8 Building an optical tweezers*

It is finally time to build our first optical tweezers. In this chapter, we shall do just that: we will build the optical tweezers shown in Fig. 8.1, proceeding step by step and explaining all the details and tricks. In a nutshell, a simple optical tweezers is a laser beam coupled to an optical microscope, which can be either commercial or homemade. For completeness, we will first proceed to realise a homemade microscope. Then we will couple it to a laser beam, appropriately prepared. Along the way, we will explain all the necessary tricks and tips, and we will also present some basic information on how to achieve steerable and multiple optical tweezers. By the end of this chapter, we will have built the optical tweezers shown in Fig. 8.1, apart from the position detection part, which will be explained in Chapter 9, and we will be able to trap and manipulate microscopic particles. In the following chapters, we will explain how to acquire and analyse optical trapping data [Chapter 9], how to measure nanoscopic forces and torques [Chapter 10], and how to realise more complex optical trapping configurations using holographic optical tweezers [Chapter 11] and alternative trapping geometries [Chapter 12].

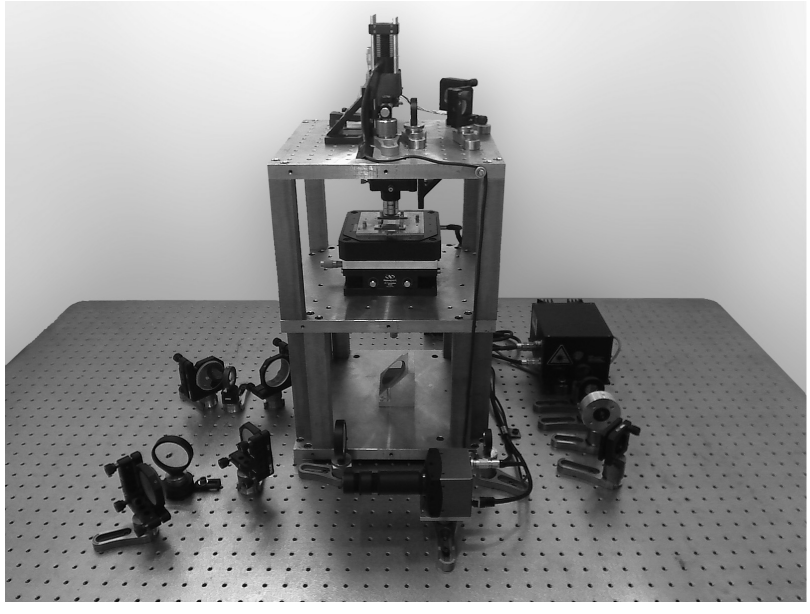

Figure 8.1 Homemade optical tweezers: Photograph of the optical tweezers that we will build in this and the following chapters.

* This chapter was written together with Giuseppe Pesce.

https://doi.org/10.1017/CBO9781107279711.011 Published online by Cambridge University Press

8.1 The right location

In the realisation of an optical tweezers, the first crucial step is to choose an appropriate location. In fact, this choice will largely determine the stability of the set-up and, ultimately, the quality of the experimental results. For simple experiments that do not require very high stability or reproducibility, e.g., experiments where the optical tweezers are used to trap and displace cells, it can be sufficient to mount the optical tweezers in a standard optical lab on a standard optical table. For more complex experiments, e.g., experiments where the optical tweezers are used to measure or exert forces, it might be necessary to choose a laboratory with stabilised temperature and humidity; in most cases, it is also a good idea to use a laboratory located on the ground floor, as this contributes to limiting the building vibrations transmitted to the optical set-up. The optical table should also use a passive or active vibration-insulation system.[1] For even more demanding experiments, e.g., the accurate measurement of forces generated by biomolecules, the highest level of experimental stability and reproducibility can be achieved by eliminating one of the greatest sources of noise in any optical lab: the human factor. Thus, it might be necessary to perform the experiment on a remote-controlled platform in a closed and acoustically insulated room.

Suggestion: Eliminate noise sources. Common sources of vibrations are table-mounted devices (e.g., the fan of the laser head), as well as vacuum pumps and other acoustic sources present in the laboratory. The best way to reduce these vibrations is to eliminate them at the origin, using baffles on air ducts, compliant pads under instruments, and acoustic enclosures.

Suggestion: Enclose the set-up. The presence of air flow, dust, or thermal gradients can affect the laser pointing stability. Thus, enclosing the set-up within a thermally, acoustically and electrically insulated box greatly increases both the stability of the laser and the quality of the acquired signals. We have done this with our set-up, although for clarity the enclosure is not shown in the pictures.

8.2 Inverted microscope construction

Once an appropriate location has been identified, we can start building an inverted microscope. The microscope will be built on several levels. We start by constructing a two-level structure, as shown in Figs. 8.2a and 8.2b. The lower level is fixed directly to the optical table; here is where the optical components necessary to prepare the optical beam and to

[1] An alternative idea is to place the optical table on a bed of sand, because this effectively damps the transmission of ground vibrations to the optical bench.

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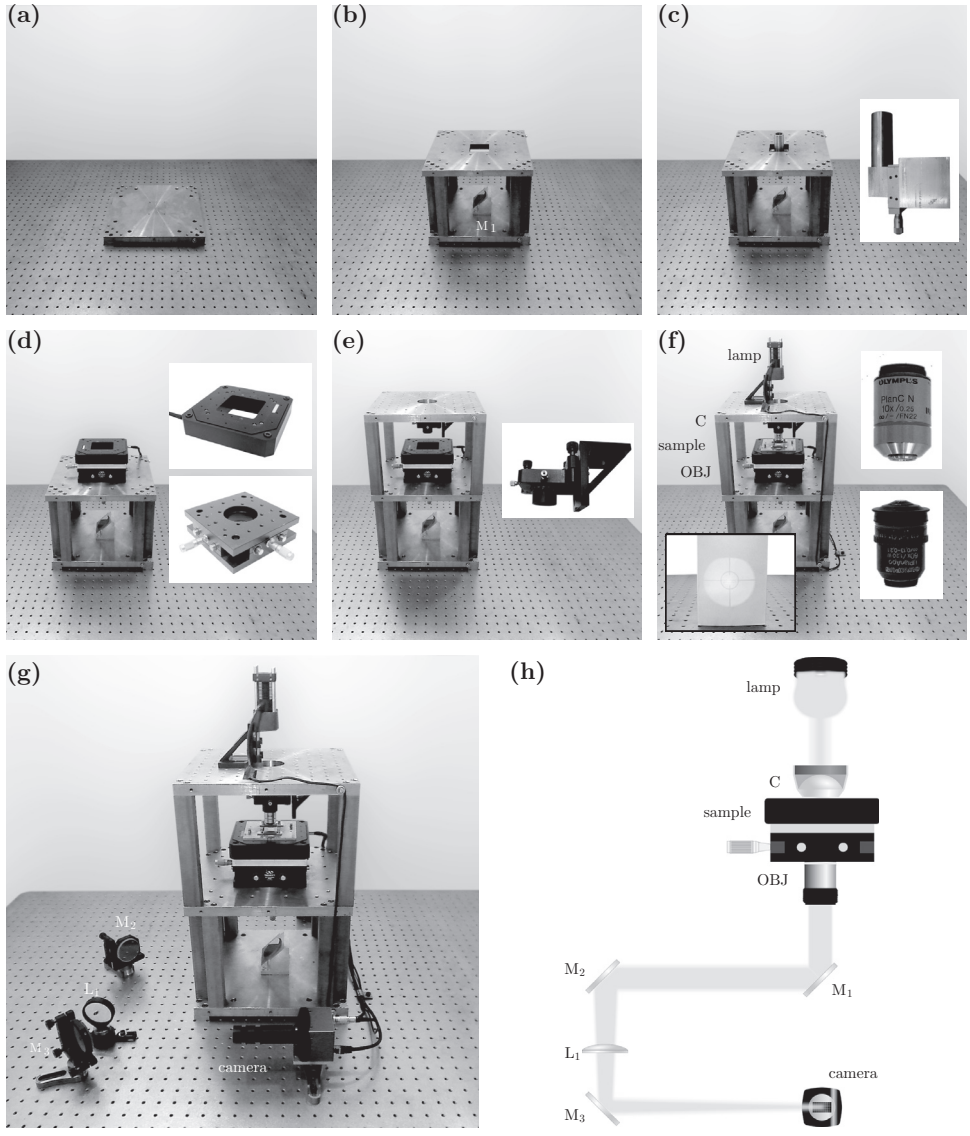

Figure 8.2 Homemade inverted microscope. Construction: (a) the base is firmly fixed to the optical table; (b) a 45° mirror M_1 is fixed on the base and a second stage is built; (c) the vertical translation stage (inset) for the objective is fixed; (d) a manual translation stage (lower inset) for rough alignment and a piezoelectric automatised translation stage (upper inset) for fine adjustment of the sample are fixed on the second stage; (e) the lateral translation stage (inset) for the condenser is fixed; (f) the objective (OBJ; lower right inset) and the condenser (C; upper right inset) are placed on the respective stages, a calibration glass slide is placed on the sample holder and the illumination LED lamp is fixed on the third level (lower left inset: image of the sample on a screen placed on the optical table); (g) the image is focused on a camera using lens L_1 and mirrors M_1, M_2 and M_3. (h) Corresponding schematic of the microscope.

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focus the sample image on the camera will be housed. The second level is a breadboard supported on four columns; here is where the stages to hold and manipulate the sample will be housed. It is important that both these levels are stable and horizontal, as can be verified during their construction using a level. The translation stage to control the vertical position of the objective with respect to the sample is also attached to the lower side of the second-level breadboard, as shown in Fig. 8.2c.

Suggestion: Objective position control. For the control of the objective, it can be often sufficient to use a one-axis (vertical) manual translation stage, as the fine adjustment of the relative position between the objective and the sample can be realised using the piezoelectric translation stage on which the sample is to be placed. However, for some set-ups, it might be useful to have more degrees of freedom for lateral alignment and/or for tilting. In other cases, it might be better to fix the position of the objective in all directions, e.g., to maintain the alignment between objective and condenser when interferometric measurements are performed [Section 9.2].

We can now mount the sample translation stage, which permits us to position the sample in three dimensions, on the second-level breadboard, as shown in Fig. 8.2d. This translation stage can be either manual or automatised. Manual translation stages typically offer precision down to about a fraction of a micrometre over a range that can easily reach several millimetres. Automatised translation stages, which are often driven by mechanical or piezoelectric actuators, permit one to achieve subnanometre position precision over a range that typically does not exceed some tens or hundreds of micrometres[2] and have the advantage that they can be controlled remotely. Often, a good approach is to combine a manual translation stage for the rough positioning of the sample and an automatised translation stage for fine adjustment; such an approach is implemented in our set-up [Fig. 8.2d].

At this point, we proceed to build a third level, as shown in Fig. 8.2e, where we will host the illumination and detection components. Furthermore, also shown in Fig. 8.2e, we fix a translation stage on which to mount the condenser. This stage permits us to align the condenser position along the lateral (horizontal) directions and, therefore, to centre the position of the collected beam.

Finally, we can now proceed to mount the objective (OBJ), which will permit us to collect the image of the sample (and later to focus a laser beam into an optical tweezers), and the condenser (C), as shown in Fig. 8.2f. The main parameters that describe an objective are discussed in Subsection 8.2.1. To start, it is convenient to use a low-magnification (e.g., $10\times$ or $20\times$) objective because it permits a simpler alignment.

Because a microscope serves to magnify the image of a small object, it is appropriate to start by choosing a simple object to look at. For the moment, we can use a calibration glass slide.[3] This sample will be mounted on the second-level sample stage, as shown in Fig. 8.2f.

