Muscle contraction on the molecular level. Actin-myosin interaction studied in an in vitro motility assay
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In vitro motility assay

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2.1 From proteins to an \textit{in vitro} motility assay

2.1.1 Protein preparations

For experiments with skeletal muscle myosin and actin, the M. erector spinae was excised from a male New Zealand White rabbit. For experiments with cardiac muscle myosin, hearts were excised from five 6-week-old male Wistar rats.

Skeletal muscle myosin was prepared according to the method of Margossian and Lowey [1982] and cardiac muscle myosin was prepared according to the method described by Sata et al. [1993]. Heavy meromyosin (HMM) was prepared by chymotryptic digestion of myosin according to Kron et al. [1991]. Myosin was stored at -25 °C in 50% \(\gamma\) glycerol or quickly frozen in liquid nitrogen and stored at -80 °C. HMM was stored at -80 °C after quick freezing in liquid nitrogen. ATPase activities were measured using the malachite green assay according to Kodama et al. [1986].

Globular actin (G-actin) was prepared according to the procedure of Pardee and Spudich [1982] and stored at -80 °C after quick freezing in liquid nitrogen. In presence of KCl (50 mM) and ATP (1 mM) G-actin was polymerised to filamentous actin (F-actin) during 3 hours on ice.

For visualisation by fluorescence microscopy, F-actin (2 \(\mu\)M) was labelled with 4 \(\mu\)M RhPh (rhodamine-phalloidin) [Kron et al., 1991] in AB-buffer (see Solutions). Reconstitution of thin filaments from (labelled) actin, tropomyosin and troponin was done by mixing the proteins at concentration of respectively 400, 100 and 80 nM in a modified regulated filament buffer (98.5 mM KCl, 10 mM MOPS, 6 mM MgCl\(_2\), 1 mM EDTA and 5 mM dithiothreitol (DTT), pH 7.4 and ionic strength 125 mM) [Gordon et al., 1997].

More details about animals used and protein isolations are described in the \textquoteleft Materials and Methods\textquoteright sections of Chapter 3, 4, and 5.

Protein concentrations were measured by absorption spectroscopy or by using a protein assay (Bio-Rad). The molecular masses and extinction coefficients used for myosin were 520 kDa and 0.55 (mg/ml)\(^{-1}\)cm\(^{-1}\) and for HMM 260 kDa and 0.60 (mg/ml)\(^{-1}\)cm\(^{-1}\), both at 280 nm. The molecular mass and extinction coefficient used for actin were respectively 42 kDa and 0.62 (mg/ml)\(^{-1}\)cm\(^{-1}\) at 290 nm. Myosin, HMM, and all other protein preparations were also analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). A typical example of a SDS-gel is shown in figure 2.1.
2.1.2 Solutions [Kron et al., 1991]

Motility assays were carried out either with actin filaments (unregulated) or with thin filaments (regulated) diluted in AB-buffer (pH 7.4, ionic strength 50 mM and pCa 10). The ionic strength was adjusted by adding KCl. Before use 35 U/ml Creatine Kinase (Boehringer Mannheim, Germany) was added to AB-buffer.

Movement of F-actin was induced by AB-buffer containing 2 mM MgATP and variable calcium concentrations. Appropriate amounts of CaCl₂ were added to obtain pCa's between 10 and 4, while maintaining the ionic strength. Scavenger solutions were used to minimise photooxidation and photobleaching [Kishino and Yanagida, 1988].

A computer program based on data of Fabiato and Fabiato [1979] was used to calculate the composition of the solutions so as to maintain ionic strength.

More details about the solutions are described in the 'Materials and Methods'-sections of Chapter 3, 4, and 5.
2.1.3 Slide preparation

The surface to which the myosin units are attached plays an important role in determining the velocity in the assay. The exact nature of the contact of the myosin with the surface is largely uncharacterised. The connection must be flexible to allow the myosin to reach the actin filament in the solution above and yet have enough resistance so that the force generated by the actomyosin motor can be transferred to the substratum. Also the myosin-coated surface must be prepared so that most of the actin and thin filaments will move at nearly the same sliding velocity as that of thin filaments in muscle during maximum shortening at zero load. And myosin has to be bound to the surface with its activity unchanged [Harada et al., 1990].

