

# Molecular Force Probe — MFP-1D™ For Precision Force Curve Measurements

## Introduction

Nanotechnology has become one of the fastest growing areas of research in the scientific community. It combines fields as diverse as molecular biology and silicon-based micro-machining. Since molecules are the building blocks of nanotechnology, understanding the forces that occur between and within molecules is fundamentally important. Instrumentation for nanoscale force measurements has been difficult to use and, at times, imprecise. Now there is an instrument that can make these measurements with high sensitivity and accuracy, with a flexible, easy-to-use interface.

## Superior Optics and Design

The MFP-1D, (Figure 1), is a single axis force curve tracer designed specifically for soft samples. The technology is based around a

the tip and sample, the MFP-1D acts as a nanoscale elasticity measurement device. The MFP-1D gives a bottom-up view of the tip and sample

through a transparent sample support such as a microscope slide or a petri dish. With diffraction-limited detection optics, the MFP-1D is compatible with current and next generation smaller microfabricated cantilevers (pg.4). The near infrared superluminescent diode avoids interference with optical measurements such as fluorescence.

Z-axis quantifies the distance between the cantilever and sample (Figure 2) while reducing or eliminating the effects of drift over long time scales. This allows longer duration experiments than is possible with competing AFMs. The novel optical lever geometry, and a low coherence light source optimizes sensitivity and minimizes interfering reflections from the sample (Figure 3). Integrated cantilever spring constant measurements use state-of-the-art thermal methods for calibrating the stiffness of the cantilever (Figure 4a) and set the industry standard.

## Powerful Software

The MFP-1D software interface is flexible and easy-to-use. Based in IGOR Pro (Wavemetrics), the user interface allows unprecedented flexibility and control over data acquisition and analysis. The interface may also be “tuned” to the user, from something as simple as single push-button operation to detailed and precise control of every aspect of the data acquisition process (Figure 4b).

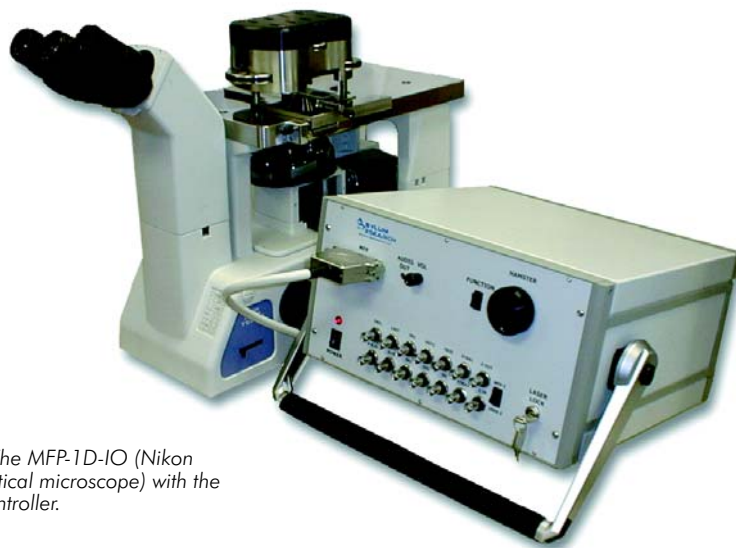


Figure 1: The MFP-1D-IO (Nikon inverted optical microscope) with the MFP-1D controller.

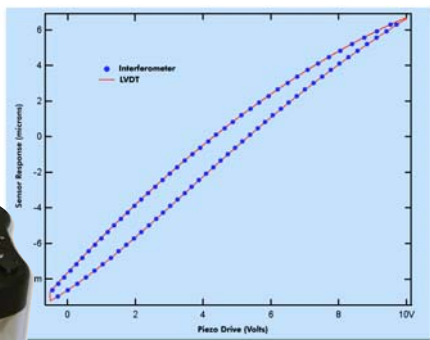


Figure 2: The NPS™ sensor (red) measures piezo creep and hysteresis. As part of our extensive quality control, each NPS is measured against an optical interferometer (blue dots.)

