

# Application of Optical Traps In Vivo

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## ***I. Introduction***

A number of groups have combined sophisticated optical-trapping setups with bead assays, to study the function of single molecules *in vitro* in exquisite detail. Such experimental setups have been well documented in a number of review articles<sup>1,2,3</sup>. However, much less has been written about using an optical trap to make quantitative measurements *in vivo*, the topic of this review.

## ***II. What is an optical trap?***

An optical trap results when a high-numerical aperture lens is used to focus a laser beam to a diffraction-limited spot (see fig. 1). When a small dielectric object such as a vesicle, lipid droplet, or polystyrene or silica bead is placed in the beam, it feels a force pulling it towards the beams' focus. The magnitude of the exerted force is a function of a number of parameters: the size and shape of the object, the difference between the index of refraction of the object and the surrounding medium, and the gradient in the intensity of the laser beam. The Force,  $F$ , the trap exerts on an object is a linear function of the object's displacement,  $x$ , from the trap center, i.e.  $F=-kx$ , where  $k$  is the trap stiffness. Deviations from this linear relationship are observed at the very edges of the beam, typically approximately 200 nm from the beam's center. The applied force increases linearly with laser power. The significance of object size and index of refraction differences are discussed below. Typically, a force of 10's of pN's can be applied to a small (half micron) bead, though it is possible to exert more than 100 pN of force on a few  $\mu\text{m}$  bead using a high-powered laser. While in principle any laser can be used to make an optical trap, in practice infra-red lasers are employed, because they cause the least optical damage (see below).

## ***III. Basic trap design***

The basic optical trap is quite simple: a single-mode laser with Gaussian beam profile ( $\text{TM}_{00}$ ) is passed through a beam expander (so that the beam fills the back aperture of the microscope objective, in order to use the entire lens, which generates the largest gradient in the focused beam), and then steered with a combination of mirrors and lenses into the microscope objective. This objective focuses the light in the sample plane of the microscope to a diffraction limited spot, forming the trap. The construction of such a basic optical trap is discussed in detail in a review article by Steve Block<sup>4</sup>.

## ***IV. Typical uses of an optical trap in vitro***

*In vitro*, optical traps are used to study polymers such as microtubules<sup>5</sup> or DNA<sup>6</sup>, and to measure properties of single molecules such as Titin<sup>7</sup>, or molecular motors like Kinesin<sup>8,9</sup>. Most of these experiments are conceptually similar: attach a glass or

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  4. S. M. Block, in "Cell Biology: A Laboratory Manual" (D. L. Spector, R. Goldman, and L. Leinwand, eds.). Cold Spring Harbor Press, 1998.
  5. H. Felgner, R. Frank, and M. Schliwa, J Cell Sci 109, 509 (1996).
  6. M. D. Wang, H. Yin, R. Landick, J. Gelles, and S. M. Block, Biophys J 72, 1335 (1997).
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polystyrene bead to the molecule or polymer of interest, and use this bead as a ‘handle’ to manipulate the molecule. To be able to exert force on the molecule or polymer of interest, the molecule in question is also attached independently to an immobilized binding partner. As well as studying the strength of molecular interactions<sup>10</sup>, less traditional molecular motors, such as RNA polymerase have also been studied<sup>11</sup>. Most of the optical traps used in these experiments are quite sophisticated. In particular, they involve quadrant-photodiode detection setups to precisely measure the displacement of the trapped bead from the center of the trap to determine the force that the bead is applying on the molecule or polymer being studied. Further, many of these setups include a feedback loop, so that the force on the trapped object can be kept constant (either by modulating the laser power, or the position of the laser relative to the trapped object)<sup>1,2,11</sup>.

### ***V. Typical uses of an optical trap in vivo***

Many *in vivo* applications of optical traps have also been discussed, though the majority of studies have employed traps in a non-quantitative manner. In this regard, optical traps have been used to manipulate the relative position of biological objects. For instance, cells have been sorted<sup>12</sup>, fibroblast cells brought into contact with a surface to investigate adhesion<sup>13</sup>, sperm have been brought into contact with eggs for *in vitro* fertilization, and chloroplasts and other internal organelles have been displaced intracellularly to investigate their mobility, and also the importance of the location of the organelle on its function.