Very clean glass, nitrocellulose-covered surfaces, siliconised, and lysine-coated surfaces have all been used to observe myosin-induced motility. For the experiments reported in this thesis, nitrocellulose-coated surfaces were selected, because these surfaces give good quality of movement and are easy to prepare. Nitrocellulose-films were created by placing two drops of 1% NC in high purity amyl acetate on a (cool distilled) water surface in a round flat jar with a diameter of 10 cm. Cover slips were placed onto the film and excess film was removed by forceps. Another quick and easy method used is to put a drop of 0.1%-nitrocellulose solution on a cover slip and spread it out with the tip of a Pasteur pipette [Kron et al., 1991]. Yanagida and co-workers [Harada et al., 1987, 1990] have used primarily silicon-treated surfaces. Since the silicone-treated surface is hydrophobic, the hydrophobic part of myosin (light meromyosin) is probably bound to it by a hydrophobic interaction. Warrick et al. [1993] examined a large number of siliconising reagents and found them unsatisfactory, producing very variable and poor movement with the exception of dichlorodimethylsilane (DowCorning, Z1219). The cover slips were prepared by dipping them in a freshly prepared solution of 2% dichlorodimethylsilane in chloroform for a few seconds and then allowing them to dry completely before use. The binding between lysin-coated surfaces and myosin is based on ionic interactions. Ohichi et al. [1993] showed that on a lysine-coated surface covered with HMM and S1 actin filaments moved with similar velocity as compared to the movement on nitrocellulose surfaces. Best results have been obtained with cover slips cleaned with ethanol. These cover slips were placed onto a drop of 0.4 mg/ml poly-L-lysine (MW 20.5 kDa) in 10mM TrisHCl pH 8.0 for 30 minutes and then washed with distilled water and extensively dried.
Electron microscopy and atomic force microscopy were used to characterise the attachment of myosin to the coated glass surface, and of actin to myosin. Electron microscope images performed by Jan van Marie (Department of Advanced Electron Microscopy, University of Amsterdam, AMC, The Netherlands) were obtained by negative staining of HMM molecules using uranyl acetate. With low density of HMM the individual molecules could be seen. At high, saturating density, the surface looks homogeneously covered with HMM. When actin filaments were added to the HMM-saturated surface they seemed to have disappeared, whereas they were clearly seen at non-saturated density. Therefore, actin filaments were likely not to slide over the tip of heads on the surface but to move with their sides surrounded by the heads, as was already proposed by Harada et al [1990]. This is schematically shown in figure 2.2a.

A typical result of low density of myosin on a nitrocellulose-coated copper grid is shown in figure 2.2b. Also atomic force has been used to study the structure of rabbit skeletal muscle myosin [Hallett et al., 1995]. A typical result obtained with atomic force microscopy (AFM) in co-operation with John van Noort (Faculty of Applied Physics, University of Twente, The Netherlands) of low density HMM molecules and F-actin on a nitrocellulose coated glass surface is shown in figure 2.2c. A single HMM molecule on a nitrocellulose coated mica surface obtained with atomic force microscopy (AFM) in cooperation with Jim Flach (This Scientific, Sliedrecht, The Netherlands) is shown in figure 2.2d.

Figure 2.2a. Schematic drawing of possible interaction between myosin and actin filament (redrawn from Harada et al. [1990]).

Figure 2.2b. Electron microscope image (120 x 90 nm) of HMM molecule on a nitrocellulose-coated copper grid.
The construction and filling procedure of a flow cell are described in detail in Chapter 3, 4, and 5. A schematic drawing of the flow cell is shown in figure 2.3.

### 2.1.4 Unloaded experiments

#### Unloaded velocity.

Unloaded velocity. As described in Chapter 1 unloaded velocity is an important parameter in studying muscle contraction. At different laboratories different velocities have been found for preparations that seemed equal at first sight. The question arose if unloaded velocity is really unloaded. Unloaded velocity in our *in vitro* motility assay is the velocity of actin filaments that are allowed to move freely over myosin molecules. This velocity is the result of equilibrium between driving force and friction, both internal forces. However, if an external force, probably a friction or drag, acts on the proteins, the measured velocity is no longer unloaded.
Haeberle and Hemric [1995] have found evidence that photo-illumination of flow cells results in a mechanical load by oxidation of myosin, which reduces interaction rate of actin and myosin. The extent of this mechanical loading is dependent on a number of experimental conditions including oxygen content of buffers, intensity of illumination, concentration of reducing agent in motility buffers, concentration of other proteins in motility buffers, duration of illumination, flow cell temperature, oxidation state of proteins when added to buffers, and concentration of the individual components of any oxygen scavenger systems and the type of oxygen scavenger used. They proposed a much-simplified motility buffer, containing no oxygen scavenger system, high concentrations of DTT (10-100 mM), and a carrier protein (1-5 mM BSA). And if used in conjunction with reduced illumination and digital image processing, this buffer would essentially eliminate photobleaching, illumination-dependent protein oxidation and myosin-dependent mechanical loading [Haeberle and Hemric, 1995]. In addition, the coating of the cover slips, or the orientation of the myosin with respect to actin can be sources of load.