## Increased Sensitivity for More Precise Measurements

The MFP-1D is engineered for maximum sensitivity and increased flexibility over other instrumentation such as many Atomic Force Microscopes (AFMs). The MFP-1D is five times more sensitive than the best competing AFM, due largely to more stable DC performance. The sensed

micro-machined flexible cantilever with a sharp tip that deflects in response to the small forces between the cantilever tip and a sample. When a single molecule is tethered between



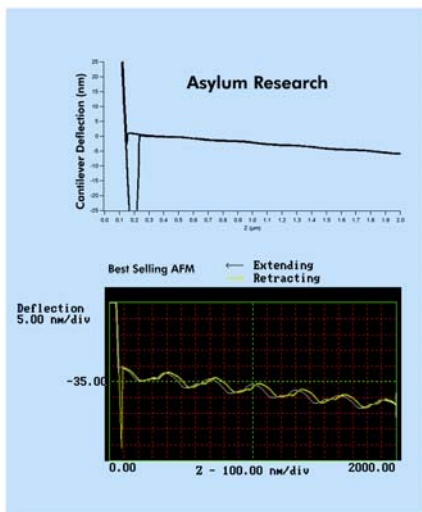


Figure 3: Optical interference is reduced 10X compared to competing AFMs.

## Two Configurations Available

The MFP-1D-IO (Inverted Optical) operates on the top of an inverted optical microscope such as the Olympus IX-70/71 Series (Figure 5), Nikon TE200, TE2000, TE300, or the Zeiss Axiovert 200, allowing very high resolution optical imaging and epi-fluorescence measurements to be made simultaneously with force measurements. The MFP-1D-IO replaces the stage of the commercial microscope and includes two sub-micron mechanical stages. One is used to align the cantilever with the optical axis and the other positions the sample beneath the cantilever tip.

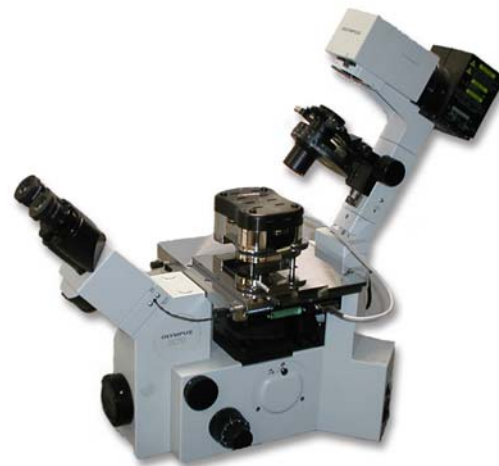


Figure 5: MFP-1D-IO is ideal for doing simultaneous fluorescence and force measurements.

The MFP-1D-SA (Stand Alone) is a stand-alone instrument utilizing the same head as the MFP-1D-IO (inset Figure 2), but operating on its own stand-alone base. This base includes a two-dimensional submicron mechanical stage for positioning the sample as well as an inverted optical microscope with two magnifications for viewing the sample and tip. Although the MFP-1D-SA has reduced optical capabilities, it offers a much smaller footprint and higher immunity to external vibration. Both models are compatible with different sample supports including glass slides, coverslips, or petri dishes from 35mm to 85mm in diameter.

## DNA Stretching

The familiar double-stranded helix structure of DNA was first proposed by Watson and Crick in 1953.<sup>1</sup> The Watson-Crick conformation (labeled "B-DNA") with its characteristic 10.5 base-pairs per helix turn is only one of numerous possible conformations. Many factors can contribute to the conformation of DNA including chemical denaturants, pH, temperature and force. In 1996, Smith et al. stretched a strand of B-DNA that was attached to a glass slide and a magnetic bead. They discovered that applying a force of 65pN resulted in a characteristic plateau in the force vs. distance curve consistent with a structural reordering.<sup>2</sup> Figure 7

