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Optical trap setup for measuring microtubule pushing forces

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We present an optical trap design for force measurements of polymerizing microtubules. These stiff, filamentous cell components contribute to dynamic processes by generating pushing forces, for example during cell division. Although single traps are widely used for molecular pulling processes, studying pushing by flexible filaments requires extra measures. We introduce multiple, asymmetric traps for directional stabilization and bracing of the microtubules for enhanced rigidity. Our method performs in a force range which was inaccessible so far, namely near the stall force of a polymerizing microtubule. The described methods open the way to the study of other polymerizing biomolecular systems as well. © 2003 American Institute of Physics. [DOI: 10.1063/1.1629796]

Optical traps are a common tool to study piconewton forces generated by a wide range of biological systems, such as motor proteins, DNA polymerases, and unfolding proteins.1 Typically, a laser with a continuous power of $\sim 10^2$ mW is focused tightly into a diffraction-limited spot of about 1 $\mu$m. In the focus, small particles can be trapped, provided their relative refractive index is sufficiently high.2 Typical trap experiments involve biochemically prepared beads, linked to specific proteins or cell components. Small ($\sim 10^2$ nm) out-of-center displacements of a trapped bead cause a proportional restoring force, and thus a calibrated trap serves as a force sensor with stiffness $k$ ($\sim 10^{-1}$ pN/nm).3 In general, optical tweezer experiments involve molecules that exert a pulling force on a trapped bead.1,4 However, there are also biomolecular systems where pushing plays a central role. Examples are actin polymerization during Listeria propulsion5 and nuclear positioning by microtubule polymerization in fission yeast cells.6 In this letter, we present a special optical trap for measuring such pushing forces, in this work those of polymerizing microtubules. The setup is schematized in Fig. 1(a).

A microtubule (MT) consists of 8 $\alpha\beta$-tubulin dimers, forming $\sim 13$ strands that polymerize into a hollow tube of approximately 25 nm diameter, see Fig. 1(b). The tube length ranges from a few up to $\sim 10^4$ dimers. In the living cell, apart from providing mechanical support as part of the cytoskeleton, MTs play an active role in dynamic structures such as the mitotic spindle.7 At the basis of the active role of MTs lies dynamic instability:5 under the influence of GTP hydrolysis, a single MT alternates repeatedly between states of prolonged growth (polymerization) and shrinkage, both in a cell (in vivo) and with purified tubulin outside a cell (in vitro).9

In vivo and in vitro experiments have confirmed that polymerizing MTs apply $\sim$ pN pushing forces,10 large enough to play a role in cellular dynamics.6,7 A decrease in growth velocity under increasing load was found in vitro by buckling experiments,10 where MTs grow from nucleation sites against rigid barriers. Both the force and the growth rate are inferred from curvature analysis of the elastic MT. Eventually, growth should stop at the stall force. The magnitude of this stall force is an important input parameter for MT tip structure models.11,12 However, the stall force can only be reached if the growing MT does not buckle before under the increasing load. This implies that inferring a stall force by buckling analysis is impossible. Further, the buckling method relies on a known rigidity of the MT. This parameter requires elaborate analysis to determine13 and may vary considerably depending on the growth conditions.14

As an alternative we employed a multiple optical trap,15 with a “construct” of two beads biochemically connected16 to a MT segment, as in Fig. 1(a). One bead in a single trap would not suffice, as the pushing filament would tend to slide along the barrier. Buckling of the rather flexible segment between the two beads, as schematized in Fig. 2(a), is prevented by an asymmetric “keyhole” shaped trap potential, shown in Fig. 2(b): in a trapped construct, one bead is tightly trapped, while the other is only constrained in its sideways motion. As a result, the keyhole trap opposes pushing only with the bead closest to the barrier, and no buckling can occur between the two beads. We realized this keyhole trap with a single laser, time-shared between different spatial positions by means of software-driven acousto-optical deflec-
tors. The frequency of the deflector input signal determines the laser spot position. Therefore, a power spectrum [Fig. 2(c), thin line] of this input signal indicates also the relative occupancy time of the laser for the various positions. At high enough time-sharing frequency, a bead will experience an effective, time-averaged potential from the trap, approximated by weighted summation of single laser potentials [Fig. 2(c), thick line].

A second measure we introduce to prevent buckling is to stiffen the construct itself, as is shown later on. Then, only the “free” growing end of a MT can contribute significantly to buckling.

