Prediction of bending stiffness and deformed shape of non-axially compressed microtubule by a semi-analytical approach

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Abstract The bending stiffness of a microtubule is one of the most important parameters needed in the analysis of microtubule deformation. In this study, a semi-analytical approach is developed to predict the bending stiffness and deformed shape of a non-axially compressed microtubule in an explicit closed form. By using the solution presented in this paper and the experimentally observed values given in the literature, both the deformed configuration and bending stiffness of a single microtubule are determined. The proposed method is validated by comparing the obtained results with available data in the literature. The comparison shows that the present semi-analytical formulation provides the same accuracy with reduced numerical effort.

Keywords Microtubule · Bending stiffness · Deformed shape · Semi-analytical method

1 Introduction

The mechanical properties of a eukaryotic cell are mainly determined by its cytoskeleton. The cytoskeleton is a system of protein filaments: mostly microtubules, actin filaments and intermediate filaments. Among the three types of cytoskeletal filaments, microtubules are the most rigid. The bending stiffness of microtubules is about 100 times that of intermediate and actin filaments [1]. Microtubules are hollow cylindrical tubes with ~25 nm outer

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diameter and tens of microns long, which are built from tubulin dimer [2]. Microtubules resist various internal and/or external forces to maintain cell shape and play an important role in many fundamental physiological processes in the cell such as providing mechanical stability for the cell, acting as railways along which motor proteins transport vesicles or organelles or separating chromosomes during cell division [3–5]. In all these physiological processes, the mechanical properties of microtubules are very important to the accomplishment of their function. Thus, the mechanics of microtubules have become a subject of primary interest in recent studies [6–10]. Specifically, buckling and vibration of microtubules have attracted considerable attention in the last decade. The buckling of microtubules in living cells has been studied in [11]. Recently, a mechanical model has been developed in [12] to study microtubule buckling in living cells modulated by the surrounding viscoelastic cytoplasm.

A comprehensive study of microtubule vibration has been carried out in [7] based on an isotropic continuum model. The free vibration of microtubules using the finite element method with radial deformation has been investigated in [13]. A two-dimensional lattice model has been developed for longitudinal and transverse vibration modes of microtubules [14]. Recently, dynamic behaviors of microtubules in cytosol have been studied in [15]. It should be noted that the bending stiffness of the microtubule is one of the most important parameters needed in quantitative analyses of microtubule mechanics. So far, different methods have been investigated for determining the bending stiffness or Young's modulus of the microtubules.

The microtubule stiffness was first estimated using the statistical measurement of microtubule curvature in electron microscopic images [16]. Since then, microtubule stiffness has been further estimated from dynamic video images by using four methods: (1) buckling force measurement using optical traps and beads [2, 16]; (2) image analysis of the relaxation process following microtubule bending [6]; (3) image analysis of microtubule bending via hydrodynamic flow [17] and (4) image analysis of thermal fluctuations of microtubule shapes in solution [1, 4, 18].

In the present paper, we focus on the first method of bending stiffness measurement. The bending stiffness of a single microtubule has already been measured using an optical trap and immobilized beads by Kurachi et al. [2]. The buckling force measurements with optical traps have been used to determine the bending stiffness of individual microtubule beads [16]. To optimize the accuracy of the measurement, they used two optical traps and antibody-coated beads to manipulate each microtubule and applied an analytical model assuming eccentrically loaded buckling. They used a non-linear differential equation and boundary conditions describing single microtubule buckling. As stated by the authors, the problem and boundary conditions were non-linear and since they did not have a closed form solution for that, they solved the problem numerically using the finite-difference method.

In spite of notable researches in the area of microtubule mechanics, there has been no attempt to tackle the problem described in the present paper. As the main novelty of this paper, we focus on presenting a useful semi-analytical and practical formulation which can easily be used in determining the microtubule bending stiffness.

In this paper, the basic non-linear governing equations of non-axially compressed microtubule are presented and a semi-analytical approach is proposed to obtain a closed form solution for the non-linear problem. Using the closed form solution presented in this paper and the experimentally observed values found in the literature, both the bending stiffness and the deformed configuration of a non-axially compressed microtubule can easily be determined; and there is no need to solve the problem numerically.

The proposed method uses the equilibrium equations of a microtubule element in the deformed configuration and employs the geometrical relationships when the compressing force applied at both ends exceeds the critical load. Moreover, two polystyrene beads attached to a single microtubule are modeled as circular sections. In this analysis, we assume that the microtubule length is constant during the process. Finally, the validity of the present analytical approach is confirmed by comparing the results with the available results in the literature. The results of comparison show that the presented analytical formulation provides the same level of accuracy with less numerical effort.