[2] In order to achieve subnanometre position *accuracy* and *reproducibility*, these stages need to be actuated with closed-loop position control, as in open-loop they are subject to significant hysteresis.

[3] Alternatively, one can use a sample of microspheres stuck to a coverslip or a glass coverslip to which some colour has been applied on one side, e.g., using an indelible marker.

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Table 8.1 Objective specifications

Manufacturer	Parfocal length	Tube lens	Thread
Leica	45 mm	200 mm	M25 × 0.75
Nikon	60 mm	200 mm	M25 × 0.75
Olympus	45 mm	180 mm	RMS[a]
Zeiss	45 mm	165 mm	M27 × 0.7

[a] The RMS (Royal Microscopical Society) standard for the objective thread was first set down in 1866 and revised in 1896. The RMS standard is a 55° Whitworth thread, with a diameter of 0.7652–0.7982 in. (19.44–19.51mm) at the top of the objective lens thread and 36 turns per inch (TPI), i.e., a pitch of 0.706 mm. Often the RMS thread diameter is quoted as 0.8 in., which refers to the diameter at the bottom of the female thread in the microscope turret.

To capture the image of the sample, an illumination source is necessary. This can be realised in a first simple configuration using a LED lamp focused on the sample by the condenser, as shown again in Fig. 8.2f. It is possible to see the resulting image with the naked eye by placing a screen along the path of the light collected by the objective. At this point it is possible to optimise the illumination train in order to maximise the illumination intensity by adjusting the condenser position. For infinity-corrected objectives, it is necessary to add a tube lens (whose focal length is typically about 150 to 200 mm [Table 8.1]) in order to form an image of the sample. More advanced illumination configurations are also possible and will be discussed in Subsection 8.2.2.

Troubleshooting: I cannot mount the objective at the right distance to image the sample. Start by aligning your system using objectives with low magnification (e.g., 10× or 20×). These objectives can be aligned much more easily because they have a longer working distance and focal depth. Only once your system is aligned and working should you switch to higher magnification objectives (e.g., 40×, 60× water-immersion, 100× oil-immersion). Note that, as long as you use objectives with the same parfocal distance, you can switch objectives, keeping the sample in focus so that, when moving to higher-magnification objectives, you do not need to adjust the vertical focal position.

Troubleshooting: I cannot focus on the sample. If you cannot focus on your sample, it might be that the coverslip is too thick for the working distance of the objective. In this case, you will also see that the objective pushes the coverslip or that (for spring-loaded objectives) the objective spring is pushed. Use a thinner coverslip, or reverse the coverslip and look at its lower side. The coverslip thickness is typically indicated by the coverslip number, as shown in Table 8.2.

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Table 8.2 Microscope coverslip thickness

Number	Thickness
0	0.085 to 0.13 mm
1	0.13 to 0.16 mm
1.5	0.16 to 0.19 mm
1.5H	0.17 to 0.18 mm
2	0.19 to 0.23 mm
3	0.25 to 0.35 mm
4	0.43 to 0.64 mm

We have finally built our first inverted microscope. We only need to add a camera at the image plane to start observing our sample. The final set-up is shown in Fig. 8.2g and the corresponding schematic in Fig. 8.2h.

8.2.1 Objectives

In an optical tweezers, the objective serves the dual function of imaging the sample and focusing the laser beam in order to generate the optical trap. For optical manipulation applications, a high-numerical-aperture objective is used in order to generate the tight focal spot needed to trap a particle in three dimensions. Such objectives are readily available from various manufacturers.[4] A schematic of a typical objective is shown in Figs. 8.3a and 8.3b. It consists of a series of lenses carefully oriented and tightly packed into a tubular brass housing, which is encapsulated by the objective barrel and, as is the case with most oil-immersion objectives, is equipped with a spring-loaded retractable nosecone assembly to protect the front lens elements and the specimen from collision damage.

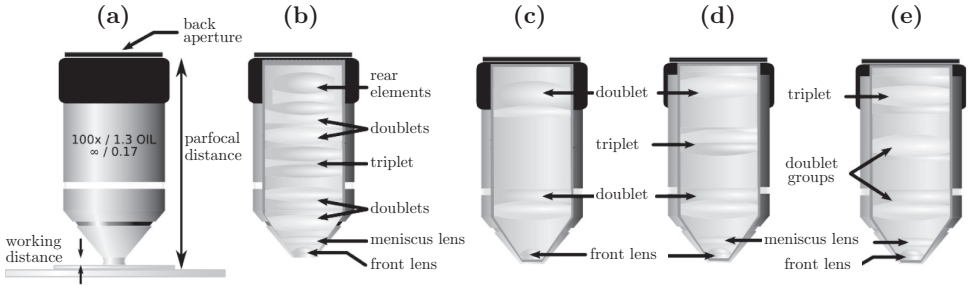

Figure 8.3 Objectives: (a) Oil-immersion infinity-corrected $100\times$ apochromat objective showing the definitions of working distance, parfocal distance and back aperture. (b) Cutaway diagram showing the lens system inside a typical $100\times$ apochromat objective. (c)–(e) Cutaway diagrams showing the increasing complexity of the lens system inside a $10\times$ objective for higher degrees of correction: (c) achromat; (d) fluorite (semi-apochromat); (e) apochromat.

[4] The major manufacturers of microscope objectives are Nikon, Olympus, Zeiss and Leica.

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The specifics of any given objective are imprinted or engraved on the external portion of the barrel. A list of abbreviations for the specifications commonly found on microscope objectives is shown in Table 8.3. In the following, we discuss the objective characteristics that are most important for optical manipulation applications:

- **Numerical aperture.** For optical manipulation, the single most important number characterising an objective is its numerical aperture [Eq. (2.22)]. This is a critical value that indicates the light acceptance angle, which in turn determines the light-gathering power, the resolving power and the depth of field of the objective. Although the numerical aperture of objectives working in air is naturally limited to about 0.95, the numerical aperture can be dramatically increased by designing the objective to be used with an immersion medium, such as water (typically 1.20) or oil (typically 1.30).[5]
- **Immersion medium.** Although most objectives are designed to image specimens, with air as the medium between the objective and the cover glass, in order to attain higher numerical apertures, many objectives are designed to image the specimen through another medium that reduces refractive index differences between glass and the imaging medium. High-resolution plan apochromat objectives can achieve numerical apertures up to 1.40 when the immersion medium is a special oil with a refractive index of about 1.51. Other common immersion media are water (refractive index 1.33) and glycerol (refractive index 1.47). Objectives designed for special immersion media usually have a colour-coded ring inscribed around the circumference of the objective barrel, as listed in Table 8.4. Some important properties of immersion oils are described in Box 8.1.

Suggestion: Check your immersion oil. The advantages of oil-immersion objectives are severely compromised if the wrong immersion oil is used. It is advisable to employ only the oil intended by the objective manufacturer and not to mix immersion oils between manufacturers to avoid unpleasant artefacts such as crystallisation or phase separation. Also, always check the expiry date of your immersion oil.

- **Working distance and coverslip thickness.** The working distance is the distance between the objective front lens and the top of the cover glass when the specimen is in focus, as shown in Fig. 8.3a. It is often inscribed on the barrel as a distance in millimetres. In most instances, the working distance of an objective decreases as the magnification increases. The working distance should be matched to the employed coverslip thickness [Table 8.2], which is typically 0.17 mm in most optical manipulation applications, i.e., a coverslip No. 1.5,[6] which is compatible with the working distances of immersion objectives

[5] Some objectives specifically designed for transmitted light fluorescence and dark-field imaging are equipped with an internal iris diaphragm that allows adjustment of the effective numerical aperture. Objectives designed for total internal reflection microscopy have numerical aperture higher than that of the medium where the focus is generated (typically water, $n_m = 1.33$) in order to produce evanescent illumination.

[6] There is often some variation in thickness within a batch of coverslips. For this reason, some of the more advanced objectives have a correction collar for adjustment of the internal lens elements to compensate for this variation. Optical correction for spherical aberration is produced by rotating the collar, which causes two of the lens element groups in the objective to move either closer together or farther apart.

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Table 8.3 Objective abbreviations

Achro, Achromat	Achromatic aberration correction
Fluor, Fl, (Neo)fluar, Fluotar	Fluorite aberration correction
Apo	Apochromatic aberration correction
Plan, Pl, Achroplan, Plano	Flat field optical correction
EF, Acroplan	Extended field (field of view less than plan)
N, NPL	Normal field of view plan
Plan Apo	Apochromatic and flat field correction
UPLAN	Olympus universal plan[a]
LU	Nikon luminous universal[a]
L, LL, LD, LWD	Long working distance
ELWD, SLWD, ULWD	Extra-long, super-long, ultra-long working distance
Corr, W/Corr, CR	Correction collar
I, Iris, W/Iris	Adjustable numerical aperture (with iris diaphragm)
Oil, Oel	Oil immersion
Water, WI, Wasser	Water immersion
HI	Homogeneous immersion
Gly	Glycerin immersion
DIC, NIC	Differential or Nomarski interference contrast
CF, CFI	Chrome-free, chrome-free infinity-corrected (Nikon)
ICS	Infinity colour-corrected system (Zeiss)
RMS	Royal Microscopical Society objective thread size
M25	Metric 25 mm objective thread
M32	Metric 32 mm objective thread
Phase, PHACO, PC	Phase contrast
Ph 1, 2, 3, etc.	Phase condenser annulus 1, 2, 3, etc.
DL, DM	Phase contrast: dark low, dark medium
PL, PLL	Phase contrast: positive low, positive low low
PM, PH	Phase contrast: positive medium, high contrast[b]
NL, NM, NH	Phase contrast: negative low, medium, high contrast[c]
P, Po, Pol, SF	Strain-free, low birefringence, for polarised light
U, UV, Universal	UV-transmitting ($\gtrsim$ 340 nm) for epifluorescence
M	Metallographic (no coverslip)
NC, NCG	No coverslip
EPI	Oblique or epi illumination
TL BBD, HD, B/D	Bright or dark-field (hell, dunkel)
D	Dark-field
H	For use with a heating stage
U, UT	For use with a universal stage
DI, MI, TI	Interferometry, noncontact, multiple beam (Tolanski)

[a] Bright-field, dark-field, DIC and polarised light.
[b] Regions with higher refractive index appear darker.
[c] Regions with higher refractive index appear lighter.

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Table 8.4 Immersion medium colour codes

Immersion medium	Colour code
Oil	Black
Water	White
Glycerol	Orange
Special	Red

Box 8.1 Immersion oil properties

Usually, the refractive index of an immersion oil is specified for one of the spectral lines given in the following table:

Fraunhofer symbol	Element	Wavelength
e	Mercury	546.1 nm
D	Sodium	589.3 nm
d	Helium	587.6 nm
F′	Cadmium	480.0 nm
F	Hydrogen	486.1 nm
C′	Cadmium	643.8 nm
C	Hydrogen	656.3 nm

DIN specification for immersion oil is a refractive index of $n_e = 1.5150 \pm 0.0004$ (at 23°C) and a dispersion given by the Abbé V-number $v_e = 44 \pm 5$ (at 23°C), where $v_e = \frac{n_e - 1}{n_F - n_C}$.