We compared the trap approach with the established buckling analysis in a pilot experiment shown in Fig. 3(a). From a trapped construct, two MTs were growing long enough to buckle against a barrier long before stalling would occur. Differential microscope images show the increasing buckling of both MTs (the left straight microtubule is an image enhancement artifact). We used the microscope images to infer the pushing forces in two ways. First, motion tracking of the lower bead provides us both with a bead displacement vs. time and, via the trap stiffness, with a force acting on the MT tips together. The absence of buckling inhibits data acquisition (before image 3). Thus, for the range where both methods are applicable, we find the same qualitative behavior.

As noted before, stall forces cannot be measured with buckling analysis. With the trap, they can be, as we show in a next pilot experiment (Fig. 4). Here, no keyhole trap was used, but instead a bundle of ~20 cross-linked MTs was used for the construct. The enhanced rigidity of this bundle prevents buckling of the segment between the two beads. The preparation method of these bundles causes only a few of these MTs to nucleate. As shown in Fig. 4(a), the bundle was kept at ~5 μm from a barrier, in this case a large, corrugated silica bead attached to the substrate. The short distance of the free growing MTs to the barrier excludes buckling of these ends.

In this particular experiment, only the left bead in Fig. 4(a) was tightly connected to the bundle, which implies that the total pushing force could be inferred from the displacement of this bead only, as shown in Fig. 4(b). In the first curve, we observe two persistent increases in force, followed by distinct plateaus. We interpret this as an event where the first one growing MT touches the wall, followed by a second MT joining in at t~650 s. From there, we measure two synchronized MTs. After full retraction of the whole construct, the force returns to zero after which a second contact event is observed.

The white squares in Fig. 4(b) indicate binned averages (over 8 s intervals), which were subsequently used to yield a pilot force-velocity curve, Fig. 4(c). The data converge to two stall forces at ~1.2 and ~3 pN for the single- and double-MT case, respectively. The low initial velocities of
The trap measurements are an experimental artifact, presumably caused by possible “settling” of the MT on the barrier or some deformation of the construct itself. We expect that for shorter free growing ends, or in a trap with lower stiffness, also the low-force range will give an accurate view of the growth velocity.

The stall force is expected to be proportional to the number of microtubules pushing. Then, the high-force tails for one single point on the zero force axis, which is indeed suggested by the data (guide lines in the plot). As a comparison, results from buckling experiments performed at higher initial growth velocities are also plotted. Again, such buckling analysis can only yield estimates of a stall force, which ends up somewhat higher than those directly measured with the trap. The difference is presumed to originate from the different initial, unloaded growth velocities in these experiments (1.2 and ~0.4 μm/min, respectively).

In conclusion, we have measured stall forces of pushing microtubules with an optical trap. This proof of principle experiment also opens the way to study microtubule growth dynamics with much larger spatial and temporal resolution than previously possible, as a trap in principle allows for high-frequency data acquisition.

The trap method is complementary to the buckling analysis method: the latter more naturally applies to large length increases at low forces, while the trap measures stall forces on short length and time scales. In addition, the trap allows for “force clamp” techniques. From the present work it appears that for such studies, a rigid and stable construct is crucial. The two methods we introduced, an asymmetric “keyhole” trap and “bracing” of cross-linked filaments, may also allow to study force generation of much less rigid polymers such as single actin filaments.

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12 We trap with a Nd:YVO₄ 1064 nm laser (Spectra Physics). The trapped bead motion is detected with a He–Ne 633 nm laser and a quadrant photodiode for calibration purposes. We infer the stiffness from the roll-off frequency in the bead motion spectrum. The trap potential was measured by capturing beads in viscous glycerol, yielding a near-Gaussian profile with a width of app. 0.8 μm.
13 We grow MTs in vitro from tubulin (Cytskeleton Inc.) and GTP in MRB80 buffer (80 mM Pipes, 1 mM EGTA, 4 mM MgCl₂, pH 6.8) from GMPCPP-stabilized seed MTs or bundles (MTs clustered with avidin). Streptavidin beads are either connected to biotynilated MTs or attached via biotynilated, immobilized AMP–PNP–kinesin as an intermediate linker. The barriers are either predeposited SiO₂, walls or silica porous beads (Asahi Glass Comp.).
14 Parallel experiments (Janson, Ph.D. thesis) predict a catastrophe within ~30 s from the onset of stalling. The extended lifetime in this particular experiment is attributed to residual stabilizing agents in the growth solution.