There has also been progress in using optical traps to address quantitative questions *in vivo*. The majority of these studies have been conceptually similar to *in vitro* studies which use a bead as a handle to manipulate an attached protein or other object. In such cases, the beads are attached to proteins or antibodies that interact with the cell’s exterior to examine cellular response<sup>14</sup>, or investigate externally accessible properties such as the tension of the cell membrane<sup>15</sup>, or the lateral mobility of membrane-bound proteins<sup>16</sup>. Cells’ responses to externally applied forces have also been examined<sup>17</sup>. Because the manipulated bead is in the extra-cellular buffer and of known size and shape, these applications are effectively *in vitro* experiments with regard to the optical trap; the trapping setups described for *in vitro* work are all more than sufficient to make such measurements.

Finally, there have been some attempts to use optical traps to make quantitative measurements where the trapped object is itself in the cell. In these cases, one can directly trap and manipulate a cellular organelle such as a mitochondrion<sup>18</sup>, or lipid

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  18. A. Ashkin, K. Schutze, J. M. Dziedzic, U. Euteneuer, and M. Schliwa, Nature **348**, 346 (1990).

droplet<sup>19</sup>, or in principle introduce appropriately coated beads into the cell through endocytosis or microinjection. This review will discuss issues that arise as a result of attempting these measurements: local heating, optical damage, the importance of the index of refraction of the cytoplasm, and determination of the force applied to the trapped object.

## VI. Heating

When focusing an intense laser beam into a small volume of the cell, the first question to be addressed is how much the area will be heated, and concurrently, how much optical damage the laser will cause. Both of these concerns are to some extent dependent on the exact details of the cellular environment, but there are a few general guidelines.

Typically, to minimize absorption of the laser light by either the proteins or the water in the cell, a laser in the near infrared (800-1100 nm) is used. Because water is relatively transparent to light in this part of the spectrum (see<sup>20</sup> for the absorption versus wavelength), heating is relatively mild. The localized heating due to an optical trap has been measured experimentally and the general rule of thumb for a laser operating at 1064 nm is to expect laser-induced heating of 1.45 deg-C for every 100mW of laser power entering the sample<sup>21</sup>. The temperature rise is very rapid (about 1 ms). Only a small amount of the input laser power is absorbed; the rest passes through the sample. The absorption is not a linear function of the incident power, and can be calculated using

Beer's law which is  $A = \epsilon bc = \log_{10} \left( \frac{P_{in}}{P_{out}} \right)$ , where A is the absorption,  $\epsilon$  is the Lambert

absorption coefficient,  $b$  is the path length of the light,  $c$  is the concentration of the absorber (in this case set to 1 for water),  $P_{in}$  the incident laser power, and  $P_{out}$  the power in the beam after it exits the sample. From this law, we calculate that the power absorbed

by the sample is  $P_{abs} = (P_{in} - P_{out}) = P_{in} \left( 1 - \frac{1}{10^A} \right)$ . As an example, using the

experimental data from 1064 nm, let us calculate the expected heating from 830 or 980 nm lasers. From Palmer and Williams<sup>20</sup> we see that at a laser wavelength of 830 nm,  $\epsilon = 0.03 \text{ cm}^{-1}$ , for 980 nm  $\epsilon = 0.5 \text{ cm}^{-1}$  and for a laser operating at 1064 nm,  $\epsilon = 0.14 \text{ cm}^{-1}$ . We assume that in the experimental measurements that determined a heating of 1.45 degrees/100 mW (at 1064 nm), the path length was  $10^{-3} \text{ cm}$  (10  $\mu\text{m}$ ). Then,

$$\frac{P_{abs,1064}}{P_{abs,830}} = \frac{\left( 1 - \frac{1}{10^{0.14 \times 10^{-3}}} \right)}{\left( 1 - \frac{1}{10^{0.03 \times 10^{-3}}} \right)} = 4.67, \text{ so the Beer-Lambert law suggests we should expect}$$

4.67 times as much heating from a 1064 nm laser than from a 830 nm laser, i.e we expect a temperature rise of roughly 0.31 deg C/100 mW at 830 nm. However, the absorbance of

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21. Y. Liu, D. K. Cheng, G. J. Sonek, M. W. Berns, C. F. Chapman, and B. J. Tromberg, *Biophys J* **68**, 2137 (1995).