The viscosity of an immersion oil is also an important parameter. Low-viscosity oils are less apt to retain small bubbles and may be preferred when the distance from the cover glass to the objective is very small. Higher-viscosity oils fill larger gaps more satisfactorily and are also reusable in that a slide may be removed and replaced with another to make contact with the oil drop that remains on the objective lens. The kinematic viscosity of some representative immersion oils is given in the table below in the cgs unit of centistokes (cSt), where $1\,\text{cSt} \equiv 10^{-6}\,\text{m}^2\text{s}$:

Oil	Viscosity
Cargille type A (low viscosity)	150 cSt
Cargille type B (medium viscosity)	1250 cSt

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Table 8.5 Objective magnification colour codes

Magnification	Colour code
0.5×	Not assigned
1×, 1.25×, 1.5×	Black
2×, 2.5×	Brown
4×, 5×	Red
10×	Yellow
16×, 20×	Green
25×, 32×	Turquoise
40×, 50×	Light blue
60×, 63×	Cobalt blue
100×, 150×, 250×	White

- **Trasmission efficiency.** Most objectives are designed to work within the visible range of optical wavelengths. Typically, their transmission efficiency is about 60% within this range for high-numerical-aperture immersion objectives. However, it can become much less at longer wavelengths in the near infrared for some objectives. Because these wavelengths are often employed in optical manipulation applications, e.g., to prevent photodamage to biological samples, one should pay attention to this issue in order not to lose too much laser power.
- **Magnification.** There exist commercial objectives with magnifications ranging from 0.5× to 250×. The most commonly employed for three-dimensional optical trapping applications are 100× oil-immersion objectives and 60× water-immersion objectives. We must remark, however, that the magnification is not the most important parameter for optical trapping applications. To help with the rapid identification of the magnification, microscope manufacturers label their objectives with colour codes, which are given in Table 8.5.
- **Optical aberration corrections.** Objectives are corrected to reduce their chromatic and spherical aberrations [Box 2.2]. Depending on the objective corrections, the internal system of lenses becomes more and more complex (and the objective correspondingly more and more expensive).
  - The simplest objectives are *achromatic objectives* with the least amount of correction [Fig. 8.3b]. Achromats are corrected for axial chromatic aberrations at two wavelengths, typically blue (486 nm) and red (656 nm), and for spherical aberrations in the green (546 nm). This limited correction can lead to substantial artefacts when specimens are examined and imaged with colour microscopy; for example, if the focus is chosen in the green region of the spectrum, images will have a reddish-magenta halo (residual colour). Nevertheless, these objectives can be a good compromise for optical tweezers application when only one wavelength is employed and there is no specific need for imaging the sample at high quality.

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Table 8.6 Objective aberration corrections

Objective type	Spherical aberration	Chromatic aberration	Field curvature
Achromat	1 colour	2 colours	Uncorrected
Plan achromat	1 colour	2 colours	Corrected
Fluorite	2–3 colours	2–3 colours	Uncorrected
Plan fluorite[a]	3–4 colours	2–4 colours	Corrected
Apochromat	3–4 colours	4–5 colours	Uncorrected
Plan apochromat	3–4 colours	4–5 colours	Corrected

[a] Plan fluorite is sometimes referred to as plan semi-apochromat.

- The next level of correction is found in *fluorite objectives*[7] or *semi-apochromat objectives* [Fig. 8.3c]. The lenses of fluorites are produced from advanced glass formulations that contain materials such as fluorspar or newer synthetic substitutes, which allow greatly improved correction of optical aberrations. Fluorites are corrected chromatically for red and blue light and spherically for two or three colours. The superior correction of fluorites compared to achromats enables these objectives to be made with a higher numerical aperture.
- The highest level of correction is found in *apochromatic objectives* [Fig. 8.3d]. Apochromats are corrected chromatically for three colours (red, green and blue), almost eliminating chromatic aberrations, and are corrected spherically for either two or three wavelengths. Although the lens design is similar to that for fluorites, they employ more advanced materials. Apochromats are the best choice for colour photomicrography in white light. Because of their high level of correction, apochromat objectives usually have, for a given magnification, higher numerical apertures than achromats or fluorites. The best high-performance fluorite and apochromat objectives are corrected for four (dark blue, blue, green, and red) or more colours chromatically and four colours spherically.

All three types of objectives suffer from pronounced field curvature and project images that are curved rather than flat, an artefact that increases in severity with higher magnification. To overcome this inherent condition, optical designers have produced flat-field corrected objectives, which yield images that are in common focus throughout the viewfield. Objectives that have flat-field correction and low distortion are called *plan achromats*, *plan fluorites*, or *plan apochromats*, depending upon their degree of residual aberration. An overview of the main objective characteristics is given in Table 8.6.

- **Parfocal distance.** The parfocal distance is measured from the nosepiece objective mounting hole to the point of focus on the specimen, as shown in Fig. 8.3a. This is another specification that can often vary by manufacturer. The major manufacturers

[7] They are named after the mineral fluorite, which was originally used in the manufacture of their lenses.

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produce objectives that have a 45 mm or a 60 mm parfocal distance [Table 8.1]. Most manufacturers also make their objective nosepieces parcentric, meaning that when a specimen is centred in the field of view for one objective, it remains centred when another objective is brought into use.

- **Mechanical tube length.** This is the length of the microscope body tube between the nosepiece opening, where the objective is mounted, and the top edge of the observation tubes, where the oculars (eyepieces) are inserted. The tube length is usually inscribed on the objective in millimetres (typically between 160 and 200 mm depending on the manufacturer, as shown in Table 8.1) for fixed tube lengths or with the infinity symbol (∞) for infinity-corrected tube lengths.
- **Objective screw threads.** Historically, the mounting threads on almost all objectives conformed to the Royal Microscopical Society (RMS) standard in order to enable universal compatibility. This standard is still used in the production of infinity-corrected objectives by the manufacturer Olympus. Zeiss, Nikon and Leica have broken from the standard with the introduction of new infinity-corrected objectives that have a wider mounting thread size, making these objectives usable only on their own microscopes [Table 8.1].
- **Specialised optical properties.** Microscope objectives often have design parameters that optimise performance under certain conditions. For example, there are special objectives designed for polarised illumination signified by the abbreviations P, Po, POL, or SF (strain-free and/or having all barrel engravings painted red), phase contrast (PH and/or green barrel engravings), differential interference contrast (DIC), and many other abbreviations for additional applications. A list of several abbreviations, often manufacturer specific, is presented in Table 8.3.

8.2.2 Illumination schemes

Proper illumination of the specimen is crucial for obtaining the best-quality imaging in a microscope, and impacts both the ease of operation of an optical tweezers experiment and the efficacy of subsequent analysis by, e.g., particle detection, counting and tracking algorithms. Here, we review the basics of achieving suitable illumination in the microscope and more advanced techniques that can be used to improve the contrast, particularly of biological samples.

Bright-field microscopy

Bright-field illumination is the simplest and most common scheme used in transmitted light microscopy: the illumination light passes through the sample to be collected by the objective and imaged onto a camera. This is the illumination scheme used in the inverted microscope we built [Figs. 8.2g and 8.2h]. Contrast in the image is produced by refraction, which is induced by local changes in refractive index and absorption in the specimen. Control of the illumination can be achieved by regulation of two diaphragms, shown in Fig. 8.4: the *condenser diaphragm*, which is placed at the condenser aperture to adjust the

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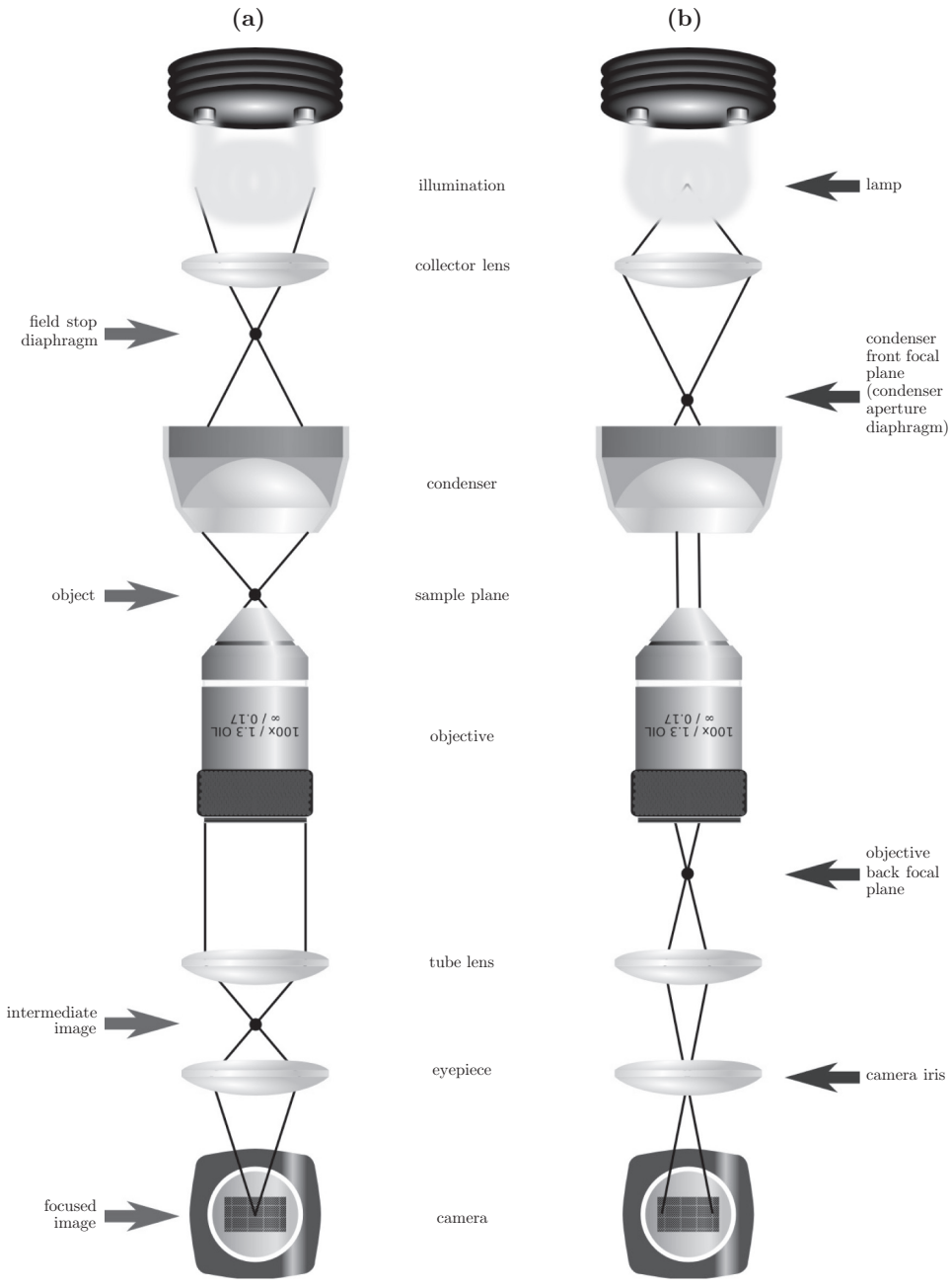

Figure 8.4 Köhler illumination: Location (a) of the field planes, conjugate with the field stop aperture, and (b) of the aperture planes, conjugate with the lamp filament.

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effective numerical aperture of the condenser lens and controls, in combination with the objective, the resolution of the system; and the *field stop diaphragm*, which controls the width of the bundle of rays reaching the condenser and is used to eliminate excess light scattering that may degrade the contrast in the image.