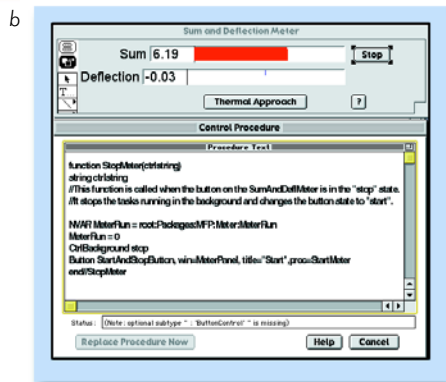
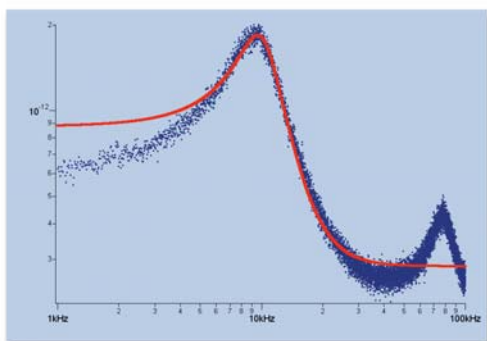


Figure 4: a) Thermal calibration of spring constants are easy and precise; b) Custom code can be easily written while in the MFP software.

## Applications

Designed specifically for researchers, the MFP-1D is ideal for studies in bioscience, polymer science, biomaterials, material science, food science, surfactants and more. Specific measurements include single molecule experiments for protein folding/unfolding and stretching/melting of DNA; chemical sensing, ligand-receptor bindings, colloidal forces, adhesion studies, energy dissipation and many more.

## Protein Folding

Titin is a giant protein found in muscle tissue. It provides one of the first spectacular examples of the utility of cantilever-based "force pulling" for studying the mechanical properties of domains in proteins. As illustrated in Figure 6, the MFP-1D is used to study domains in synthetic molecules constructed from tandem repeats of a single Titin domain.

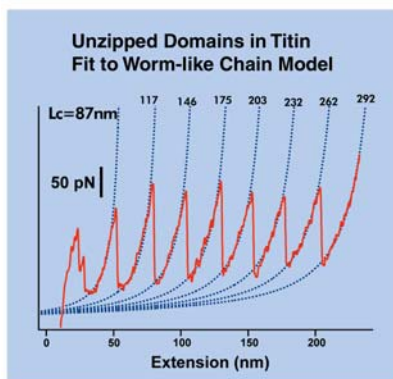


Figure 6: Force curves taken with the MFP-1D with a protein molecule composed of tandem repeats of a Titin module tethered to the cantilever. The red curve shows the experimental data and the purple shows the fit to the Worm-like chain model. The numbers next to the fits indicate the contour length obtained from the fit. Data courtesy of Dr. J. Clarke, S. Fowler and A. Steward of Cambridge University, UK.

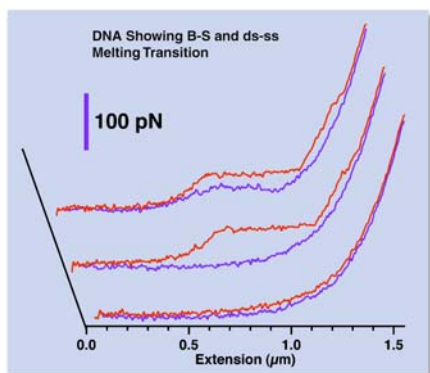


Figure 7: MFP-1D force measurement of a single tethered molecule of Lambda Digest DNA showing the B-S and the melting transition. During the extension of the molecule (red trace), the DNA first goes through the B-S transition (the plateau), and then melts to single-stranded DNA (ssDNA) at a higher force. During relaxation (purple trace), the DNA doesn't re-anneal so the curve is a simple freely-jointed-chain curve indicative of ssDNA. Traces were made at a pulling speed of 1  $\mu\text{m}/\text{second}$ . Sample courtesy of H. Claussen-Schaumann and R. Krautbauer, Gaub Lab, Ludwig Maximilians University.

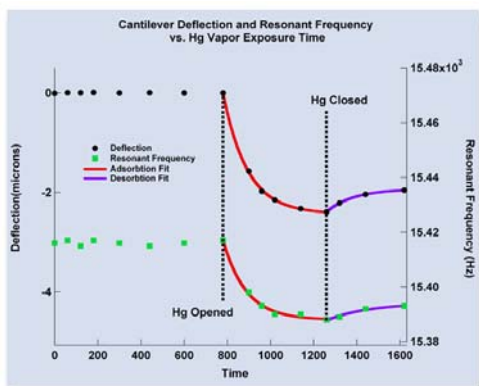


Figure 8: Mercury sniffing using the MFP.