water is significantly higher at 980 nm than 1064, and we calculate  $\frac{P_{abs,980}}{P_{abs,1064}} = 3.54$ ,

implying that a 980 nm laser should cause roughly 3.5 times the heating of a 1064 laser, or approximately 5.1 deg C/100 mW. Note that the quoted laser power of 100 mW is the power in the sample, not the power at the laser itself. Since a good 100X objective only transmits about 50-60% of the incident light, including other optical elements between the laser and the sample, typically approximately 45% of the laser light enters the sample. Thus, trapping with a (nominal) 200 mW laser functioning at 1064 nm in practice causes a rise of roughly 1.3 deg C in the sample. The heating due to either a 1064 nm or 830 nm laser is probably irrelevant, but heating due to a 980 nm laser may be an issue, depending on how sensitive the process being studied is to the local temperature.

### ***VII. Optical Damage: choice of laser wavelength***

While any laser can be used to make an optical trap, a few wavelengths are preferred due to minimized optical damage. A number of studies have investigated optical damage, primarily using two types of assays to measure cell health. In the first, the efficiency of cloning was measured as a function of exposure to the optical trap<sup>22</sup>. In the second, *E. coli* bacteria were tethered to a surface by single flagella, and their attempt to swim resulted in regular rotations of the bacteria. The rate of rotation was used as an indicator of bacterial health<sup>23</sup>. Both studies came to the same conclusion: lasers at 980 nm do the least damage. 830 nm is second best, with the commonly used 1064 nm wavelength a distinct third. On a scale where the damage at 980=1, the damage at 830=1.27, and the damage at 1064=2.5<sup>22,23</sup>. Thus, an 830 nm laser does roughly 30% more damage than a 980 nm laser, while a 1064 nm laser does 2 and a half times as much damage.

It is surprising that these independent studies came to the same conclusion with regard to optical damage since they are presumably sensitive to different factors: in the cloning efficiency study the laser is focused on the nucleus, and damage presumably occurs inside the nucleus, while in the second the laser is focused on a part of the bacteria, and appears to affect metabolic processes. One possibility<sup>23</sup> is that the mechanism of damage is relatively generic, e.g. due to the generation of free oxygen radicals that damage whatever they encounter.

Additional studies by Liu et. al<sup>24</sup> used a number of different probes to investigate whether optically trapping cells (at 1064 nm) had effects on DNA structure, cell viability, and intracellular pH. No effect on either DNA structure or intracellular pH was observed at up to 400 mW. Some loss of viability for low-motility sperm was observed when they were held for extended periods (more than 2 minutes) at 300mW.

### ***VIII. Are there ways to decrease optical damage?***

Given that damage has been minimized to the extent possible by appropriate choice of laser wavelength, how can damage be decreased? If possible, the most practical

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  23. K. C. Neuman, E. H. Chadd, G. F. Liou, K. Bergman, and S. M. Block, *Biophys J.* **77**, 2856 (1999).
  24. Y. Liu, G. J. Sonek, M. W. Berns, and B. J. Tromberg, *Biophys J.* **71**, 2158 (1996).

solution is to increase the size of the trapped object—using the same power, a 0.5  $\mu\text{m}$  bead feels significantly less force than a 1  $\mu\text{m}$  silica bead (see below). The presence of oxygen is also a key factor in determining optical damage: in the bacterial assay<sup>23</sup>, eliminating oxygen using an oxygen scavenging system (introduced just before trapping) resulted in a 3-6 fold decrease in damage. While the direct use of such a scavenging system is possible in vitro and when studying anaerobic organisms, it is of little utility when studying eukaryotic organisms. However, adding quenchers of singlet oxygen to the growth media<sup>23</sup> (e.g. antioxidants, etc.) might be helpful in this latter case, but to date there is no reported evidence supporting the efficacy of such additives.

### ***IX. How much power can be used?***

The amount of power that can be used depends to some extent on the experiment. The bacterial assay suggests that optical damage is a gradual (rather than catastrophic) process, so minimizing the length of laser exposure is important. The assay using the efficiency of cell cloning as a readout for optical damage (trap focussed on the nucleus) found that a 1 minute exposure to 176 mW (in the sample) had no effect on cloning efficiency at 830 nm or 980 nm, and only reduced cloning efficiency by 10% at 1064 nm. A three minute exposure of 176 mW had more effect, reducing the cloning efficiency by 50% at 830 nm, 20% at 980 nm, and 70% at 1064 nm. So for manipulation of nuclei, it is probably reasonable to use up to approximately 200 mW power (in the sample) as long as a 830 or 980 nm laser is used, and exposure times are kept relatively short.