Köhler illumination

The Köhler illumination scheme, first introduced by August Köhler in 1893, produces optimum illumination of the sample, achieving high contrast by illuminating the sample evenly. The principle is illustrated by the ray paths shown in Fig. 8.4. Fig. 8.4a shows the *field planes*, i.e., the planes that are conjugate with the field stop diaphragm: the object in the sample plane, an intermediate image between the tube lens and the eyepiece (or the lens which focuses the light onto the camera), and the camera sensor where the (magnified) image is formed. Fig. 8.4b shows the *aperture planes*, i.e., the planes that are conjugate with the filament of the illumination lamp: the front focal plane of the condenser, the back focal plane of the objective and, in this case, the iris of the camera. This arrangement ensures that the structure of the filament is not imaged onto the sample or the camera, so that it is prevented from causing significant variation in intensity across the image.

The microscope can be configured for Köhler illumination following these steps:

1. observe a specimen (a sample of microspheres stuck to a coverslip is useful for this purpose) under simple bright-field illumination with both field diaphragm and condenser aperture diaphragm fully open;
2. close down the field stop diaphragm and translate the condenser lens (this can be done by using the focusing control knob in a commercial microscope) until the edges of the diaphragm are in focus;
3. adjust the transverse position of the condenser to achieve maximum intensity through the closed-down field diaphragm;
4. open the field stop diaphragm;
5. adjust the aperture diaphragm to illuminate the sample and achieve the best balance between contrast and resolution.

We note that following these steps should properly configure the microscope for Köhler illumination for the objective with which the procedure is carried out, whereas changing the objective will require re-adjustment of the condenser aperture and field diaphragms for optimum illumination.

Phase contrast

The phase contrast technique, invented by Frits Zernike in 1934,[8] is best applied to thin specimens that have little absorption and small refractive index difference from the surrounding medium and, hence, have poor visibility in standard bright-field microscopy. Its

[8] Zernike was awarded the Nobel Prize in Physics in 1953 'for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope'.

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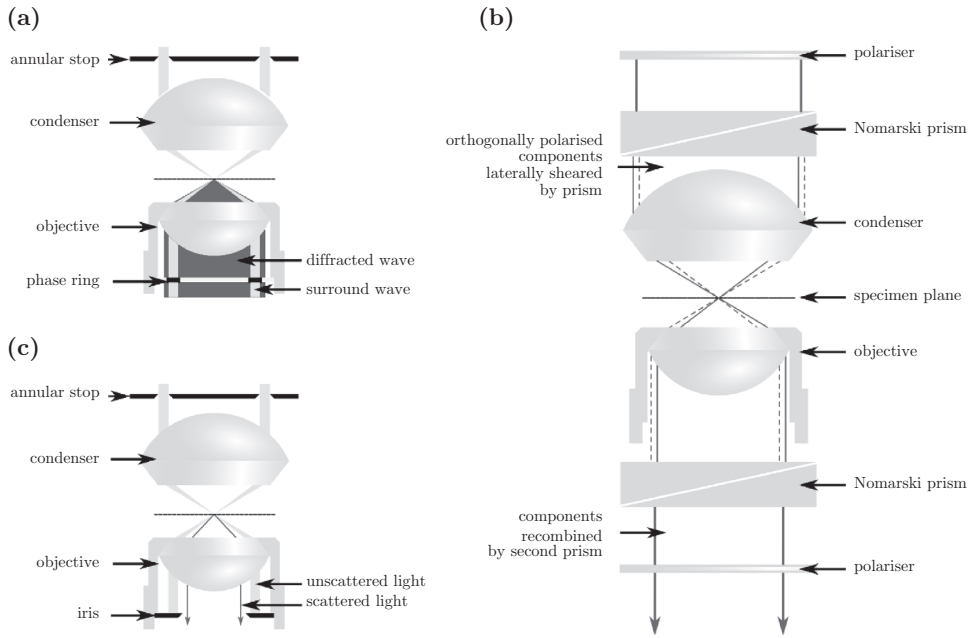

Figure 8.5 Contrast enhancement techniques: (a) phase contrast; (b) dark-field illumination; (c) differential interference contrast (DIC).

principle of operation is illustrated in Fig. 8.5a. Phase contrast microscopy requires the addition of an *annular stop* at the condenser aperture and the use of a corresponding *phase contrast objective*, denoted by the legend Ph1, Ph2 or Ph3 on the objective barrel [Table 8.3], where the number refers to the diameter of the phase stop annulus. Under conditions of Köhler illumination, the specimen is illuminated by plane waves emanating from the condenser annular stop. Waves that do not interact with the specimen (sometimes called *surround waves*) are brought to a focus in the back focal plane of the objective, which is conjugate with the condenser aperture, as shown in Fig. 8.4b. For a weakly scattering specimen, i.e., one with a low refractive index contrast, the scattered waves (sometimes called *diffracted waves*) have a small amplitude compared to that of the surround waves but, crucially, acquire a small phase shift in their interaction with the sample; for example, for a low-contrast biological sample with a thickness of ≈ 5 μm, this phase shift is about $\pi/2$ for light in the green part of the optical spectrum. The diffracted waves occupy the whole extent of the pupil, as shown in Fig. 8.5a. The phase and amplitude of the surround waves, which would otherwise overwhelm the small-amplitude diffracted waves, are controlled by a phase ring in the back focal plane of the objective, which acts predominantly on the surround waves by advancing their phase by $\pi/2$ and by attenuating them. Eventually, the surround and diffracted waves interfere at the image plane and the contrast of the specimen over the background illumination is greatly enhanced compared to that with bright-field illumination.

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Dark-field microscopy

Dark-field microscopy also uses an annular condenser stop to illuminate the specimen at oblique angles. In the dark-field configuration, though, unscattered waves are either simply not collected by a low-numerical-aperture objective or, as shown in Fig. 8.5b, stopped by an adjustable iris inside a specialised high-NA objective. The effect is to increase contrast, as only light that has been scattered by the specimen is collected, resulting in a bright image on a dark background.

Differential interference contrast

Differential interference contrast (DIC), sometimes called Nomarski interference contrast after its inventor, Georges Nomarski, increases contrast by converting gradients in optical path difference into intensity modulation. In DIC, initially linearly polarised light is separated into two orthogonally polarised beams with a small displacement by a modified Wollaston (Nomarski) prism, as illustrated in Fig. 8.5c. If the specimen presents gradients in the optical path experienced by the two beams become of gradients in thickness and/or refractive index, then the two beams acquire a relative phase shift. The lateral shear between the two beams is removed by a second Nomarski prism and an analyser (polariser) projects the polarisation of the two beams onto the same axis. Gradients in optical path in the sample are then converted into gradients in intensity in the interfering beam, resulting in improved contrast, although as one side of a feature in the specimen appears brighter than the opposite side, the features often present a shadowed, pseudo three-dimensional appearance. DIC microscopy requires the use of a specialised condenser and prism system. Unlike phase contrast and dark-field objectives, the objective is not modified internally, but is designed to be used with an external Nomarski prism.

8.3 Sample preparation

We are now at the point where we need to prepare a sample with some trappable objects. The range of trappable objects is quite large, as we have seen in Fig. 1.3. However, to start it is simplest to use some synthetic microparticle in aqueous solution. Particles with very good characteristics, e.g., sphericity and size variance, are widely available from commercial providers.[9] The ideal size to start with lies in the intermediate size regime, i.e., from about 0.5 to 2 μm radius, where optical forces are maximised, as we have seen in Fig. 5.1. It is also possible to use some simple living organisms, e.g., yeast cells, which can be obtained easily and safely from a bakery. At first, one can simply place a droplet of the solution on top of a glass slide placed on the microscope sample stage and then slightly raise the objective (or lower the sample) in order to have the focal spot inside the droplet and be able to observe

[9] Some providers of microparticles are Duke Scientific, Bangs Labs, Microparticles.de, Polysciences and Kisker Biotech.

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Table 8.7 Microparticle material properties

Material	Refractive index	Density
Melamin resin (MF)	1.68	$1.51\,\mathrm{g\,cm^{-3}}$
Polystyrene (PS)	1.59	$1.05\,\mathrm{g\,cm^{-3}}$
Poly(methyl methacrylate) (PMMA)	1.48	$1.19\,\mathrm{g\,cm^{-3}}$
Silica (SiO_2)	1.42 to 1.46	$1.8\,\mathrm{g\,cm^{-3}}$ to $2.0\,\mathrm{g\,cm^{-3}}$

the objects in the suspension. Of course, we will soon need a more professional sample. In fact, the sample prepared as just described is not stable; e.g., it is subject to evaporation. In the following, we provide some recipes for how to prepare a sealed sample. These should be treated as starting guidelines to perfect one's own sample. Indeed, it is safe to say that there are at least as many approaches to sample preparation as researchers, because the specific sample to be employed strongly depends on the experiment to be conducted and also on the preferences of the experimentalist preparing it.

The sample preparation starts by suspending microparticles in a solution (often an aqueous solution) at an appropriate concentration. A series of typical microparticle parameters is given in Table 8.7. Microparticles are usually supplied by manufacturers at high concentration. The number density, i.e., the number of particles per millilitre of liquid, can be found as

$$n = \frac{w \times 10^{12}}{\frac{4}{3}\pi a^3 \rho}, \tag{8.1}$$

where w is the particle concentration in mg/ml, ρ is the particle material density in g/cm^3 and a is the particle radius in μm. For example, $a = 1\,\mu\mathrm{m}$ radius polystyrene spheres may be sold at 2.5% solids (w/v) or $w = 25\,\mathrm{mg/ml}$, i.e., 25 mg of solid material per millilitre of solution, giving a number density of 4.6×10^{10} particles/ml. A solution at workable concentration typically requires diluting this concentration by several orders of magnitude, e.g., in the ratio of one part microparticle solution to $\approx 10^6$ parts solution.[10]

We now need a sample cell to host our solution on top of the microscope sample stage. A possible preparation process is shown in Fig. 8.6. The process starts with a carefully cleaned microscope glass slide [Fig. 8.6a], on top of which two stripes of parafilm (about 100 μm thick) are placed [Fig. 8.6b]. A thin coverslip is then placed on top of the parafilm [Fig. 8.6c] and this sample is heated so that the parafilm sticks to the glass surfaces. Finally, after removal of the excess parafilm [Fig. 8.6d], the chamber is filled with sample solution using a pipette, sealed using vacuum grease [Fig. 8.6e], placed upside down on the sample stage and gently fixed with some plastic screws [Fig. 8.6g]. It is now possible to observe the sample and to apply techniques such as digital video microscopy [Section 9.1] to study the Brownian motion of particles.

[10] This is best achieved by subsequent dilutions of the commercial solution. For example, it is possible to make a mother solution by diluting 100 to 1000 times the commercial solution and then dilute the mother solution further to create the solutions to be employed in the actual experiments.

