Optical detection system for probing cantilever deflections parallel to a sample surface

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To date, commercial atomic force microscopes have been optimized for measurements of forces perpendicular to the sample surface. In many applications, sensitive parallel force measurements are desirable. These can be obtained by positioning the cantilever with its long axis perpendicular to the sample: the so-called pendulum geometry. We present a compact optical beam deflection system which solves the geometrical constraint problems involved in focusing a light beam onto a cantilever in the pendulum geometry. We demonstrate the performance of the system on measurements of forces imparted by a muscle myofibril, which is in-plane to a high-magnification objective of an optical microscope. © *2011 American Institute of Physics*. [doi:10.1063/1.3527913]

I. INTRODUCTION

The most evident advantage that atomic force microscopy (AFM)[1] boasts over all other microscopic and nanoscopic imaging methods is its unique ability to probe forces. A cantilever acts as the force transducer; its interaction with a sample causes a deflection which can be measured and calibrated into a force. The optical beam deflection (OBD) method[2] is typically used to measure the deflection of the cantilever, although interferometry[3] can also be used.

The versatility of AFM is highlighted by the fact that the same instrument can be used for probing the piconewton forces of a single covalent bond,[4] as well as studying the forces exerted by cells in the micronewton range. However, all commercial AFMs are optimized for measuring forces which are perpendicular to the sample surface, which becomes a limitation when the forces of interest are parallel to the sample surface. This has led many researchers to design home-built systems to overcome this problem by the use of the pendulum geometry:[5,6] the cantilever is positioned with its long-axis perpendicular to the sample surface, such that forces in-plane can be measured with high sensitivity.

The pendulum geometry prevents the snap-to-contact problem that afflicts soft cantilevers in a regular AFM. Very soft cantilevers enable attonewton force sensitivity which is necessary, for example, in the detection of single spins in magnetic resonance force microscopy (MRFM).[5,7] At the microscopic length scale, the studies of cellular or subcellular forces which are parallel to the imaging plane of an optical microscope have also precipitated the development of home-built OBD systems[8] due to the demand for high sensitivity force measurements at sampling frequencies beyond available video rates.

The difficulty in implementing the pendulum geometry lies in the constraint imposed by the focused incoming light or the diverging outgoing light which easily interferes with the sample surface. Here, we describe an OBD system which overcomes this problem. The geometrical restriction is solved by the key component of our system—the optical periscope (Fig. 1).

II. THE OPTICAL PERISCOPE

The optical periscope is a custom machined optical component which allows the guiding of the laser light toward and away from the cantilever in constraining configurations. The laser light must be redirected away from the sample surface immediately after reflection from the cantilever because the beam spreads due to diffraction. Figure 1 illustrates how the optical periscope achieves this task. The benefit of this particular design is that the exposed optical surface—the periscope aperture—is composed of hard glass, which can easily be cleaned and kept scratch-free. On the other hand, the delicate reflective surfaces are internal to the periscope and therefore impervious to damage. Most importantly, this design does not require any acute angles which are prone to chipping during handling. This ensures that the reflective surface is free of defects to the very edge of the periscope, allowing to the laser be brought very close to the sample surface where it is needed. One noteworthy advantage of this design for use in liquid environments is that the light path inside the liquid is minimized to below 1 mm. This feature reduces the total interference caused by contaminants and air bubbles present inside the liquid, which can degrade the signal quality.

The specific context for the development of our OBD system was the probing of mechanical properties of skeletal muscle myofibrils, composed of individual sarcomeres arranged in-series—the smallest intact muscle structure that still retains the tri-dimensional lattice intact. The novel configuration of our system allows for high-speed and high-resolution measurements of forces parallel to the microscope's imaging plane, while simultaneously imaging the myofibrils tethered to the cantilever apex roughly 10 μm above the microscope

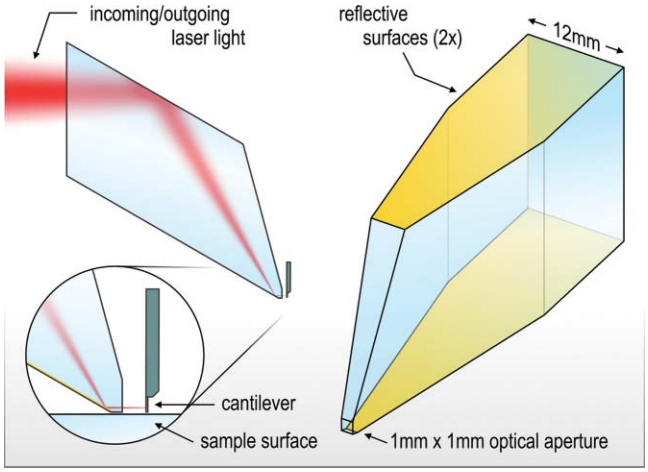

FIG. 1. (Color online) Side-view with zoom-in and isometric view of the optical periscope which allows a light beam to be focused onto a cantilever that is perpendicular to a surface. The two reflective surfaces are parallel such that the light paths entering and leaving the periscope remain parallel even if the periscope is rotated to adjust its height above the sample. Notice that four of the periscope faces must be optically polished, and two of which must be metal-coated. The rest can simply be ground rough.