As far as manipulating mitochondria or other organelles, the amount of power that can be used again depends on wavelength. At 1064 nm, this has been measured directly: using up to 340 mW (measured after the objective, but before the sample) to manipulate and hold an organelle causes no obvious damage, even when the organelle is held for a number of minutes<sup>25</sup>. However, damage starts to appear at powers greater than 370 mW, though again only when the object is trapped for a number of minutes<sup>25</sup>. This result is consistent with the work of Ashkin et. al., who found that a 220 mW trap (the maximum power they required to stop all moving mitochondria, with the power of the 1064 nm laser measured at the sample plane) did no apparent damage to moving mitochondria<sup>18</sup>-- motion of the organelles was the same before and after brief periods of immobilization in the trap.

Since both the bacterial health and the cloning assays show that 830 nm is significantly preferable to 1064 (by roughly a factor of 2) and heating at 830 nm is less than at 1064 (by a factor of 4.67, see above), I estimate that at 830 nm one can probably use approximately double the maximum power possible at 1064, i.e. 680 mW at the sample. Of course, the shorter the exposure, the better. For many experiments the manipulated object needs to be trapped only for seconds rather than the minutes of exposure used in the damage experiments.

### ***X. How much power is really needed?***

In practice, I have generated forces of more than 8 pN on 0.5  $\mu\text{m}$  diameter lipid droplets<sup>26</sup> using a (nominal) 200 mW 830 nm laser, of which only approximately 35%

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26. S. Gross, M. Welte, S. Block, and E. Wieschaus, J. Cell Biol. 148, 945 (2000).

made it through the lens (i.e. approximately 70 mW at the sample). Using 220 mW (at the sample), Ashkin et. al<sup>18</sup> were able to stop all moving mitochondria. The typical molecular motor exerts a force somewhere between 1 and 6 pN, and the limited experimental data in vivo suggests that at most approximately 5 motors function together on the same cargo<sup>18,19</sup>. Thus, the largest force one would estimate would be required for studies of cargo transport, to be able to stop all moving cargos, would be 30 pN. Since maximum stalling force is linearly proportional to laser power, assuming cargos similar to the lipid droplets, this would require approximately 300 mW at the sample, well within the range of what can be used at 830 nm. Since many motors require less than 6 pN to stall, this 300 mW is more or less an upper limit on the power required after the lens, for organelle-type studies. Thus, in general it should be possible to use enough power to stall any molecular-motor driven cargo in vivo. However, a priori it is not possible to make a definite estimate of the amount of power needed for a specific application because the force that can be applied in vivo will strongly depend on the optical properties of the trapped object, e.g. its size and refractive index. Thus, it may not be possible to effectively manipulate very small (200 nm) vesicles.

### ***XI. Which laser to chose?***

A number of factors must be considered in choosing which wavelength laser to use. If small laser powers will be used, it probably does not matter much. If large powers are likely, optical damage and heating become more of an issue. If the paramount concern is optical damage, 980 nm is best. However, if quantitative measurements are being made which could be affected by heating, it is probably better to use an 830 nm laser, trading a small increase in optical damage for a large decrease in heating. However, working at 830 potentially involves high cost: currently, the highest power single-mode (TM<sub>00</sub>) 830 nm diode is 200 mW, sold by Melles-Griot (around 6000 US\$, including controller). The only way to get more power at 830 nm is to use a Ti:Sapphire laser, which typically costs approximately 60,000 US\$, including a good pump laser. While a reasonable alternative, Ti:Sapphire lasers tend to be more fussy to use than the other diode lasers available at 830, 980, and 1064 nm. At 980 nm, the choice of lasers is much greater; a 1 W MOPA laser (980 nm) can be had for approximately 10,000 US\$. Finally, there a number of outstanding lasers at 1064 (Nd:YVO<sub>4</sub>), with good beam stability and an excellent beam profile. One of the best of these lasers is made by Spectra-physics (Topaz), has a 3 W output, and costs roughly 30,000 US\$. Of course, at 1064 there are many less expensive alternatives as well. Even though 830 nm is probably preferable, for many studies the difference is insignificant—in the majority of cases one will end up using significantly less than the maximum 340 mW possible at 1064 nm.