**Actin filament movement.** The rate of movement of actin filaments is largely independent of the density of myosin heads bound to the surface. According to Toyoshima et al. [1990] a change of HMM concentration of 5 till 50 μg/ml has no effect on the sliding velocity. Harris et al. [1993] showed that at a myosin concentration of 100 μg/ml, where there were 50 or more heads available per filament, the sliding velocity was maximal. This means that, over a wide range of myosin concentrations introduced into the flow cell, the velocity is constant. At very low myosin density however, the rate of movement of actin filaments does decrease dramatically as the density is lowered further. This observation was exploited by Uyeda et al. [1991] in an attempt to determine the distance that myosin translocates an actin filament per ATP hydrolysed (see Chapter 1). The rate of movement is not dependent on the length of actin filaments [Toyoshima et al., 1989; Morel & Bachouchi, 1990; Harada et al., 1990; Takiguchi et al., 1990]. The direction of movement of an actin filament is determined by the inherent polarity of the actin filaments (Chapter 1) [Takiguchi et al., 1990; Harada et al., 1990].

Each myosin isoform has its own characteristic rate of translocation of actin filaments. Reported actin translocation rates measured under the same ionic conditions for various myosins, vary widely, ranging from 0.04 μm/s for brush border myosin I or phosphorylated human platelet myosin II in the absence of tropomyosin to a high value of
about 6µm/s for native clam thick filaments. Table 2.1 shows actin filament velocities for different myosin (sub)fragments from rabbit cardiac and skeletal muscle.

<table>
<thead>
<tr>
<th></th>
<th>$v_0$ (µm/s) ± s.d.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit cardiac muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myosin molecules</td>
<td>0.51</td>
<td>[Margossian et al., 1991]</td>
</tr>
<tr>
<td>Chymotryptic S1</td>
<td>0.37</td>
<td>[Margossian et al., 1991]</td>
</tr>
<tr>
<td>Rabbit skeletal muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myosin molecules</td>
<td>3.9 ± 0.8</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
<tr>
<td>myosin filaments</td>
<td>3.5 ± 0.6</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
<tr>
<td>Chymotryptic HMM</td>
<td>4.6 ± 0.4</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
<tr>
<td>Tryptic HMM</td>
<td>7.5 ± 1.1</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
<tr>
<td>Papain Mg$^{2+}$ S1</td>
<td>1.8</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
<tr>
<td>Papain EDTA S1</td>
<td>1.7 ± 0.4</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
<tr>
<td>Chymotryptic S1</td>
<td>0.91 ± 0.28</td>
<td>[Toyoshima et al., 1987]</td>
</tr>
</tbody>
</table>

Table 2.1. Actin filament velocities for rabbit skeletal and cardiac muscle at 30 °C.

Effect of biochemical conditions. The rate at which any given myosin translocates actin filaments is a function of the ionic conditions, pH and temperature of the assay, as shown in figure 2.4 and 2.5.

Figure 2.4. Effect of pH on filament velocity. Data are redrawn from Homsher et al. [1992]. Filament velocity at pH 7.35 was assigned value of 1.0 and the filament velocity observed at different pH was expressed relative to this value. Line is a 3rd-order best-fit polynomial.
Chapter 2

Figure 2.5. Effect of ionic strength and temperature on actin filament velocity produced by chymotryptic HMM. Values are given as means ± SD. Each data point is average of 6-63 filaments. Lines through data points are 2nd-order polynomial least-square fits to data. Data are redrawn from Homsher et al. [1992].