cover slip. The proximity to the cover slip is necessary for enabling simultaneous high-magnification (150×) optical imaging of the myofibrils. The problems associated with this proximity to a surface were solved by the optical periscope.

Figure 2 illustrates the integration of the optical periscope into our compact OBD unit; herein referred to as "the Penguin" for its uncanny resemblance to a penguin diving into the water, as seen in Fig. 2(e). The Penguin mounts onto the rear column of an inverted optical microscope. Its tapered shape and location prevents it from obstructing all the hardware necessary for the manipulation of the cantilever, the myofibril, and all the microfluidics necessary to controlling the biological environment.[8–10]

The remainder of the optical system, based on a previous design,[11] will now be explained in detail with reference to Fig. 2. The *laser light* necessary for the detection of cantilever deflection enters the system via an optical fiber connector. It is immediately collimated to a beam diameter of 3.1 mm and is reflected by *two rotating mirrors* toward the cantilever. These two mirrors can be manually rotated by *micrometers* for positioning the focused laser light onto the cantilever before the experiment; the positioning sensitivity is 1 μm in both directions. The *focusing lens* position is adjusted such that its focal plane coincides with the microscope's *optical axis*, where the cantilever is always roughly positioned.

The focused laser light enters the *periscope*, where two reflective surfaces direct the light toward the cantilever. For convenience, both surfaces were made parallel such that the entering and exiting light beams of the periscope remain parallel even if the periscope is rotated, which is necessary for adjusting its height above the *glass cover slip*. A *height adjustment knob* allows to fine tune the periscope height above the cover slip; ideally it is within a few tens of microns away without touching the cover slip. After reflection from the can-