### ***XII. Importance of particle size and index of refraction***

For a trap based on a TM<sub>00</sub> laser beam (Gaussian beam profile), the axial force (along the beam path) is weaker than the lateral force (pulling the object in towards the beam center). However, for both axial and lateral forces, at a given displacement from the trap center, the force a trapped object feels is a function of its size: up to about 4 μm, the larger the object, the more force it feels. Theoretical calculations for objects whose size is small relative to the wavelength of the trap light (Rayleigh particles) suggest a cubic

dependence ( $\text{Force}_{\text{max}} \sim d^3$ ), where  $d$  is the objects size<sup>27</sup>. However, for trapped particles of the same approximate size as the light's wavelength (e.g. 500 nm vesicles in an 830 nm trap), this dependence is somewhat weaker, in general somewhere between linear and quadratic. Empirically, for trapped latex beads, the maximum lateral force applied on a 1.02  $\mu\text{m}$  bead was 7 times that on a 0.3  $\mu\text{m}$  bead, and the maximum force on a 2.97  $\mu\text{m}$  bead was 2.47 times more than on the 1.02  $\mu\text{m}$  bead<sup>28</sup>.

The applied force is also a function of  $n$ , the relative index of refraction defined as  $n=n_1/n_2$ , where  $n_1$  is the index of refraction of the trapped object and  $n_2$  is the index of the surrounding medium. A good discussion of this can be found in Ashkin's review article<sup>29</sup>. For lateral forces, the larger  $n$ , the larger the applied force. Up to a point, this is also true for axial forces. By way of a typical example, consider how much the applied force changes when an object (e.g. a lipid droplet,  $n_1=1.52$ ) is in water ( $n_2=1.33$ ) versus in cytoplasm ( $n_2=1.39$ ). Using Ashkin's published values (and a bit of linear interpolation) we find that going from  $n=(1.52/1.33)=1.14$  to  $n=(1.52/1.39)=1.09$  results in a decrease of the applied lateral force by approximately 20%. So, relatively small corrections are needed to enable calibrations to be done in vitro, and actual measurements to be done in vivo.

### ***XIII. Particle-cover slip distance***

While in vitro the trapped object is manipulated very close to the cover slip, controlling the object-cover slip distance is often not possible in vivo. High NA Oil-immersion microscope lenses are corrected to decrease spherical aberration, but only close to the cover slip. As the lens is focused deeper into the sample, aberration increases, which results in a decrease in image quality, and more importantly, a decrease in trapping power. In practice, the extent of the change depends on the properties of the trapped object: in vitro, the effect is significantly more pronounced for polystyrene as opposed to silica beads. As a rough estimate, as the distance between the coverslip and the trapped object increases from 5  $\mu\text{m}$  to 30  $\mu\text{m}$ , there is a roughly 50 % decrease in the applied trapping force, for the same laser power (see Felgner et. al for experimental data<sup>28</sup>).

While spherical aberration is important when working at depth, the extent of object-cover slip viscous coupling must be considered when the trapped object is relatively close to the coverslip. This effect does not actually alter the maximum force that the trap can apply, but does alter the way the trapped object moves, because its motion results from its exposure to both viscous and trapping forces. Thus, if one attempts to move the trapped object rapidly relative to the coverslip, the apparent trapping force is lower than might be expected if the object is close to the coverslip, because the viscous force opposing motion is high. Stokes law (discussed below) can be corrected to reflect this increased viscous drag; the functional form of the correction is given in Felgner et. al<sup>28</sup>. Experimentally, given the same relative motion a 0.5  $\mu\text{m}$  bead feels twice as much viscous drag at 1.5  $\mu\text{m}$  from the coverslip than at 4  $\mu\text{m}$ <sup>30</sup>.

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27. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, *Optic Letters* 11, 288 (1986).

28. H. Felgner, O. Muller, and M. Schliwa, *Applied Optics* 34, 977 (1995).

29. A. Ashkin, in "Laser Tweezers in Cell Biology" (M. P. Sheetz, ed.), Vol. 55, p. 1. Academic Press, San Diego, 1998.

<sup>30</sup> J. Dai and M. P. Sheetz, in "Laser Tweezers in Cell Biology" (M. P. Sheetz, ed.), Vol. 55, p. 157. Academic Press, San Diego, 1998.