The velocity of the travelling actin filaments depends strongly on the free adenosine triphosphate (ATP) concentration; below 2-4 μM ATP, filament movement is abolished. However, after a brief exposure to 1 mM ATP, actin filament motility persists down to nanomolar ATP concentrations [Kellermayer and Pollack, 1996].

Addition of MgADP decreased the velocity of actin filaments on both cardiac and skeletal myosins in the in vitro motility assay system. Experiments on HMM at 25°C and 50 mM ionic strength have shown that 1 mM MgADP inhibits the unloaded velocity to half of its value. A cross-bridge model in which MgADP competes with MgATP for the substrate site on myosin can explain the inhibition of the velocity by MgADP. MgADP may be a modulator of muscle contraction, acting as a competitive inhibitor of cross-bridge detachment [Yamashita et al., 1994; Homsher et al., 1992].

Myosin. The isolated head fragment, subfragment-1 (S1), contains the ATPase and actin-binding activities of myosin. Hynes et al. [1987] showed that beads coated with heavy meromyosin (HMM) can move on Nitella actin filaments. Using the myosin-coated surface assay of Kron and Spudich [1986], Harada et al. [1987] and Toyoshima et al. [1987] showed that single-headed myosin filaments bound to glass support movement of actin at nearly the same velocity as intact myosin filaments. These studies show that the terminal portion of the rod and the two-headed nature of myosin are not required for movement. Because myosin-S1 is sufficient to move actin filaments in vitro this work
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supports the idea that the site of active movement in muscle is within the myosin head. The in vitro rates of movement directed by S1 and HMM were 1-2 μm/s and 7 μm/s respectively [Toyoshima et al., 1987]. In contrast, the maximum actin-activated ATPase activity of these preparations of S1 and HMM were equal. Therefore, the difference in the rates of movement directed by S1 and HMM in this assay cannot be explained by differences in the turnover rates of ATP. One possible explanation for the faster rate of movement of HMM than S1 is that a double-headed myosin fragment is inherently better at producing movement than a single-headed fragment. However, Toyoshima et al. [1987] also showed that nearly homogeneous single-headed HMM supported the sliding movement of actin filaments at a velocity higher than HMM or myosin. Harada et al. [1987] suggest that the double-headed structure of myosin may be important for generating a large force with high efficiency, by increasing affinity of myosin for actin. But co-operative interaction between the two heads is not essential for inducing the sliding movement.

The different rates of movement of actin filaments on S1 and HMM could also be caused by a difference in attachment of these molecules to the nitrocellulose surface. HMM may bind to the nitrocellulose via its S2 portion, allowing a productive interaction of many of the heads with actin. S1 may not bind to nitrocellulose with such a favourable orientation; some of the S1 can bind to actin but may not be able to move, causing a restraining force. The resulting balance of forces could result in the relatively slower rate of movement of S1. In experiments of Winkelmann et al. [1995] in which the mode of attachment is studied, fast skeletal muscle myosin monomers were bound to the surface through specific interaction with monoclonal antibodies. Each of these antibodies produced a stable myosin coated surface that supported uniform motion of actin over several hours. Attachment of myosin through the anti-S2 and anti-LMM monoclonal antibodies yielded a significantly higher velocity (10μm/s at 30 °C) than attachment through anti-LC2 (4-5μm/s at 30 °C). For each antibody, a characteristic value of the myosin density for the onset of F-actin motion and a second critical density to reach maximum velocity were observed. The specific mode of attachment influenced the velocity of actin filaments and the characteristic surface density needed to support movement. The precise mode of myosin attachment influenced motion in two ways: the average velocity is reduced when the myosin heads are too close to the supporting surface, and the myosin density for the onset of actin filament motion is dependent on the...
flexibility of attachment. The best overall motion was achieved when the mode of attachment most closely approximates that of myosin in muscle thick filaments, i.e., tethered through the anti-S2 monoclonal antibody with much of the S2 region of the rod free.