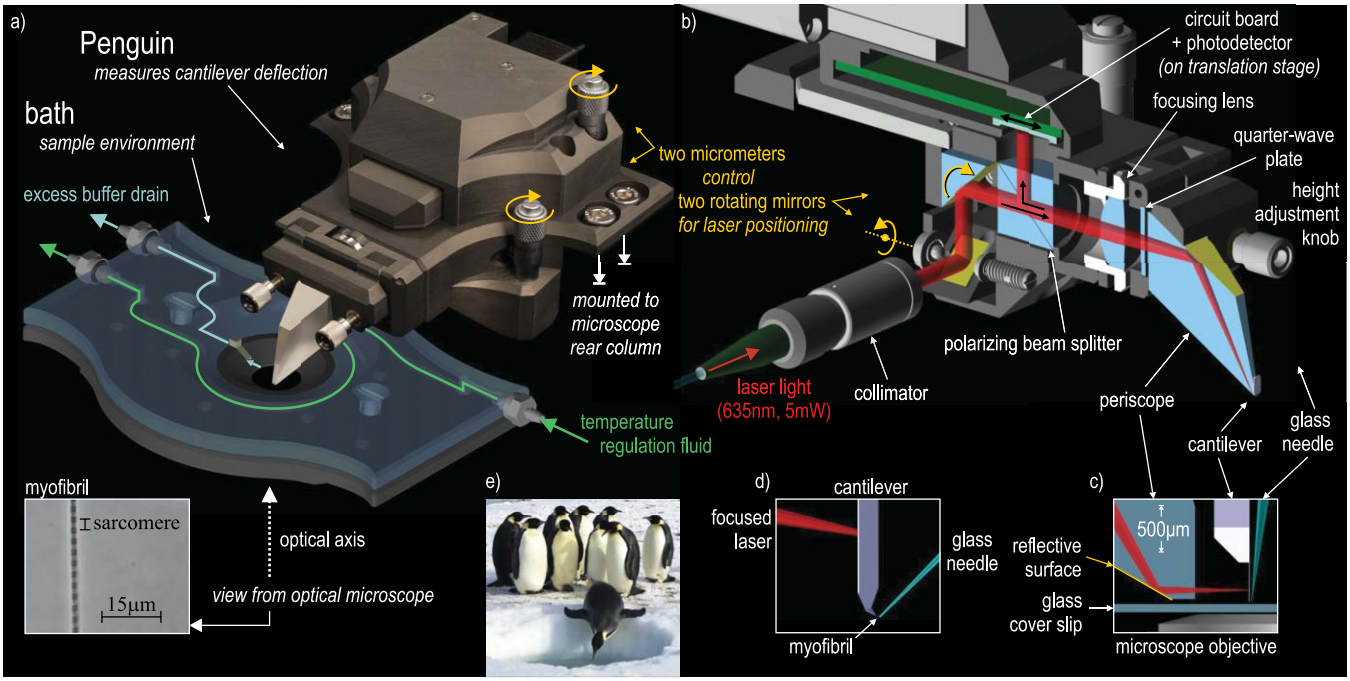

FIG. 2. (Color online) (a) Photograph of the Penguin with a digitally rendered bath, which is used for regulating the temperature of the sample environment. The view through the optical microscope shows the myofibril under investigation. (b) Side-cut of the Penguin showing the path travelled by the laser light. The position of the focus on the cantilever is controlled by two micrometer screws, which rotate two gold mirrors, as shown. The optical periscope from Fig. 1 guides the light to and from the cantilever which is roughly 10 μm above the glass cover slip. The returning light is redirected toward the photodetector by the polarizing beam splitter, such that angular changes of the cantilever can be measured. The measured cantilever deflections are proportional to the force exerted by the myofibril in its changing environment. (c) Zoom-in near the optical aperture of the periscope, showing how the laser light is deflected away from the cover slip before diverging. (d) Zoom-in of the cantilever. The myofibril is tethered in between the cantilever apex and a rigid glass needle. (e) Photograph explaining our choice for naming this system "the Penguin" (photo by Emily Stone courtesy of the National Science Foundation).

tilever, the light retraces its incoming path, and is redirected toward the *photodetector* by the *polarizing beam splitter*. This preferential reflection toward the photodetector is enabled by the *quarter-wave plate*, described in detail elsewhere.[12] Briefly, it allows the incoming light beam to directly traverse the polarizing beam splitter, while the light returning from the cantilever is redirected onto the photodetector. This well-known polarization trick used in all CD/DVD readers allows for a much more compact optical system because the same optical components are used for the incoming and reflected light beams.

A change in the cantilever angle, due to a force imparted by the myofibril, translates into a difference in optical power ΔP impinging on the top and bottom section of the photodetector. This difference in optical power ΔP is computed by analog electronics (LT1214) and immediately amplified by an instrumentation amplifier with digitally programmable gains (AD8251), on the backside of the photodetector *circuit board*. Before leaving the Penguin, the deflection signal is antialiased at ~1/3 the sampling frequency by a digitally programmable eighth order low-pass filter (LTC1564). Finally, the deflection signal is read out by an analog-to-digital converter, controlled by home-built Labview™ acquisition software. The gains and the antialiasing cut-off frequencies are remotely selected by the same acquisition software. This software also controls a motorized *translation stage* (miCos GmbH, VT-21) which centers the photodetector onto the laser spot before the experiment such that zero volts correspond to zero force. These features are necessary to prevent touching the Penguin and optical microscope once a myofibril is successfully strung between the cantilever and the glass needle.

III. EXPERIMENT

The experiment designed to test the new system was performed with a myofibril isolated from the rabbit psoas muscle, following an established protocol.[9] Briefly, a myofibril was suspended between an atomic force cantilever (ATEC-CONTPt, Nanosensors™, nominal stiffness 0.2 N/m) and a rigid glass needle, both connected to three-dimensional micromanipulators. The temperature of the solution surrounding the myofibrils was controlled and maintained constant at 15 °C.

The myofibril was initially immersed in a bath containing a resting solution with low Ca^{2+} concentration ($pCa^{2+} = 9.0$) and the sarcomere length and myofibril diameter were measured under 90× magnification (Nikon Plan Fluor 60×, N.A. 0.70), using a charge-coupled device (CCD) camera (Go-3, QImaging, USA; pixel size: 3.2 μm × 3.2 μm), with an additional internal microscope magnification of 1.5×.Activation of the myofibril was achieved by quickly exchanging (<10 ms) the solution surrounding the myofibril using a double barreled pipette, attached to a multichannel perfusion system (VC-6M, Harvard Apparatus, USA) as previously performed in our laboratory.[9]

When the myofibril is surrounded by an activating solution with high Ca^{2+} concentration ($pCa^{2+} = 4.5$), it contracts and exerts a force on the cantilever, measured by the Penguin.