Thus, for quantitative measurements, it is important to pay close attention to the distance of the trapped object from the cover slip. If the sample geometry is such that one needs to work at depth, it is worth considering using a high NA water-immersion lens, which is designed to minimize spherical aberration over a much larger range of focusing depths. However, unless absolutely necessary these objectives are not optimal for trapping, because they have a slightly lower numerical aperture(1.2), so that the gradient in the focused beam (and hence trapping strength) is decreased. They are also quite expensive (~10,000 US\$).

#### ***XIV. Determination of the applied force***

In general, there are two ways that optical traps are used to determine stalling forces. In one approach, the ‘position determination method’, a high laser power is used to stop all moving beads (or other objects), and then the force required to stop the bead is determined from the position of the bead in the trap, having previously calibrated the relationship between bead displacement and applied force. The second method instead looks at the ‘escape force’. In this approach, the initial calibration determines how much force it takes to remove the object entirely from the trap, as a function of laser power. Then, force measurements on a specific object are made by fixing the laser at a given power, and by scoring whether at that power (and hence applied force) the object is able to escape from the trap. This was the method used by Gross et. al.<sup>26</sup>. As a variation on this method, if the object’s behavior is not rapidly changing, initially a high laser power can be used such that all the objects are trapped. Then, the laser power is gradually reduced to find the power at which the object escapes.

There are advantages to both methods. The ‘position determination method’ is typically used in vitro, and has the advantage of extremely high spatial and temporal resolution. However, it requires precise determination of the location of the trapped object relative to the trap center, usually to within a few nanometers. Such determination usually involves a sensitive detection setup that involves a quadrant photodiode and good electronic amplifiers, which can be somewhat involved. The quadrant photodiode can be placed either in the back focal plane of the microscope<sup>2</sup>, or used in an imaging mode<sup>1</sup>. When operating in the back focal plane, the deflection of the laser beam is measured after it passes through the sample. This method has the advantage that it does not measure the global position of the trapped object, but rather is sensitive only to the displacement of the object relative to the trapping beam. This often turns out to be impractical for in vivo work, where there can be numerous large scattering centers in the sample but after the trap’s focus. These additional scattering centers cause additional (random) beam deflections, so that it is impossible to determine which contributions result from the trapped object alone. Thus, for most in vivo applications the quadrant photodiode should be imaged. One drawback of imaging the photodiode is that only a very small part of the field of view is imaged, so the trapped object to be measured must be carefully positioned. Thus, for such an approach to be practical in vivo requires an accurate computer controlled x-y translational stage to move the object to the appropriate location.

Instead of using a quadrant photodiode, particle tracking can be used to determine the particle’s position so that the position-determination method can be employed without sophisticated electronics. With a good image, current software can

determine the location of the center of a 0.5  $\mu\text{m}$  bead to within 8  $\text{nm}$ <sup>26</sup>. For a trap which produces a maximum of 10 pN of force, the trap stiffness constant is approximately  $k=(10 \text{ pN}/200 \text{ nm})=0.05 \text{ pN/nm}$ , so an uncertainty of 8 nm corresponds to an uncertainty of 0.4 pN in determination of the stalling force. Relative to the use of a quadrant photodiode, particle tracking is slow (maximum of 30 Hz) and less precise, but is easier to set up.

The advantage of the ‘escape force’ approach is that the object can be trapped anywhere in the field of view, and it does not require detection electronics: the object is trapped, and whether or not it escapes at a given force can be scored by eye. A relatively precise measurement of the minimum force required to stall the object can be made by slowly decreasing the laser power, and noting at what point the object escaped. The disadvantage of this method is that it is slow, and it is impractical to slowly lower the laser force for objects whose behavior is rapidly changing. Thus, rather than measuring the stalling force for each object, one often ends up measuring a ‘population’ stalling force: what was the average force required to stop the majority of moving objects, or what force was required to stop all moving objects (though many were stopped at lower powers). Both works describing *in vivo* measures of stalling forces<sup>18, 19</sup> used variants of escape-force measurements.

Regardless of which method is used, the force the laser applies to the objects of interest must be calibrated. This is done *in vitro*. If beads are used as handles, the calibration is done on the appropriately sized bead. If a biological object (e.g. a vesicle) is the object being studied, these must be biochemically purified, so that calibration can be done on typical examples, again *in vitro*. All measurements are made at typical cover slip-bead working distances.

