VALIDATION OF AN OPTICAL TWEEZERS/INTERFEROMETER SYSTEM FOR MECHANICAL TESTS OF SINGLE COLLAGEN MOLECULES

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Introduction: Collagen molecules are the essential building blocks of connective tissues, and play critical roles in mechanical functions. Direct assessment of the structure-function relationship at the molecular level, from where the disorders originate, will provide significant insight on the mechanism of degeneration and mutations such as osteoarthritis and ligament loosening. In order to perform such mechanical characterization, a testing system based on an optical tweezers/interferometer was developed. In this study, the accuracy and resolution of such system were evaluated.

Methods: The principle has been described for the mechanical tests of collagen molecules using this system [Luo *et al*., 1997]. Briefly, a solution of mixed collagen molecules, small polystyrene beads and large polystyrene beads is prepared, and placed upon a coverglass which is mounted on and moves with a nanometer resolution piezo-stage (P-731.20, Polytec PI, Inc., Auburn, MA). The concentration of molecules and beads is carefully adjusted to have a high incidence of one-to-one binding, i.e., one terminus of the molecule attached to a small bead, and the other terminus attached to the coverglass via a large bead. (Fig. 1). The small bead is trapped by the optical tweezers and the molecule is stretched by slowly moving the stage. The deformation of a molecule is measured as the displacement of the small bead center from the large bead center. Because the trap is mechanically equivalent to a spring, the trapping force is determined from the trapping stiffness and the displacement of the small bead from the trapping center [Svoboda and Block, 1994]. The stiffness of the molecule is then calculated from the molecular deformation and the force applied on the molecule which is equal to the trapping force. A custom-made interferometry system (Micro Development, Inc., Zimmerman, MN) was built on top of the optical tweezers to monitor the trapped small bead motion.

Two critical validations - small bead displacement and trapping stiffness - were performed in this study to ensure that the system has the sensitivity at the level of nanometer displacement and piconewton force necessary for measuring the molecular stiffness. Polystyrene beads of 0.49 μm diameter were used (Bangs Laboratories, Inc., Carmel, IN). The displacement noise level was first validated on the beads immobilized on the lower coverglass to determine the relationship between the interferometer output and the bead displacement [Svoboda and Block, 1994]. The laser was carefully focused at the center area of the bead. The interferometer output was recorded while the piezo-stage was moved from –1 to 1 μm. Determination of the trapping stiffness was based on three complementary methods [Svoboda and Block, 1994]: (1) the power spectrum estimation of Brownian motion of the bead under the trapping confinement, (2) equipartition theorem, and (3) a given fluid flow, and in this study, a sinusoidal fluid motion. This oscillation method also provided a convenient way to determine the linear range of the trapping stiffness. By testing the oscillation with different amplitudes, one should either obtain a constant trapping stiffness, if the oscillation was within the linear range, or a varying one if the oscillation was beyond the linear range. In this study, a series of amplitudes ranging from 1 to 5 μm were applied. A LabVIEW® code was created and used to control and monitor the piezo-stage, and to monitor the interferometer output. A sampling rate of 1000 Hz was used. A total of 20 beads were tracked and analyzed for each experiment, and each bead was tested at least four times.

Essential results: The interferometer tracked the bead displacement up to 220 nm (Fig. 2). The linear response was up to about 120 nm. The noise level calculated from the difference between the experimental data and the fitted curve was 0.8 nm root mean square.

The trapping stiffness measured from all three different methods shows close results. The stiffness determined by the power spectrum was $3.14\pm0.29\times10^{-3}$ pN/nm. The corresponding mean displacement gives the trapping stiffness of $3.02\pm0.37\times10^{-3}$ pN/nm. When the oscillation was applied, the stiffness was calculated as $3.16\pm0.29\times10^{-3}$ pN/nm. The linear range was up to about 120 nm (Fig. 3). The average trapping stiffness of the three methods was about 3.1×10^{-3} pN/nm.

Discussion: This study quantitatively measured the trapped bead displacement and the trapping stiffness of the optical tweezers. This was also the first validation of the interferometry built on the commercial available optical tweezers. With this validation, we believe that the optical tweezers system is capable and suitable for the measurement of biomechanical properties of single collagen molecules.

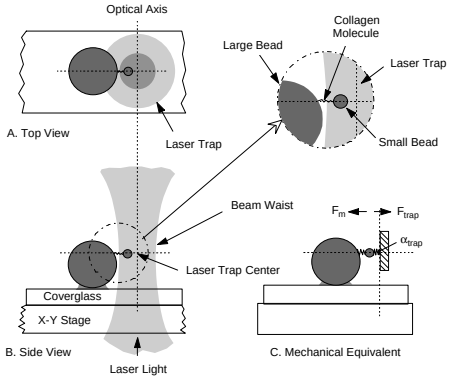

Figure 1: An illustration of the essential features of biomechanical testing of single collagen molecules.

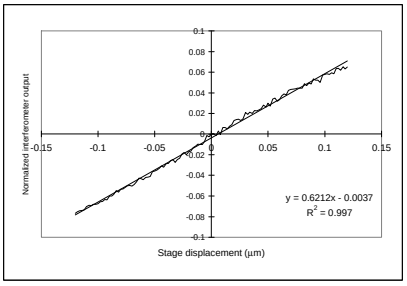

Figure 2: A typical output from the interferometer via the bead displacement. The linear response range was up to 120 nm.

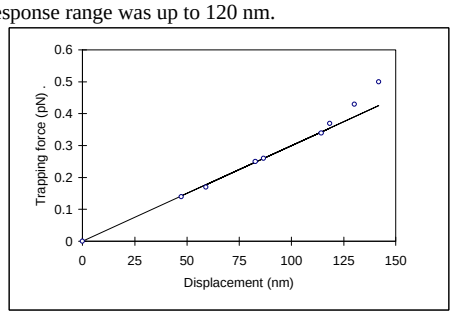

Figure 3: A typical result showing the trapping force as a function of bead displacement from the trapping center. The linear response is up to 120 nm.

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Svoboda, K. and Block, S.M. (1994) Force and velocity measured for single kinesin molecules. *Cell* **77,** 773-784.

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