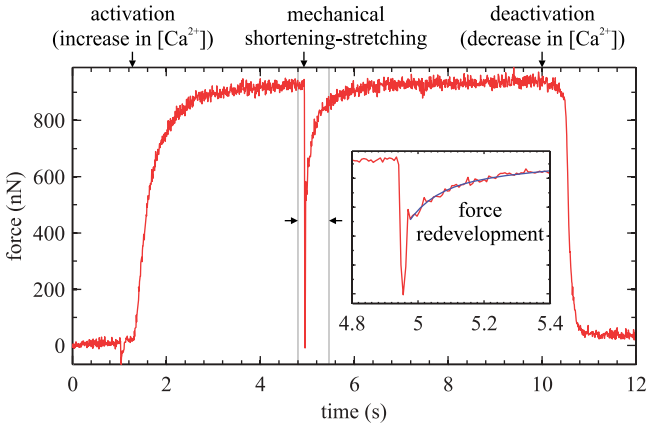

FIG. 3. (Color online) An example force record from a myofibril activated in a solution with pCa^{2+}=4.5, at an initial sarcomere length of 2.4 μm. Upon activation, the sample was allowed to achieve a stable force, and then a quick shortening-stretch maneuver was implemented just before 5 s. Once a stable force was achieved after force redevelopment, the sample was subjected to a relaxing solution at around 10 s. Inset: Zoom-in of the shortening-stretch phase with the fitted force redevelopment exponential.

Furthermore, since the glass needle is connected to a computer controlled motor arm, changes in myofibril length can be made rapidly allowing for an accurate biomechanical characterization.

Figure 3 shows a contraction produced at a sarcomere length of 2.4 μm. Upon activation, the force increased rapidly to achieve a steady-state level. The force and the rate of force development (K_{act}) upon activation produced by this myofibril before shortening–stretching is comparable to the levels observed previously in other laboratories.[8,10] When the myofibril is rapidly shortened and restretched to the initial length, there is rapid force redevelopment. The rate of force redevelopment (K_{tr}) is commonly used for probing the mechanisms of interaction between myosin and actin—the two major contractile proteins present in striated muscles.

The relaxation phase upon myofibril deactivation can be fitted with a linear function (K_{lin}) and an exponential function (K_{rel})—associated with the detachment of myosin–actin interactions and consequently sarcomere relaxation.[13] Given the characteristics of force production for activation and relaxation of these myofibrils, it is apparent that our newly developed OBD system adequately adapts the pendulum geometry such that forces can be measured parallel to the plane of the sample.

IV. SUMMARY

Of fundamental importance is the general applicability of this OBD system. Although we tested it with muscle myofibrils, this system is exportable within an extensive field of AFM-like applications. It carries prominent potential in many force sensing applications in the fields of biology, surface science, experimental molecular mechanics, and especially for MRFM. As a result, it has far-reaching implications.

[1]G. Binnig, C. F. Quate, and C. Gerber, Phys. Rev. Lett. **56**, 930 (1986).
[2]G. Meyer and N. M. Amer, Appl. Phys. Lett. **53**, 1045 (1988).
[3]Y. Martin, C. C. Williams, and H. K. Wickramasinghe, J. Appl. Phys. **61**, 4723 (1987).

[4]M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann, and H. E. Gaub, Science **283**, 1727 (1999).
[5]D. Rugar, R. Budakian, H. J. Mamin, and B. W. Chui, Nature (London) **430**, 329 (2004).
[6]S. Rast, U. Gysin, P. Ruff, C. Gerber, E. Meyer, and D. W. Lee, Nanotechnology **17**, S189 (2006).
[7]C. L. Degen, M. Poggio, H. J. Mamin, C. T. Rettner, and D. Rugar, Proc. Natl. Acad. Sci. **106**, 1313 (2009).
[8]I. A. Telley, J. Denoth, E. Stüssi, G. Pfitzer, and R. Stehle, Biophys. J. **90**, 514 (2006).
[9]D. E. Rassier, Proc. R. Soc. B: Biol. Sci. **275**, 2577 (2008).
[10]N. Piroddi, C. Tesi, M. A. Pellegrino, L. S. Tobacman, E. Homsher, and C. Poggesi, J. Physiol. **552**, 917 (2003).
[11]A. Labuda, W. Paul, B. Pietrobon, R. B. Lennox, P. H. Grutter, and R. Bennewitz, Rev. Sci. Instrum. **81**, 083701 (2010).
[12]T. E. Schaffer, J. P. Cleveland, F. Ohnesorge, D. A. Walters, and P. K. Hansma, J. Appl. Phys. **80**, 3622 (1996).
[13]R. Stehle, J. Solzin, B. Iorga, and C. Poggesi, Pflügers Arch. Eur. J. Physiol. **458**, 337 (2009).