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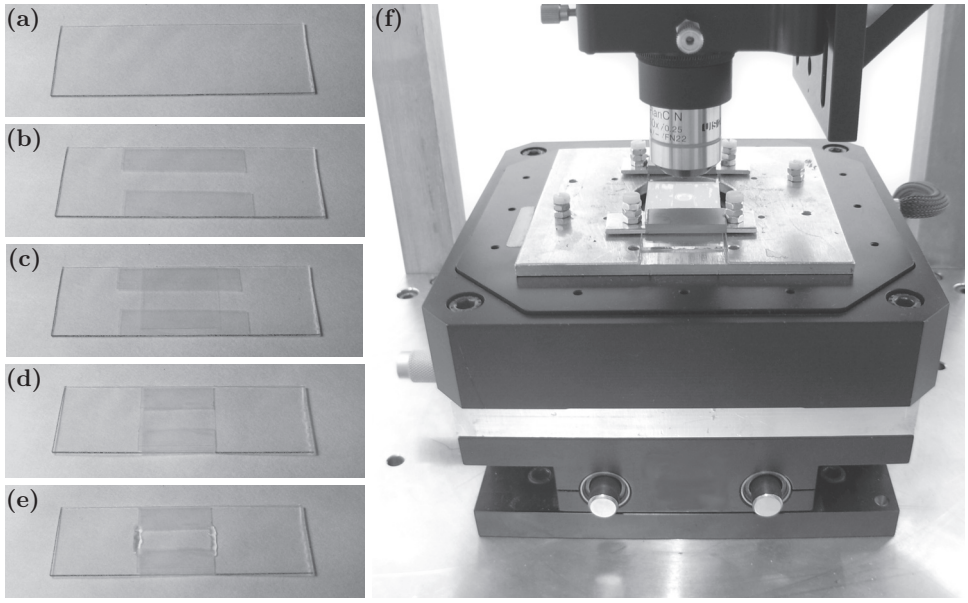

Figure 8.6 Sample preparation: (a) a glass slide is carefully cleaned; (b) two stripes of parafilm (about 100 μm thick) are placed on the glass; (c) a coverslip is placed on top of the parafilm, and the sample is heated in order for the parafilm to stick to the glass surfaces (not shown); (d) the excess parafilm is removed; (e) the chamber is filled with sample solution (e.g., microparticles suspended in an aqueous solution) and is sealed using vacuum grease; (g) the chamber is placed upside down on the sample stage and is gently fixed with some plastic screws.

Starting from the recipe explained, several variations are possible in order to build a sample cell. In particular, it is possible to use microscope slides having a well. Standard 76 mm × 26 mm slides with a 15 mm-diameter recess in one face can be obtained readily from suppliers. The process involves the following steps: fill or slightly overfill the recess with 80 μl of liquid (a calibrated pipette is useful for this purpose); place a coverslip on the slide adjacent to the recess and then slide it over while maintaining downward pressure; finally, fix the coverslip in place, using epoxy or even nail polish. Another alternative is to use adhesive spacers, such as Invitrogen SecureSeal. A convenient size is the 9 mm-diameter spacer available from Fisher Scientific (Product Code 10634453), which may be stuck to a plain slide, filled with a small volume of the sample solution and sealed using a coverslip.

Troubleshooting: My particles get stuck to the coverslip and/or between themselves. This is most likely due to electrostatic interactions between the particles and/or the coverslip. When working with an aqueous solution, you can perform one or more of the following operations:

- use deionised water to increase the screening length and, therefore, the electrostatic repulsion between particles;

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- use a hydrophilic coverslip to increase the electrostatic repulsion between the coverslip and the particles (the coverslip can be made hydrophilic by plasma cleaning it or by leaving it for several minutes in a 1 mM NaOH solution);
- add a small amount of surfactant to the solution, e.g., sodium dodecyl sulphate (SDS) or Triton-X.

Troubleshooting: I took the sample out and put it back. Now the image on the camera looks terrible. If you have an oil-immersion objective, the act of removing and replacing the slide may have created small bubbles in the oil so that there is no longer a continuous medium between the objective and coverslip. Clean both the objective and the coverslip, and start again with fresh immersion oil.

8.4 Optical beam alignment

We are now ready to transform our inverted microscope into an optical tweezers by coupling a laser beam to the set-up we realised in the previous sections. The first crucial step is to choose an adequate laser, which depends strongly on the foreseen application. The most important considerations to be kept in mind are the following (a more in-depth overview of lasers is given in Subsection 8.4.1):

- **Laser power.** Typically, a laser with a power of about 10 to 100 mW is more than sufficient to generate a single optical trap capable of manipulating microscopic particles. As a rule of thumb, one can expect the optical stiffness to have a value similar to the ones reported in Fig. 5.1. Note that, because optical losses occur at the objective (which can be as large as 50%) and because of the necessary overfilling, the laser power at the focus, which is the laser power that ultimately determines the stiffness of the optical tweezers, might be significantly lower than the original one.
- **Wavelength.** In many cases, the wavelength of the laser light plays a crucial role. In particular, it is important to consider the potential for photodamage to the specimen, because the power density in even a low-power optical beam brought to a diffraction-limited spot can exceed 10^6 W/cm^2. For example, for biological applications it is important to choose a wavelength that minimises absorption (to prevent local heating) and photodamage (to maintain the biological samples' viability). Because most biological samples are in an aqueous medium, it is useful to keep in mind the absorption coefficient of water, shown in Fig. 8.7a.[11] Low photodamage is typically achieved for wavelengths in the near infrared,

[11] The absorption coefficient of a material, e.g., α_w for water shown in Fig. 8.7a, is expressed in cm^{-1}. According to Beer's law, the intensity of an electromagnetic wave penetrating a material falls off exponentially with distance from the surface, i.e., $I(d) = I_0 e^{-\alpha_w d}$, where $I(d)$ is the light transmitted at a distance d (expressed in cm) from the surface and I_0 is the incident intensity.

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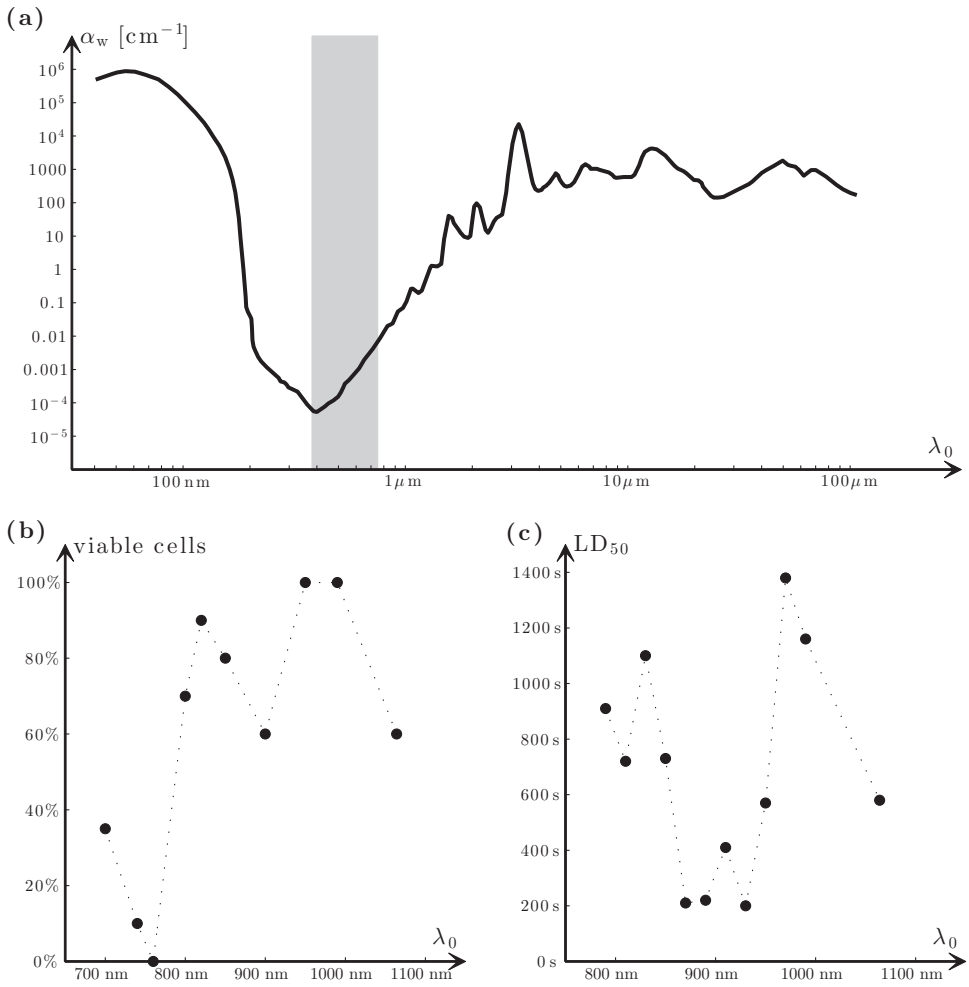

Figure 8.7 Wavelength dependence of water absorption and photodamage to biological samples: (a) Absorption coefficient of water α_w as a function of the vacuum wavelength λ_0. The shaded area represents the visible portion of the spectrum. (b) and (c) show the photodamage to biological material held in optical tweezers as a function of wavelength: (b) percentage of Chinese hamster ovary cells that remain viable after 5 minutes of trapping at 88 mW power (Liang et al., 1996); (c) time after which the rotation rate of the flagella of *E. coli* bacteria held in an optical tweezers at 100 mW trapping power decreases to 50% the initial rate (Neuman et al., 1999).

as is shown for the case of Chinese hamster ovary cells in Fig. 8.7b and for the case of *E. coli* bacteria in Fig. 8.7c. Furthermore, one should consider the transmission properties of the objective lens at the wavelengths being used, as the transmission across the near infrared is considerably less than 100% and, for some objectives, drops dramatically for wavelengths above ≈ 850 nm.[12]

[12] It is advisable to check carefully the transmission characteristics supplied by the manufacturer of an objective not only at the trapping wavelength but also at any other wavelengths that may be used, e.g., for acquisition of

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- **Beam stability and quality.** Depending on the application at hand, the stability and quality of the beam can play a crucial role. This is particularly true in applications that require high reproducibility and quantitative measurements, e.g., measurement of nanoscopic forces and torques. For more qualitative applications, e.g., the optical trapping and delivery of cells or vesicles, it might be possible to relax the requirements on beam stability and quality.

Suggestion: Start with a visible laser. When building your first optical tweezers, we advise you to use a laser emitting in the visible portion of the spectrum with relatively low power. For example, HeNe lasers at 633 nm (red) or solid state lasers at 532 nm (green) with output power about 50 mW are ideal.

Once the laser has been chosen, one has to firmly fix it onto the optical table, as shown in Fig. 8.8a. In our case, we use a solid state laser with wavelength 1064 nm. To increase the stability of the laser beam, we prefer not to change the laser output power during our experiments. Therefore, we opt for an alternative way of controlling the laser power: we place along the beam path a linear polariser, a half-wave plate and a second beam polariser; in this way, we can tune the power of the beam without altering the laser setting by rotating the half-wave plate. The next step is to direct the laser through the objective, making use of a series of mirrors, as shown in Fig. 8.8b. We first use mirror M_4 to align the beam parallel to the optical table.[13] Then we proceed to direct the laser beam through the objective using mirrors M_5 and DM_6. In doing this, we apply the basic principle of beam steering, which is illustrated in detail in Fig. 8.9 and, in a nutshell, permits one to use two steerable mirrors to get a laser beam to go along any straight line. We also note at this point that DM_6 is in fact a dichroic mirror, which permits one to split the light of the laser beam, which goes to the objective, and the light of the lamp, which goes to the camera. We also add a filter in the light path leading to the camera in order to remove any laser light that might saturate the camera.

Suggestion: Centre your beams. Optical beams should always go through the centres of optical elements, e.g., lenses and mirrors. This is because these optical elements are usually optimised for a beam going through their centres (e.g., using a lens off axis can result in the introduction of aberrations that can severely affect the qualify of focusing) and also because this makes any future correction to the beam path easier.

Suggestion: Use dielectric mirrors. It is better to use a dielectric mirror instead of a metallic mirror in order to reduce losses. In fact, whereas losses of about 5% can be expected at metallic mirrors, at dielectric mirrors these losses can be reduced to about 0.5% or less.

Raman or fluorescence spectroscopy signals. Also, the range of wavelengths over which aberrations are well corrected is important, as an objective designed for, e.g., fluorescence microscopy in the visible spectrum may have relatively poor aberration correction in the near infrared.

[13] It is also usually useful to align the beam such that it goes straight along a series of holes in the optical table.