Given a single bead or vesicle trapped in buffer, the way the force is calibrated depends on whether one plans to do ‘position determination’ or ‘escape-force measurements’. If escape-force measurements are planned, the trapped object is moved relative to the buffer, until viscous drag forces pull it out of the trap. The force at that moment can then be calculated from Stoke’s law, which says that the viscous drag force,  $F$ , on a spherical object is given by:

$$F = 6\pi\eta r v$$

where  $\eta$  is the coefficient of viscosity,  $r$  is the object’s radius, and  $v$  is the velocity of the fluid relative to the object. The actual drag is modified by the particle’s distance to the cover slip; see Felgner et. al<sup>28</sup> for details. The viscous drag is usually generated by keeping the trap fixed and using a computer controlled x-y translational stage to move the stage relative to the trapped object.

If ‘position-determination’ measurements are planned, the applied force can be calibrated in a variety of ways. As above, a constant force can be applied by moving the translational stage at a constant velocity. Then, as long as the applied force is below the escape force, the object stays in the trap, and its displacement relative to the trap center can be measured with whatever position detection scheme is being used. Using different velocities, one can experimentally determine the force-displacement curve. This is compatible with particle-detection approaches to position determination, as well as quadrant photodiode methods. Alternatively, one can use the Brownian motion of the

object in the trap to determine the traps stiffness, as long as the position-detection equipment has sufficient bandwidth<sup>31</sup>.

## ***XV. Conclusions***

The *in vivo* environment is determined by the organism rather than what is optimal for optical trapping. This leads to two sets of concerns: how to minimize optical damage, and how to calibrate the force actually being applied. For *in vivo* work, lasers operating at 830 nm are particularly good, because they both minimize optical damage and cause relatively little local heating. Since optical damage is a gradual process, it is particularly important to minimize the duration that an object is exposed to the laser. A lot of this review was devoted to optical damage, but the actual laser power required in many applications is low enough that it may not be an important design consideration.

To calibrate the applied force involves initial *in vitro* calibration, followed by corrections to compensate for the differences between working in buffer and in the relatively uncontrolled cytoplasm. The major concerns are the depth at which the measurements are made, the effective index of refraction of the cytoplasm, and the homogeneity and size of the trapped objects.