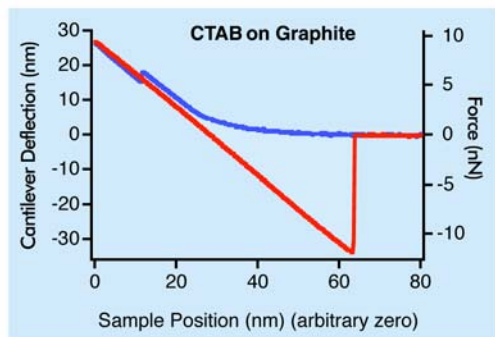


Figure 9: CTAB on graphite.

shows the force plateau measured with the MFP-1D. Because the MFP-1D can apply larger forces than optical or magnetic tweezers, the transition from double stranded (ds) to single stranded (ss) DNA is observed.

### Chemical Sensing

Cantilevers can be used as chemical or biological sensors. All the techniques rely on the binding causing a change in a mechanical property of the cantilever, whether it is stress leading to a deflection, a mass loading leading to a change in the resonant behavior, or heat released changing the cantilever temperature.

A number of years ago, Thundat et al.<sup>1</sup> showed that microfabricated cantilevers could be used as high-sensitivity mercury vapor detectors. Figure 8 shows the deflection (upper curves) and resonant frequency (lower curves) of a gold coated cantilever in the MFP-1D as a function of time. To simulate a mercury spill, a flask containing 5 grams of mercury was opened at time  $t=780$  seconds, approximately 5cm away from the cantilever. Immediately upon opening, the cantilever began to deflect and the resonant frequency dropped as mercury was absorbed onto the gold coating. Both the deflection and resonant frequencies were exponentially time dependent, (with a time constant of roughly two minutes) as shown by the red fitted curves, suggesting a diffusion limited adsorption rate. When the flask was closed again at  $t=1260$  seconds, the deflection and resonant frequency relaxed back in the positive direction but towards a new value, suggesting that some mercury was remaining trapped on the cantilever surface. The fact that the cantilever did not relax back to its original deflection or resonant frequency values suggests that it could be used as an integrating sensor that could be carried in a badge or other mobile detector.

### Colloidal Science

Colloidal science is incredibly diverse, covering areas such as detergency, food science, bioremediation, industrial ore separation, foams, and ceramic processing.

For detergency applications, surfactant molecules surround the hydrophobic dirt particles with a hydrophilic skin in water allowing them to dissolve. A model system for studying these effects is observing the forces caused by a surfactant molecule adsorbed on a very hydrophobic surface such as graphite.

CTAB (cetyltrimethylammonium bromide) is a cationic surfactant commonly used in detergents. The force curve shown in Figure 9 is between a silicon nitride tip and a cleaved crystal of graphite. The CTAB is thought to form a bilayer on the hydrophilic silicon nitride tip and forms hemimicelles on the graphite. These are structures with the CTAB's hydrophobic tails towards the graphite and its hydrophilic head facing the water. Because the CTAB is charged in solution, electrical double layer forces develop between the tip and surface. This causes the long ranged repulsive force visible in the figure. At closer distances, more repulsion develops as the two adsorbed CTAB layers come in contact with each other. Finally, attractive forces pull the tip into hard contact with the graphite.

### The Future is Smaller Cantilevers

Cantilevers provide the basis of operation for the MFP-1D and AFMs. Although cantilevers have already come a long way since the early days of hand-cut aluminum strips, decreasing the cantilever size will produce higher resonant frequencies and will allow faster measurements with higher sensitivity. Asylum Research is on the forefront of developing smaller cantilevers to enhance single molecule, small force measurements (Figure 10).

As a practical application of lower noise measurements to single molecule force measurements, Figure 11 shows a measurement of the B-S transition in  $\lambda$ -digest DNA in PBS solution.<sup>3</sup>

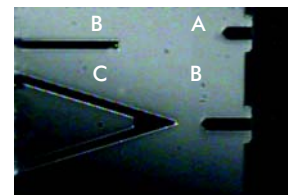


Figure 10: See Table 1 for thermal force noise measured. Levers (top to bottom, left to right), Thermo B, Thermo C, Olympus A, Olympus B.