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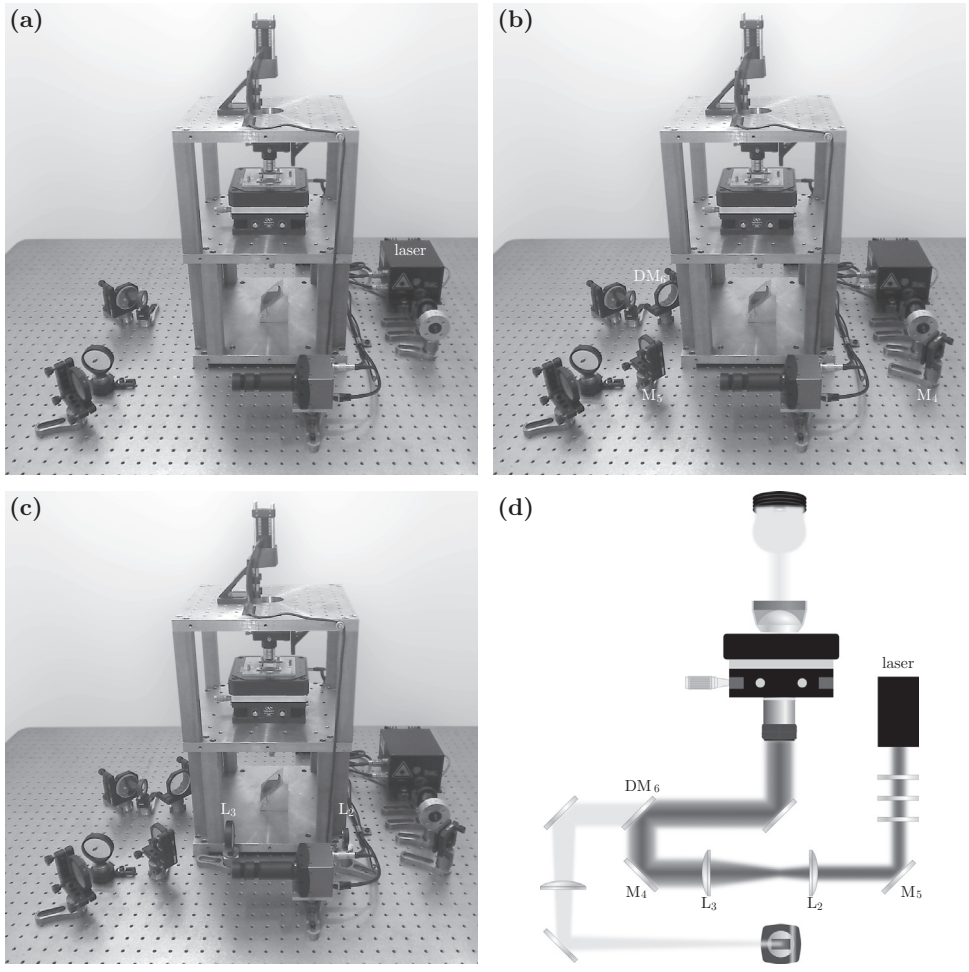

Figure 8.8 Alignment of the laser beam to generate an optical tweezers: (a) The laser head is firmly clamped on the optical table and the laser beam is prepared by using two polarisers and a half-wave plate to control the beam power without changing the laser power setting (a filter is also added in the camera path to prevent the laser light from reaching the camera); (b) a series of mirrors (M_4, M_5 and DM_6) are added to guide the beam through the objective in order to generate the optical trap; (c) a telescope (constituted by lenses L_1 and L_2) is added to increase the beam waist size and overfill the objective back aperture. (d) Corresponding schematic of the optical tweezers set-up.

Finally, as shown in Fig. 8.8c, we need to add a telescope in order to create a beam with an appropriate size to overfill the objective back aperture and to generate a strong optical trap. The beam waist can be straightforwardly measured by fitting a Gaussian profile to an image of the beam profile acquired by a digital camera (some neutral density filters might be necessary to prevent the camera from saturating).[14]

[14] Alternatively, if the beam is larger than your camera chip, it is possible to use a knife-edge and a photodiode to measure the transmitted power while cutting the laser beam transversally along a direction (x). The transmitted

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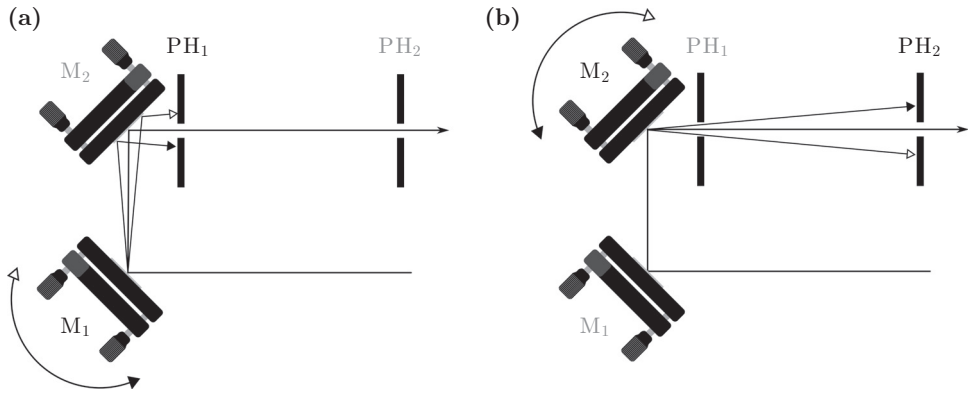

Figure 8.9 Beam alignment technique. In order to get a laser beam to go along any line (indicated by the two pinholes PH_1 PH_2), it is possible to use two manually steerable mirrors (M_1 and M_2) and follow the procedure illustrated here: (a) with M_1, centre the beam on PH_1; (b) with M_2, centre the beam on PH_2; repeat the previous two steps iteratively. The convergence of this procedure is greatly improved by placing PH_1 as close as possible to M_2 and PH_2 as far away as possible from M_2.

Finally, we can align the beam so that the focus is on the glass–air interface. This is best done using a sample without any liquid in order to increase the amount of reflected light that goes back to the camera, but can also be done with a sample filled with a solution, even though the intensity of the back-scattered light will be lower. It might also be useful to remove the filter in front of the camera. In practice, one has to approach the objective to the sample slowly, until the back-scattered pattern size becomes minimised, as shown in Fig. 8.10. The resulting pattern should be as symmetric as possible. Furthermore, the pattern should remain symmetric and with the same centre as the relative distance between focus and interface is changed.

Troubleshooting: I don't get a symmetric back-scattered light pattern. The lack of symmetry in the back-scattered light pattern is most likely due to misalignment of the beam. You should check that the beam is perfectly centred and aligned with the objective axis.

power is the integral of the cut laser beam intensity profile, i.e.,

$$P(x) = \frac{P_{\text{beam}}}{2}\left[\text{erf}\left(\frac{\sqrt{2}x}{w_0}\right) + 1\right],$$

where P_{beam} is the total beam power, w_0 is the beam waist and

$$\text{erf}(\xi) = \frac{2}{\sqrt{\pi}}\int_{-\infty}^{\xi} e^{-t^2}\,dt$$

is the *error function*. Thus, by fitting the measured power as a function of the knife-edge position, it is possible to measure the laser beam waist.

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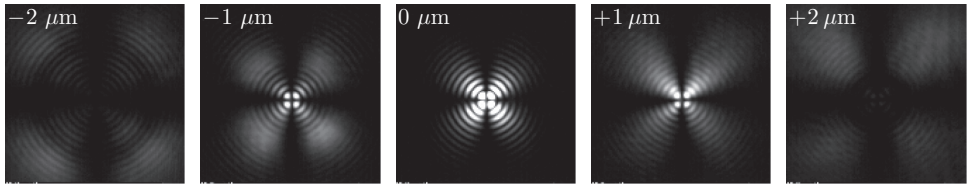

Figure 8.10 Back-scattered light patterns from a focused beam: patterns generated at a glass–air interface as a function of the focus–interface distance (z). It can be seen how the pattern becomes smaller as the focus–interface distance decreases. The cross is due to the presence of polarisation elements; often, one can observe rings instead.

Troubleshooting: As I move the objective, the back-scattered light pattern changes its centre. This is most likely due to misalignment between the beam and objective axes, i.e., the beam is tilted. Correct the vertical alignment of the beam.

8.4.1 Lasers

Laser safety

Correct safety procedures when working with lasers are of paramount importance. In fact, lasers are potentially harmful devices, which, depending on their power and wavelength, can cause eyesight impairment and other physical injuries. We will not discuss laser safety procedures in depth here, as this book should not be a substitute for rigorous laser safety training. However, we urge, all users to undergo suitable safety training before starting to work with lasers and to observe all local safety regulations, including, in particular, the use of appropriate eye safety equipment.

For reference only, we provide in Table 8.8 a summary description of the hazards by laser safety class according to the IEC (International Electrotechnical Commission) 60825-1 and ANSI (American National Standards Institute) Z136.1-2007 Laser classification schemes for lasers and laser systems operating in the range from 0.18 μm to 1 mm. The classification of a laser depends on the wavelength, output power, beam divergence and user's exposure time.

Technical characteristics

Several different lasers are available. A list of the main kinds that have been used in optical trapping experiments is given in Table 8.9. In the following, we discuss the laser characteristics that are most important for optical manipulation applications:

- **Beam quality.** The quality of the laser beam is critical to achieve the tightly focused (as close to diffraction-limited as possible) spot required for optical trapping. The quality of the laser beam is often expressed as the parameter M^2, which is the ratio between the *beam parameter product* of the laser beam and that of a diffraction-limited beam. The beam parameter product is the product of the beam waist and the beam divergence

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Table 8.8 Laser safety classification scheme

Laser class	Description of hazard
Class 1	Any wavelength. Safe under reasonable foreseeable conditions due to low emission power (e.g., < 0.39 mW for red laser) or due to total enclosure.
Class 1M	Any wavelength. Geometrical spread of beam reduces naked eye exposure to maximum permissible exposure (MPE) level, but beam is hazardous with viewing aids.
Class 2	Visible lasers only, $\lambda_0 = 400$ to 700 nm. Low power. Blink reflex affords adequate protection.
Class 2M	Visible lasers only. Moderate power where beam divergence reduces the hazard, but may be hazardous if viewing aids or optics are used.
Class 3R	Any wavelength. Moderate power: accessible emission limit is $5\times$ Class 1 limit for invisible lasers or $5\times$ Class 2 limit for visible. Risk of injury is relatively low, but prevent direct exposure to the beam.
Class 3B	Any wavelength. Direct viewing is hazardous. Upper limit can be harmful to skin and pose scatter hazard.
Class 4	Any wavelength. No upper power limit. Harmful to eyes and skin. Possible scattered light hazard.

Table 8.9 Lasers

Laser	Typical wavelength	Typical power
Diode lasers	Visible – near infrared	< 250 mW
Fibre lasers	Near infrared (e.g., 1064 nm, 1070 nm)	1 W to 10 W
Diode-pumped solid state (DPSS) lasers, e.g., Nd:YAG, Nd:YLF	Near infrared 1064 nm, 1047 nm, 1053 nm	1 W to 10 W
Frequency-doubled DPSS lasers, e.g., frequency-doubled Nd:YAG	Visible 532 nm	1 W to 5 W
Helium–neon (HeNe) lasers	633 nm	< 100 mW

half-angle, which for a diffraction-limited beam is λ_0/π. A Gaussian beam has $M^2 = 1$ and, for optical tweezers applications, a laser beam with M^2 as close as possible to this diffraction-limited performance is preferable. Typically, a spatial mode TEM_{00} will also be specified.

