.
Figure 16. Estimated versus actual position. The maximum likelihood calculation of position is not very precise. The red line represents the ideal case where the actual and estimated positions are equal to each other. At the settings in use here (0.5 μm, 0.1 mW, 3 ns fluorescence lifetime), the maximum likelihood calculation only guessed the correct polarity of position at a ratio of roughly 2:1 using ~3.5 photons per time step. Only the values between −0.3 and 0.3 μm are shown in the above figure.
Figure 17. Position versus time in particle reloading simulation. Note the three particle entries. This data set displays an initial entry which survived bleaching long enough to be trapped, a second entry that did not survive entry, and a final entry which was trapped for a longer time than the first.
Vita

William Neil Robinson was born in the Fort Campbell Blanchfield Army Community Hospital on March 3, 1983. He received his undergraduate degree attaining a double major in Physics and Mathematics at Athens State University in Athens, Alabama in May 2006. Since June 2006, he has been at UTSI working towards his Masters and Doctorate in Physics.