2 Mechanical modeling of single microtubule

The equations needed for determining the deformed configuration and the bending stiffness of a non-axially compressed microtubule are presented in this section. Figure 1a shows a video-enhanced differential interference contrast (DIC) image of a microtubule in a highly deflected state [2]. A schematic representation of a non-axially compressed microtubule is shown in Fig. 1b.

When the symmetry of the load and deformed configuration are used, the microtubule shape in the high deflected configuration can be defined by using the parametric equations $x(s)$ and $y(s)$ where s is the arc-length along the microtubule measured from point C ($s = 0$). Two polystyrene beads of radius r are attached to a single microtubule. One bead, at the bottom, is fixed to the glass surface and the other bead, on top, is trapped by a laser beam. It should be noted that the second bead is not fixed. Considering the single microtubule

Fig. 1 **a** Video-enhanced DIC image of a MAP-stabilized microtubule in highly deflected state [2]. **b** Schematic representation of a deformed microtubule

shown in Fig. 1 and in the absence of other forces, two forces, P , equal in size but opposite in direction, are applied. They ensure mechanical equilibrium.

The tangential angle of the microtubule with the horizontal direction is $\theta(s)$, the distance between the two polystyrene bead centers is y_d and the tangential angle of the microtubule at the bottom end is ψ_1 . Taking the coordinate axes as shown in the figure, we find that the exact expression for the curvature of the microtubule is $\frac{d\theta}{ds}$. Since the bending moment in the microtubule is equal to bending stiffness times the curvature, the exact differential equation of the deflection curve of the microtubule is

$$EI \frac{d\theta}{ds} = Px, \quad (1)$$

where EI is the bending stiffness of a single microtubule, E is Young's modulus and I is the geometrical cross section moment of inertia. Moreover, the geometrical relationships of the microtubule are

$$\frac{dx}{ds} = \cos \theta, \quad (2)$$

$$\frac{dy}{ds} = \sin \theta. \quad (3)$$

In order to obtain the microtubule's deformed shape and bending stiffness, the above system of differential Eqs. 1–3 must be solved. In the next sections, the semi-analytical solution of this problem is proposed.

3 Solution methodology

In this section, we construct a semi-analytical solution for Eqs. 1–3. Eliminating s between (1) and (2), the resulting equation in x is easily integrated. Using the boundary conditions at point C ($s = 0$)

$$x_{(s=0)} = r \sin \psi_1, \quad (4a)$$

$$\theta_{(s=0)} = \psi_1, \quad (4b)$$

$$y_{(s=0)} = -r \cos \psi_1, \quad (4c)$$

we obtain

$$x = \sqrt{(r \sin \psi_1)^2 + \frac{2EI}{P} (\sin \theta - \sin \psi_1)}. \quad (5)$$

For determining the y -coordinate of the microtubule, we use chain rule differentiation in (1) and substitute (3) and (5) in the resultant equation. By direct integration and using the boundary conditions at point C (4c), we obtain

$$y = \int_{\psi_1}^{\theta} \frac{EI \sin \beta}{P \sqrt{(r \sin \psi_1)^2 + \frac{2EI}{P} (\sin \beta - \sin \psi_1)}} d\beta - r \cos \psi_1. \quad (6)$$

When Eq. 5 is reintroduced in Eq. 1, a differential equation for s in terms of the variable θ is obtained:

$$EI \frac{d\theta}{ds} = P \sqrt{(r \sin \psi_1)^2 + \frac{2EI}{P} (\sin \theta - \sin \psi_1)}. \quad (7)$$

Integrating this equation gives

$$s = \int_{\psi_1}^{\theta} \frac{EI}{P \sqrt{(r \sin \psi_1)^2 + \frac{2EI}{P} (\sin \beta - \sin \psi_1)}} d\beta, \quad (8)$$

with $s = 0$ when $\theta = \psi_1$.