- **Pointing stability.** For optical tweezers applications, good pointing stability is necessary to keep the position of the optical trap steady. Fluctuations in beam-pointing direction can arise from, e.g., mechanical vibrations of optical elements in the laser resonator or thermal effects in the laser gain medium. Several different quantities are used to express the pointing stability, so care should be exercised when trying to interpret this parameter,

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paying attention in particular to the conditions under which it has been measured and to whether the quoted stability refers to the average direction of the beam or to the root-mean-square (rms) fluctuations in direction. One commonly used measure is the change in beam direction with a change in temperature, usually specified in μrad/°C.

- **Power stability and noise.** In optical trapping experiments, fluctuations in laser power lead to fluctuations in the strength of the optical trap. In laser data sheets, there are usually specified the *power stability*, i.e., the drift in average laser power measured over an extended period of time, and the *noise*, i.e., fluctuations around the average of the laser power within a specified bandwidth, typically from a few hertz to (tens of) megahertz. These quantities are often normalised by the average value during the measurement and quoted as either rms or peak-to-peak deviations from the average, expressed as a percentage.
- **Frequency stability.** In general, the frequency stability depends on the type of laser. It can range from several hundred to a few megahertz for a free-running laser. Active stabilisation through an external cavity and locking to atomic lines yields improvements of several orders of magnitude, as the linewidth of lasers locked to atomic transitions can range in the hundreds of kilohertz. Although the frequency stability of the laser is crucial for laser cooling of atoms, as we will see in Chapter 24, it is not very important for most mesoscopic optical tweezers applications.

In dealing with infrared lasers, a means of visualising the beam path is necessary. A laser viewing card can be used for this purpose. The card has a photosensitive area that glows with visible light in the spot illuminated by the laser beam, thus enabling the user to follow the beam path. Cards made with several materials with different sensitivity to different parts of the spectrum are available.

8.4.2 Lenses

Lenses are used in the optical train to manipulate the beam diameter and divergence. Factors that should be taken into account when selecting lenses include the following:

- **Material.** Optical tweezers typically use a laser with a wavelength in the visible to near-infrared portion of the spectrum. These lenses are typically made of N-BK7, a kind of glass which has a high transmission in this spectral range. Its refractive index is $n_\mathrm{d} = 1.5167$ and its dispersion is $v_\mathrm{d} = \frac{n_\mathrm{d}-1}{n_\mathrm{F}-n_\mathrm{C}} = 64$ (measured at the wavelength of the sodium D-line [Box 8.1]).[15]
- **Lens shape.** Cross-sections showing the shape of different common lens types are shown in Fig. 8.11. As most lenses readily available from commercial manufacturers have spherical surfaces, they introduce a small amount of spherical aberration, which can be minimised by turning the curved side towards the side where the beam is collimated,[16]

[15] Note, however, that although its Abbé V-number is high, indicating relatively low dispersion, at the wavelength $\lambda_0 = 1064\,\mathrm{nm}$, which is commonly used in optical tweezers, the refractive index is $n_{1064} = 1.5066$.

[16] Or towards the side where the beam has lower divergence, in case the beam is not collimated on either side.

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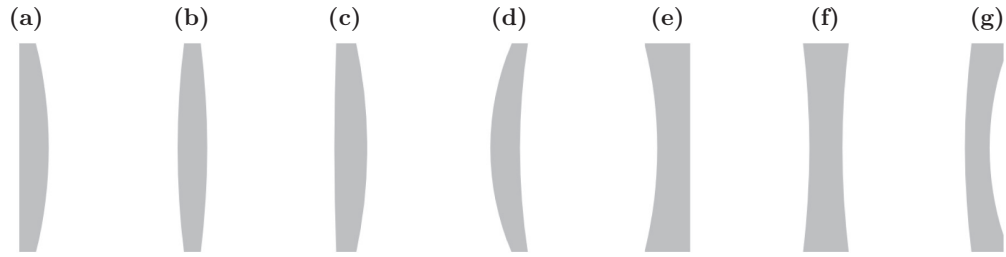

Figure 8.11 Lens shapes: (a) plano-convex; (b) bi-convex; (c) best form; (d) positive meniscus; (e) plano-concave; (f) biconcave; (g) negative meniscus. The lenses shown here have a focal length of (a)–(d) $f = +100\,\mathrm{mm}$ (converging or positive lenses) or (e)–(g) $f = -100\,\mathrm{mm}$ (diverging or negative lenses). The focal lengths are calculated assuming lenses made of N-BK7 glass and with a diameter of $25.4\,\mathrm{mm}$ (1 inch).

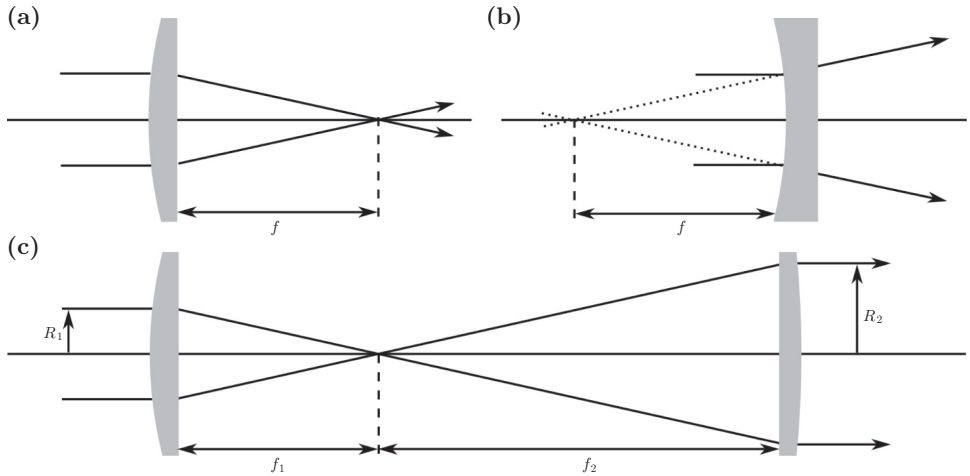

Figure 8.12 The action of lenses illustrated with ray diagrams. (a) A plano-convex (positive) lens focuses an incoming collimated beam. (b) A plano-concave (negative) lens makes an incoming collimated beam divergent. (c) A telescope expands a beam. Note that the curved side of each plano-convex lens is turned towards the side where the beam is collimated (parallel rays) to minimise spherical aberration.

as shown in Fig. 8.12. Table 8.10 describes the most common uses of the various kinds of lens.

- **Surface quality.** The surface quality of a lens is measured by a quantity known as scratch-dig. A scratch is a mark of the polished or coated surface. A dig is a small rough spot or pit on the polished or coated optical surface. Scratch-dig is reported as a pair of numbers, such as '60–40', where the first number indicates the maximum width of a scratch in micrometres and the second number is the maximum diameter for a dig in hundredths of a millimetre. A scratch-dig of 60-40 is regarded as low quality. Lenses for manipulating the laser beam for an optical tweezers should have a scratch-dig of 40-20 or better.
- **Anti-reflection coating.** A small fraction of the intensity incident on the air–glass interface at the surface of a lens will be reflected. This fraction, which can be calculated from

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Table 8.10 Lens shapes

Plano-convex	Best suited for infinite conjugate applications, e.g., focusing a collimated beam or collimating a diverging beam.
Biconvex	Most suitable for finite conjugate imaging where the ratio of the image and object distances is between 0.2 and 5.
Best form	Designed to minimise spherical aberration while still using spherical surfaces to form the lens. They provide the best possible performance from a spherical lens for collimating and focusing beams.
Positive meniscus	Most commonly paired with another positive lens to increase the numerical aperture of the system.
Plano-concave	Used to diverge a collimated beam from a virtual focus. Commonly used in Galilean beam expanders.
Biconcave	Used to increase the divergence of a converging beam.
Negative meniscus	Used in combination with another lens to decrease the numerical aperture of the system.

the Fresnel reflection coefficients [Eqs. (2.5) and (2.7)], for most glass types is about 4% (at near-normal incidence). To reduce this loss, lenses are often coated with an anti-reflection (AR) coating, which can reduce the reflection coefficient at each surface of the lens to less than 0.25%. AR coatings can be either specialised for a particular laser line or broadband to cover a portion of the spectrum (typically up to several hundred nanometres).

8.4.3 Mirrors

Mirrors are used to control the beam direction in the optical train. The surface of a mirror is made highly reflective by either a metallic or a multi-layer dielectric coating. Metallic mirrors are commonly coated with silver (reflection coefficient $R > 97.5\%$ in the visible to near-infrared), aluminium ($R > 90\%$ in the visible and ultraviolet) or gold ($R > 96\%$ in the near-infrared to mid-infrared). Dielectric mirrors are available with high-reflection (HR) coatings with reflectivities in excess of 99% both for particular laser lines and broadband. It should be noted, however, that the reflection coefficient is a function of polarisation and of angle of incidence, and performance may degrade for, e.g., angles of incidence in excess of 45°. As for lenses, the surface quality of mirrors is specified by scratch-dig, with very high quality scratch-dig 10-5 dielectric mirrors being readily available. Metallic mirrors tend to be of lower surface quality.

8.4.4 Filters

Neutral density (ND) filters are used to attenuate the intensity of an optical beam. ND filters may be either absorptive or reflective; i.e., they reduce the intensity either by absorbing or reflecting power. Absorptive ND filters are usually made of an absorbing glass, whereas reflective ND filters are constituted by a glass substrate with a metallic (reflective) coating.

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The degree of attenuation is specified by the optical density (OD) of the filter, defined as

$$\mathrm{OD} = -\log_{10}(T), \tag{8.2}$$

where T is the transmission coefficient. For example, an ND filter with OD = 0.3 transmits approximately 50% of the incident intensity. The optical density will usually be specified at a particular test wavelength, e.g., that of the helium–neon laser (633 nm), and it is advisable to verify the value of the OD at the actual wavelength being used.

Wavelength-selective filters can be used to separate or to selectively stop light of a particular wavelength (or range of wavelengths). An *edge filter* transmits wavelengths above (below) a threshold wavelength and rejects wavelengths below (above) it. A *longpass filter* transmits wavelengths longer than the threshold (known in this case as the *cut-on wavelength*), whereas a *shortpass filter* transmits wavelengths shorter than the threshold (*cut-off wavelength*). A *bandpass filter* transmits wavelengths in a specified range (the *pass-band*) around a centre wavelength, whereas a *band-stop filter* performs the inverse operation and stops wavelengths in a particular range (*stop-band*). A *notch filter* stops transmission within a much narrower range of wavelengths, typically within tens of nanometres around the centre wavelength, with very high transmission (> 90%) outside this range. Notch filters are useful in selectively blocking laser light while allowing other wavelengths to pass, e.g., in order to stop scattered laser light from reaching the imaging camera in an optical tweezers experiment. High-quality filters are made from a dielectric multi-layer stack, which operates as a Fabry-Perot etalon to transmit wavelengths only within a certain range, often with an additional broadband absorber to block out-of-band transmission. Such filters usually have a preferred orientation in the optical beam (indicated on the filter itself) such that the absorber follows the dielectric multilayer to minimise heating effects from absorption. Coloured glass filters are a lower-cost alternative to edge and bandpass filters.

Dichroic mirrors are used to combine or separate beams of different wavelengths as wavelength-selective filters do, but in doing so introduce minimum wavefront distortion into both the transmitted and reflected beams. These are, therefore, best used when the wavefront quality of both beams is important, e.g., in combining the optical tweezers trapping beam with a probe beam of a different wavelength where both beams must subsequently pass though the microscope objective to be focused on a diffraction-limited spot.