The productive interaction of actin and myosin is considered to be the result of a specific binding of the myosin head to actin. In this assay, continuous, directional actin movement occurs despite a random orientation of the myosin fragments on the surface, which implies that there must be torsional freedom within the actin-myosin system. Actin filaments are known to have considerable flexibility [Thomas et al., 1979; Egelman et al., 1982; Yanagida et al., 1984]. Flexibility about the S1/S2 junction of myosin allows freedom of rotation of the myosin heads [Mendelson et al., 1973; Thomas et al., 1975], and HMM-directed movement might be facilitated by rotational freedom at this site. Toyoshima et al. [1987] demonstrated that S1 can direct actin sliding movement while it is bound to a solid support. Several reports have shown that the heads of isolated myosin molecules are capable of large rotational movements about the subfragment-1 2 junction [Winkelmann and Lowey, 1986; Craig et al., 1980]. Similar observations have been made on myosin heads projecting from myosin filaments [Walker and Trinick, 1986, 1988]. Reedy et al. [1989] used mutated Drosophila flight muscle, in which peripheral thick and thin filaments are disarranged, and showed that myosin heads can bind with opposite rigor cross-bridges angles to flanking actin filaments. Toyoshima et al. [1989] described bi-directional movement of actin filaments along tracks of myosin heads, created by binding actin filaments, saturated with skeletal muscle HMM, to the surface of a cover slip. In addition, myofibrillar Mg$^{2+}$ATPase activity did not decrease at short sarcomere length, suggesting that actin of the opposite polarity can activate the Mg$^{2+}$ATPase activity of myosin [Stephenson et al., 1989]. Sellers et al. [1991] proposed a model in which the heads of myosin have considerable rotational flexibility. The direction of movement is determined by the polarity of actin and myosin heads can rotate perhaps 180° in order to interact with actin. Also actin filaments can move in both directions on tracks of heavy meromyosin made on a nitrocellulose surface, and, furthermore, along native thick filaments passing over their central bare zone. These observations indicate that the myosin molecule has a considerable flexibility in interacting with actin filaments. No difference in velocity of actin filaments travelling towards and from the central bare zone
of the native thick filament of crayfish claw muscles (Crustacean skeletal muscles) could be found [Toyoshima, Y.Y., 1991].

Lowey et al. [1993] showed that light chains from skeletal muscle myosin are not essential for enzymatic activity, but that light-chain/heavy-chain interactions play an important part in the conversion of chemical energy into movement. They measured a six-fold decrease in actin filament velocity for myosin without the alkali light chain (ALC), and a decrease up to ten-fold for removal of both classes of light chains.

In summary, buffer conditions, mode of attachment, myosin(-fragment) used, and light chain composition are all important in determining the 'true' unloaded velocity.

2.1.5 Loaded experiments

So far the *in vitro* motility assay enables us to measure unloaded velocity. To be able to determine force-velocity relationships we need to apply force. Therefore we developed a technique based on magnetic forces. The reasons why we developed a new technique next to the already existent force-applying techniques and the details about this technique are described in Chapter 6. To be able to exert force on an actin filament with an electromagnet we needed to attach a kind of handle to the actin filaments. Therefore we used magnetic beads.

Details about coating of these beads and further use are described in Chapter 6. A schematic drawing of a flow cell as used for loaded experiments is shown in figure 2.6.

![Figure 2.6. Schematic drawing of myosin-actin interaction on coated cover slip. Actin is attached to a (magnetic) bead held in an optical trap.](image-url)
2.2 Set up

2.2.1 Microscope

A schematic drawing of the set up is shown in figure 2.7. Actin filaments were observed through an inverted microscope (Nikon Diaphot 300) equipped with a Hg100W lamp and a Nikon fluorescence filter set (Omega Optical XF37: $\lambda_{\text{exc.}} = 546$ nm, beam splitter 560 nm, $\lambda_{\text{em.}} = 570$ nm). Actin filaments were imaged with an intensified CCD camera (Photonic Science Limited Darkstar). The standard PAL video signal from the camera, together with time/date/title signal (Blaupunkt), was recorded on an S-VHS video recorder (VCR model GV 470 S VPT Grundig) for off-line analysis. Actin filament velocity was calculated using a home written program (ActinFil), described in detail in Chapter 3.

![Figure 2.7. Schematic drawing of set up.](image)
The optical trap, used to bind beads to actin filaments, consists of a 1 W 1064 nm NdYAG-laser (Uniphase), coupled into a fibre (single mode, with a diameter of 4\,\mu m), focussed through a microscope objective (40x, N.A. 0.65), resulting in a spot of 1-2 \,\mu m. Theoretical background information on optical traps is given in the next paragraph. The electromagnet, for convenience shown as two squares marked with N and S in figure 2.7, is used to apply forces to actin filaments. Chapter 6 describes the electromagnet in detail.

