PREFACE

Over the past 10 years there has been an explosive growth in both the number of laser tweezers and the number of articles published with data using them. From sophisticated force measurements to simple placement of beads, there are a wide variety of applications for a device that enables the application of known forces to micrometer- and submicrometer-size particles even within cells. Dr. Arthur Ashkin's system for trapping bacteria was visionary and has opened the way for rapid measurements of force in a range difficult to measure otherwise. The purpose of this book is to provide the practical knowledge needed to set up a laser trap, to use it in a variety of applications, and to understand the trap limitations and potential pitfalls.

The practical range of forces that can be measured by the tweezers is from 0.2 to 200 pN for a particle approximately 1 μm in diameter. For larger forces, such as those needed to unfold most proteins, the atomic force microscope (AFM) is the current instrument of choice. Factors affecting the maximum force of the tweezers include the size of the particle (force increases approximately as the square of the particle diameter for 0.2- to 1.5-μm particles), the refractive index difference between the particle and the medium, the laser power (direct dependence), and the gradient of laser intensity in the microscope (any aberrations in beam profile will alter maximum force). In terms of understanding the theory behind the tweezers, the simplest access to the literature is through the Ashkin article (Chapter 1). For many motor and membrane experiments, the tweezers provide sufficient force and are more flexible than other system.

In terms of the practical issue of setting up laser tweezers, this book provides many useful hints, but nothing can truly replace hands-on experience. To get such experience, it is best to visit a laboratory currently working on applications similar to the one planned and see the chosen system in operation. Often a set of collaborative experiments will provide hands-on training as well as useful preliminary data. Like any other major tool, laser tweezers have limitations in each application. Before monetary and personnel resources are committed to acquiring laser tweezers, practical experience on a working system is often invaluable.

Although technology is developing at a rapid pace, the systems described in this book will be useful tools for solving many biological problems for years to come. Most cell functions have a mechanical component in either position dependence or force dependence that is difficult to probe without a tool like the laser tweezers. The one new area that will be important to watch is the overlap between laser tweezers and 2-photon confocal fluorescence. Trapped fluoresecent

beads will fluoresce, and the exciting light for 2-photon fluorescence will exert considerable force on cellular structures. The area that may improve dramatically is lens design. Because many recent light microscope applications involve infrared wavelengths (800–1100 nm), new lenses may be designed to correct for chromatic aberrations at the end of the spectrum.

A variety of applications are considered here, but the list is by no means exhaustive. The trade-offs between the applications are considerable. If endogenous cellular structures or materials within cells are trapped and moved, the calibration of the force is difficult because neighboring structures and changes in the refractive index of cytoplasm can alter the applied force. Furthermore, the strength of the trap is insufficient to break cytoplasmic filaments (even one actin filament requires a force of 400 pN to break it). With extracellular beads or *in vitro* assays, the force per nanometer of bead displacement in the trap can be readily determined, but many cellular functions are difficult to approach outside the cell. Early cell biological applications of laser tweezers have been in the study of the plasma membrane because the forces on the particles can give information about critical cell functions directly. The other major current application of laser tweezers is in the area of biophysical, molecular mechanics, which includes motor function and polymer unfolding. As intracellular functions are recapitulated in *in vitro* assays, the forces involved can be readily measured using laser tweezers. Furthermore, laser tweezers can be used to spatially organize components *in vitro* to enable the reconstitution of cellular functions that require the correct juxtaposition of components.

In considering the current limitations of the tweezers from heating and photodamage to the time required for force analyses, we see possibilities for the greatest changes in the next 10 years in the area of analyses. Digital image analysis and quadrant detector improvements will essentially allow real-time analysis of forces even in complex samples. There are no obvious ways to overcome heating and photodamage limitations in cellular systems, but optimization of laser tweezers could conceivably give four- to eightfold increases in the maximum force per particle. Optimizing the wavelength of laser tweezers to minimize damage to the cells as well as optimizing the beam profile (such as the use of TEM-01 lasers) could enable greater forces to be applied for the same level of heating and photo damage. Thus, we can expect to be able to break single-actin filaments (400 pN) with laser tweezers, but it is unlikely that fibroblast focal contacts (>2 nN) will be broken. Still, a wide variety of cellular phenomena involve forces in the range of 2 to 400 pN, and rapid force analyses can allow us to define the molecular and physical bases of these phenomena.

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