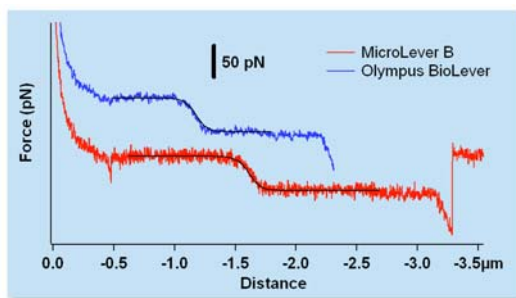


Figure 11: Noise is greatly reduced with the Olympus Bio-Lever, a small, low noise cantilever. For additional information, see our application note "Low Noise Cantilevers for Force Measurements."

The red curve "B" was made with the Microlever, and the blue curve, "A", with the Bio-Lever. Both measurements were close to being thermal noise limited. However, because the thermal noise level was higher for Microlever B, the signal to noise ratio is reduced for that lever. The background noise for the Microlever "B" was about 5pN and about 3pN for the Bio-Lever. The transition was fitted using a hill function for both transitions and found to be  $57 \pm 3$  pN for the blue curve and  $55 \pm 4$  pN for the red. The force curves were made over the same sample. It is apparent from the horizontal axis that the DNA strand length was different for the two measurements. Note that the nominal transition force of 65pN typically reported in the literature is greater than that observed here. Lower forces have been observed in the presence of higher salt concentrations. No effort was made to control the salt concentration in these measurements.

**Table 1**

Cantilever	Length	Thermal Noise*
Thermo B	200μm	5.2 pN
Thermo C	320μm	6.1 pN
Olympus A	60μm	3.6 pN
Olympus B	200μm	4.2 pN

\*Measured in fluid in 1Hz- 1kHz bandwidth.

## Specifications

<b>Optical lever noise</b>	Less than 0.3Å RMS, 100mHz to 1kHz measurement bandwidth.
<b>Z-axis instrumentation</b>	Less than 3Å RMS, 100mHz to 1kHz measurement bandwidth.
<b>Light Source</b>	Superluminescent diode is classified as a Class 1M. Viewing with an optical instrument within a distance of 100mm may pose an eye hazard.
<b>Sample Supports</b>	Petri dishes (35 mm to 85 mm), microscope slides, coverslips and others. Gold slides are available through Asylum Research.
<b>Computer</b>	Minimum Dell Dimension 4550, 2.0GHz, 1GB RAM, 40GB Hard Drive, DVD, Windows® XP Professional. Igor Pro Software (Wavemetrics). Custom configurations available.
<b>Physical</b>	MFP-1D-SA: height 152 mm (6.0"), width 140 mm (5.5"), length 200 mm (7.9"), mass 7 kg (14.4 lbs).  MFP-1D-IO: (Baseplate and head), height 130 mm (5.1"), width 230 mm (9.0"), length 300 mm (11.8"), mass 13 kg (28 lbs).

## Asylum Research— Dedicated to Nanotechnology

Asylum Research was founded in April 1999 to better serve the scientific research community with tools for nanoscale science and technology. Our instruments are designed by scientists for science, and we're dedicated to providing the highest quality instrumentation for exploring the nanoworld. We guarantee that you will be satisfied with our products. If for any reason you are not satisfied within the first six months of ownership, we will refund your money. We encourage you to send us your samples or schedule a demonstration to see why the MFP-1D is the most accurate and sensitive instrument for your force measurements.

### References

1. T. Thundat, E. A. Wachter, S. L. Sharp, and R. J. Warmack, Detection of mercury vapor using resonating microcantilevers, Appl. Phys. Lett. **66** (13) 1695 (1995).
2. T. Thundat, R. J. Warmack, G. Y. Chen, and D. P. Allison, Thermal and ambient-induced deflections of scanning force microscope cantilevers, Appl. Phys. Lett. **64** (21) 2894 (1994).

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