2.2.2 Optical trap

Ashkin et al. [1986] realised trapping of atoms and molecules with one laser beam with the advent of the single-beam gradient force optical trap. A force is imparted to a reflecting or absorbing surface in a vacuum when it is hit by a flux of photons. The change in momentum of a single photon upon absorption is $\Delta p = h/\lambda$, the Broglie relation, where $h$ is Planck’s constant and $\lambda$ is the wavelength. For a beam of light of a fixed power level, $P$, the force produced by the flux is given by $P/c$, where $c$ is the speed of light. The force is $2P/c$ in the case of reflected light, since the change in photon momentum is double for a reflected beam. This force is called the scattering force that occurs when light bounces off an object. Scattering forces tend to push objects along the direction of the beam. Optical traps however work by ‘radiation pressure’. The gradient force trap, as the name implies, derives its trapping power from a spatial gradient in light intensity, which needs not to point along the direction of the beam. The gradient force can be explained in two complementary ways, by electric field gradients and induced electric dipoles, and by ray tracing.

We will confine ourselves to the explanation by electric field gradients and induced electric dipoles: When light, which is a high-frequency electromagnetic oscillation, passes through a dielectric material, the time-varying electric field vector polarises the medium, inducing a set of fluctuating dipoles. The induced dipoles of the material are fixed in the object itself, which thereby experiences a force in the direction of the light gradient. The strength of the induced dipole moment, $p$, however depends on the polarisability, $\alpha$, of the medium (which is related to its refractive index, $n$) and on the strength of the field, introducing another power of $E$ into the field dependence. It can be shown that for a Rayleigh particle (a particle with dimensions much smaller than the
wavelength of light) of radius $r$ and refractive index $n$, immersed in a medium of refractive index $n_0$, the gradient force is given by:

$$F_{\text{grad}} = -(\alpha/2) \cdot V E^2,$$

(2.1)

where $\alpha$, the polarisability, equals $r^3 n_0^2 \{ (n^2-1)/(n^2+2) \}$. So particles that find themselves in a light gradient will experience a force pulling them towards a point where the light is brightest, for example, up into a focus. However, in addition to this gradient force, there is a scattering force, pointing along the beam direction, arising from light that is absorbed or reflected. For a non-absorbing Rayleigh particle, the scattering force is given by $P_{\text{scat}}/c$, where $P_{\text{scat}}$ is the scattered power. Tiny particles scatter light in inverse proportion to the fourth power of the wavelength $\lambda$. The exact expression is

$$F_{\text{scat}} = \frac{I_0}{c} \frac{128 \pi \lambda^6}{3 \lambda^4} \left| \frac{n_b \left( \frac{n^2-1}{n^2+2} \right)}{1} \right|,$$

(2.2)

where $I_0$, the light intensity, is proportional to $E^2$.

$F_{\text{scat}}$ and $F_{\text{grad}}$ depend in different ways on $n$, $n_b$, and $r$, and $F_{\text{scat}}$ depends on the square of the field while $F_{\text{grad}}$ depends on the square of the gradient of the field. For tiny particles, and for a sufficiently large ratio $(VE^2)/(E^2)$, a region exists where $-F_{\text{grad}}$ is everywhere greater than $F_{\text{scat}}$. This is the trapping zone. This region occurs near the waist of a highly convergent beam, e.g., near the focus of an objective of high numerical aperture. The balance between scattering and gradient forces dictates the range of particle sizes that can be successfully trapped.

Block et al. [1990] gives a rough limit for the trapping force based on experiment of $F \approx 0.03(n_b P/c)$ for a micron-sized sphere trapped by an infrared laser, i.e. about 3% of the equivalent photon pressure on an absorbing surface. The question of optical damage arises. Optical traps work by virtue of the fact that trapped particles refract light, and not because they absorb or reflect it. Too much absorption can lead to excessive heating and in the end to destruction of the material. For an irradiated sphere immersed in water, the equation for thermal conduction is

$$\Delta T = \frac{3P}{8\pi\kappa r},$$

(2.3)

where $r$ is the radius, $\kappa$ is the thermal conductivity of water, and $P$ is the power delivered to the sphere. One microwatt of absorbed power for a sphere of 1 $\mu$m in diameter, at 25°C ($\kappa = 0.6071$ W/Km) will only raise the temperature $\sim 1$°C at steady state.

The optical trap used in our set up is described in the former paragraph.
References