8.4.5 Polarisation control

The output beam from most laser systems is a TEM_{00} (Gaussian) beam with linear polarisation. Control of the state of polarisation of the beam is important both for manipulating the beam, e.g., splitting a single beam into multiple beams, and for preparing it for use with polarisation-sensitive devices, e.g., a spatial light modulator. Control of polarisation is performed using *wave plates*. Wave plates are thin slices of a transparent birefringent material such as quartz, which is cut so that the components of the electric field vector in the directions of the two crystal axes (known as the ordinary and extra-ordinary directions) acquire different phase shifts. When housed in a rotatable mount, wave plates constitute a powerful toolbox for continuous control of the state of polarisation of a laser beam.

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A *half-wave plate* (HWP) introduces a phase difference of π between the ordinarily and extra-ordinarily polarised components of the beam. For an input beam that is linearly polarised, the net effect is a rotation of the direction of linear polarisation by twice the angle that the input polarisation direction makes with the HWP extra-ordinary axis.

A *quarter-wave plate* (QWP) introduces a phase difference of $\pi/2$ between the ordinarily and extra-ordinarily polarised components of the beam. For an input beam that is linearly polarised, the output beam is elliptically polarised, with ellipticity and handedness that depend on the orientation of the QWP. In particular, an input polarisation that is linear at an angle of 45° to the QWP axes produces a circularly polarised output. For an input beam that is circularly polarised, the output beam is linearly polarised at an angle of 45° to the QWP axes.

A *polarising beam splitter* (PBS) is capable of separating a beam into two linearly polarised components. *Plate polarising beam splitters* have the beam splitter coating applied to the front face. A *PBS cube* is made from a pair of right-angle prisms cemented together with the coating applied to the hypoteneuse face of one. Both plate and cube PBS reflect light that is *s*-polarised with respect to the beam-splitting face and transmit light that is *p*-polarised.

8.5 Optical trapping and manipulation

At this point, everything is ready for prime time. We can therefore place our diluted sample of microparticles on the sample stage and proceed to trap our first particle. First of all, we need to place the sample on the stage and adjust the position of the objective and sample holder such that we can visualise the plane just above the glass–solution interface. The image recorded by the camera should show the sedimented particles and look similar to the one shown in Fig. 8.13a.[17] In order to get the image in focus, it might be necessary to adjust the lens in front of the camera. At this point, it should be sufficient to move the laser focus a few micrometres above the interface (by moving the sample stage down) and approach some of the particles. If a particle is free to move and not stuck to the glass, you will see the particle jump into the laser beam and become optically trapped, as shown in Fig. 8.13a. To double-check that you have actually optically trapped the particle, you can now try to move it around. You can first move the sample stage further down so that the optically trapped particle gets moved vertically above the glass–solution interface; as shown in Fig. 8.13b, you can see that the non-optically trapped particles on the sample cell bottom go out of focus, while the optically trapped particle remains in focus. Then you can also move the sample stage horizontally; again, as shown in Fig. 8.13c, the image of the optically trapped particle remains at the same spot within the image captured by the camera, while the background particles sedimented on the coverslip appear to be displaced.

[17] In most cases the particles have higher density than the solution. If this is not the case, the particles are to be found near the solution–glass interface at the top of the sample cell.

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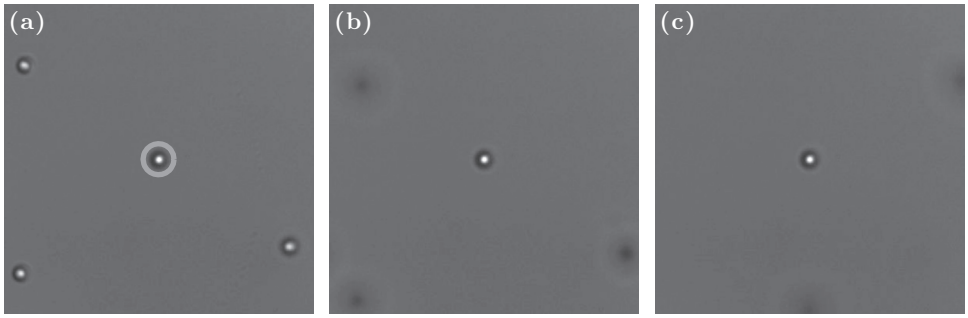

Figure 8.13 Optically trapped particle: (a) Optically trapped particle (circled particle). As the sample stage is moved (b) vertically and then (c) horizontally, the optically trapped particle appears to remain at the same spot within the image captured by the camera, whereas the background particles sedimented on the coverslip appear defocused when the sample is moved vertically in (b) and displaced when it is moved horizontally in (c).

Troubleshooting: I can see the particles in focus, but I cannot trap them. If the problem persists after you have checked that your laser beam is effectively switched on and unobstructed, it is most likely due to the fact that the camera imaging plane and the laser focusing plane are significantly different. This can be due to chromatic aberrations (especially with simpler objectives) or to a misalignment of the camera lens. To solve this problem, check that you can effectively focus your laser beam on the coverslip while observing the sedimented particles at the same time (in order to ensure that the two focal planes are the same). This alignment can be achieved by focusing the laser on the coverslip and then moving the camera lens in order to get the sedimented particles in focus. This might not be possible if the difference between the two planes is too large.

Troubleshooting: My particle is pushed away from the trap. Probably there is too much scattering force. Try to reduce the laser power and/or to increase the overfilling. You can also use particles with lower refractive index contrast or of larger dimensions (to increase the effective gravity). It is also possible that you are trying to trap too far away from the coverslip, where spherical aberrations have degraded the focal spot; in this case, try to trap closer to the coverslip.

8.5.1 Steerable optical tweezers

The optical tweezers we have built so far has only one fixed trap. As we have seen in Figs. 8.13b and 8.13c, it is possible to move an object relative to its surroundings by moving the sample. However, sometimes it is more advantageous to be able to move the trap over the field of view, i.e., to have steerable optical tweezers. To create a movable trap that also is stable, i.e., with the same amount of trapping power, regardless of the movement of the beam, whenever moved the laser beam has to (1) pivot around the entrance aperture

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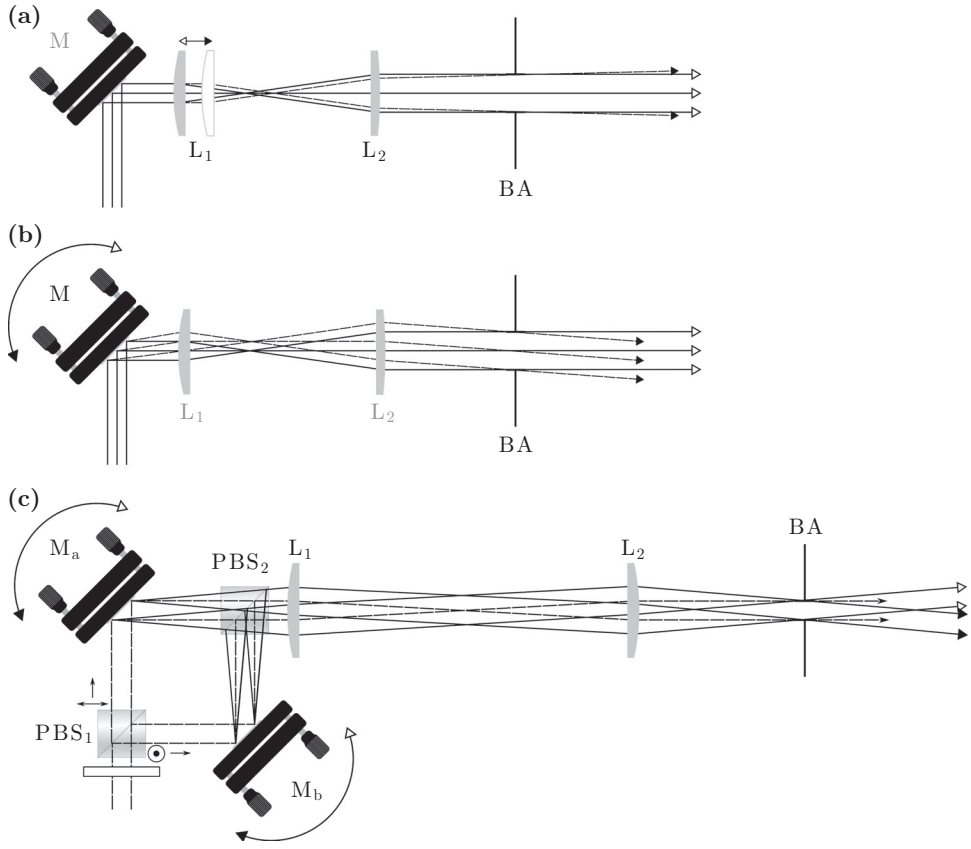

Figure 8.14 Trap steering. The position of an optical trap can be changed within the sample (a) along the vertical z-direction by displacing one of the lenses in a telescopic arrangement and (b) in the horizontal xy-plane by conjugating the plane of a steerable mirror to the objective back aperture (BA). (c) It is also possible to construct a dual optical tweezers where a beam is split into two and recombined using two polarising beam splitters (PBS_1 and PBS_2) in order to create two independently steerable optical traps; the choice of polarising beam splitters is to avoid losing optical power.

of the microscope objective and (2) retain the same degree of overfilling of the microscope entrance aperture.

Beam steering along the vertical z-direction can easily be achieved by moving one of the lenses in the telescope in order to change the curvature of the trapping beam, as shown in Fig. 8.14a. Beam steering in the horizontal xy-plane can be achieved by tilting the beam at a plane conjugated to the back aperture of the objective, as shown in Fig. 8.14b. In this way, the beam entering the objective is slightly tilted, resulting in a focus in a slightly offset position. Instead of a gimbal-mounted mirror, it is possible to use a galvometre-mounted mirror or an acousto-optic deflector to move the trap in a very controllable way. In this way it is also possible to generate multiple optical traps by time-sharing a single optical beam.

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An interesting approach to a dual-beam steerable optical tweezers was presented by Fällman and Axner (1997). The schematic is shown in Fig. 8.14c. This set-up makes use of a couple of polarising beam splitters to split a beam into two orthogonally polarised beams and then recombine them before the objective entrance. Beam steering is achieved by using two gimbal mirrors.

More advanced techniques to control the position and characteristics of optical tweezers can be implemented using wavefront engineering and spatial light modulators, as we will explain in detail in Chapter 11.

8.6 Alternative set-ups

The optical tweezers set-up we presented in this chapter is but one of infinitely many possible implementations. Several alternative approaches have been presented in the literature, based both on homemade and commercial microscopes. In particular, there are the set-ups proposed by Smith et al. (1999), Bechhoefer and Wilson (2002), Mellish and Wilson (2002), Appleyard et al. (2007), Lee et al. (2007) and Mathew et al. (2009).

Problems

8.1 Build an optical tweezers with a near-infrared laser source. Trap several different particles such as latex beads of different sizes, yeast cells, and gold nanoparticles. Find the minimum trapping power needed to have a stable trap. What does this tells you about the trapping efficiency?

8.2 How can you build an optical tweezers set-up with multiple traps? How many particles can you trap? Does the number of particles you can trap depend on their size? Why?

8.3 Design a steerable optical tweezers using a gimbal mirror or a pair of galvo-mirrors. How can you use such a set-up to move different particles in controlled patterns such as a line, a circle or a Lissajous figure?

8.4 Design an optical tweezers set-up capable of using non-Gaussian laser beams, such as Laguerre–Gaussian beams and cylindrical vector beams. What kinds of experiments can be performed with such a set-up that cannot be performed with a standard optical tweezers?

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