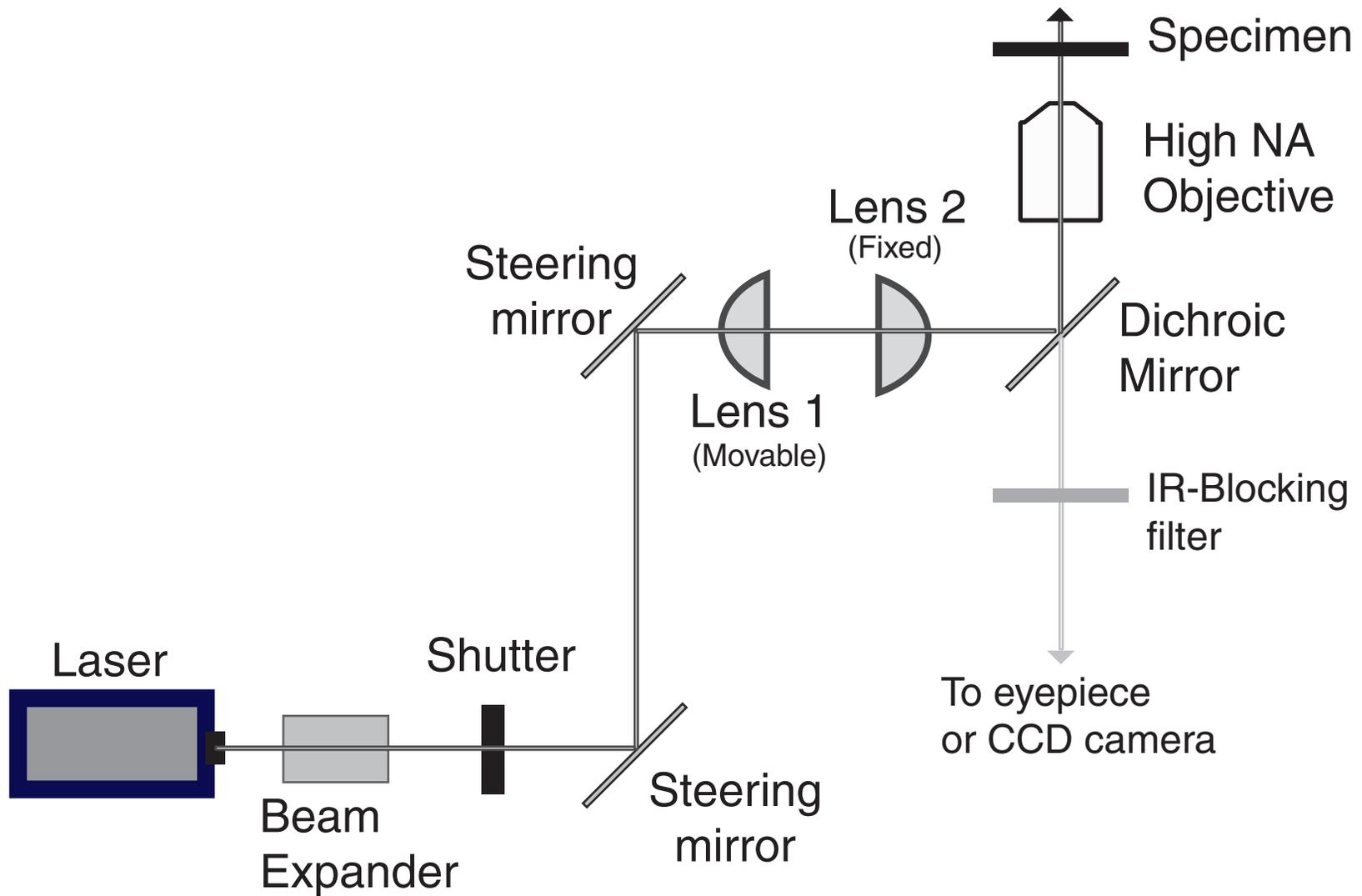
All of these difficulties notwithstanding, the laser trap is a tremendously powerful tool whose use can benefit many areas of research. With the recently completed genomes, we are starting to have a complete picture of all the proteins present in an organism, yet in many cases we still have little understanding of how these proteins function. By their very nature, process where the cytoskeleton plays an important role involve understanding forces and motion, questions for which *in vivo* optical traps are ideal tools. While the field is still in its infancy, the use of optical traps is already allowing exciting advances in such diverse fields as immunology and cargo transport.

**Figure 1:** This simple block diagram shows the path of the laser light from its origin at the laser, through its formation of an optical trap in the specimen. The beam expander (typically a 3X or 5X expander) is chosen so that the laser beam just fills the back aperture of the microscope objective: if the back aperture is overfilled, laser light is lost, and if under filled, less of the lens is used, resulting in less of a gradient in the focused beam. The shutter is used to block the laser, in order to minimize the sample's exposure to laser light when an object is not being actively trapped. The steering mirrors are used to control the orientation of the beam, to bring it into the objective. Lens 1 and 2 form a 1: 1 telescope, and moving Lens 1 relative to Lens 2 allows steering and parfocalizing of the trap. Lens 1 and 2 are identical planoconvex lenses, placed so that the distance  $D$  between the 2 is twice their focal length  $f$ , i.e.  $D=2f$ , so that parallel light entering the lenses remains parallel when it exits. This design was made popular by Dr. Block, and is discussed in his review article<sup>4</sup>. After passing through the two lenses, the laser is brought into the microscope optical path by reflecting it off of a dichroic mirror, mounted below and in line with the microscope objective. The exact position of the dichroic mirror depends on whether one plans to simultaneously use the optical trap and fluorescence. If not, one can simply replace one of the fluorescent cubes with the mirror, a very easy operation. However, in order to build a system capable of simultaneous fluorescence and trapping, it is better to avoid trying to pass the laser through the fluorescence cube, since this leads to both loss of laser power, and also interferes with the choice of fluorescent filters. In this case, it is better to modify the microscope, to position the dichroic mirror below the lens, but *above* the filter cube. Such modification can be time consuming.

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30. K. Visscher, S. P. Gross, and S. M. Block, IEEE Journal of Selected Topics in Quantum Electronics 2, 1066 (1996).

# Laser path for a simple optical trap



(Fig. 1)