Equations 5 and 6 define the shape of a non-axially compressed microtubule as a function of tangential angle of the microtubule and three constant parameters ψ_1 , EI and P . It should be noted that in actual buckling experiments, the load and deformation are the measured quantities. Hence, the magnitude of P and y_d is known and two other parameters must be calculated. In order to determine the two unknown parameters, two constraints must be imposed. The first relation can be obtained by using the y -coordinate of the microtubule at the top end,

$$y_d + r \cos \psi_1 = \int_{\psi_1}^{\pi - \psi_1} \frac{EI \sin \beta}{P \sqrt{(r \sin \psi_1)^2 + \frac{2EI}{P} (\sin \beta - \sin \psi_1)}} d\beta - r \cos \psi_1. \quad (9)$$

The other relationship is obtained from the length of the microtubule (L), as

$$L = \int_{\psi_1}^{\pi - \psi_1} \frac{EI}{P \sqrt{(r \sin \psi_1)^2 + \frac{2EI}{P} (\sin \beta - \sin \psi_1)}} d\beta. \quad (10)$$

These two relationships constitute a set of two equations with the same number of unknowns, namely EI and ψ_1 which have to be determined for a given set P , y_d , L and r . The non-linear Eqs. 9–10 can simultaneously be solved by using the Newton method [19]. Finally, the coordinates x and y of the deformed microtubules under eccentric load can be determined from Eqs. 5 and 6. It should be noted that the formulation for axial buckling can easily be obtained by using the present formulation when r tends to zero.

4 Results and discussion

4.1 Measurement of bending stiffness

In this section, the bending stiffness of a non-axially compressed microtubule is obtained by using the semi-analytical formulation presented in previous section and the experimental

Table 1 Comparison of microtubule bending stiffness, EI , calculated by using the new semi-analytical model presented in this paper and numerical results obtained by Kurachi et al. [2]

Samples	Microtubule length (μm)	Buckling force (pN)	Deflected length (μm)	Bending stiffness ($\times 10^{-23} \text{ Nm}^2$)	
				[2]	Present study
MAP-stabilised microtubule	10.5	4.9	6.0	4.3	4.27
	10.5	3.7	9.1	3.8	3.86
	19.9	3.1	9.3	9.1	9.00
	27.9	3.3	17.0	20.0	20.81
Taxol-stabilised microtubule	4.4	1.2	3.6	0.20	0.21
	11.8	0.5	9.5	0.59	0.64

measurements reported by Kurachi et al. [2] and Kikumoto et al. [16]. In the first case, the bending stiffness for MAP-stabilized microtubules and taxol-stabilized microtubules in axial buckling are estimated using the magnitudes of P and y_d measured by Kurachi et al. [2]. The numerical results for the bending stiffness of single microtubule are calculated for two types of microtubule and six microtubule lengths by the method proposed in this paper. The results are given in Table 1 and are compared with those obtained by Kurachi et al. [2].

In the second case, the bending stiffness of Paclitaxel-stabilized microtubules and Paclitaxel-free microtubules is estimated. Table 2 shows the comparison between microtubule bending stiffness obtained by using the semi-analytical method presented in this paper and numerical results presented by Kikumoto et al. [16] for the two types of microtubule and eight microtubule lengths in axial (without arm) and non-axial (with arm) buckling. The parameter r , the bead radius, is assumed to be $0.9545 \mu\text{m}$.

It should be noted that a good agreement between the semi-analytical results obtained in this paper and the numerical results given by Kurachi et al. [2] and Kikumoto et al. [16] for the bending stiffness of microtubules can be seen. It can be concluded that the present formulation is an appropriate method and a new approach to predict the bending stiffness of different types of microtubule.

Table 2 Comparison of microtubule bending stiffness, EI , calculated by using the new semi-analytical model presented in this paper and numerical results obtained by Kikumoto et al. [16]

Samples	Microtubule length (μm)	Buckling force (pN)	Deflected length (μm)	Bending stiffness (without arm)		Bending stiffness (with arm)	
				$(\times 10^{-24} \text{ Nm}^2)$		$(\times 10^{-24} \text{ Nm}^2)$	
				[16]	Present study	[16]	Present study
Paclitaxel-stabilised microtubule	8.4	0.36	3.7	1.8	1.82	1.9	1.85
	10.0	0.32	4.8	2.4	2.37	2.5	2.39
	15.7	0.11	7.6	2.0	2.01	2.0	2.03
	17.3	0.11	5.8	2.2	2.17	2.5	2.25
Paclitaxel-free microtubule	8.8	1.35	4.7	8.1	8.04	8.4	8.39
	12.2	0.58	7.0	6.7	6.83	7.1	7.17
	13.6	0.50	8.0	7.4	7.39	7.8	7.77
	16	0.43	8.3	8.3	8.38	8.5	8.54

4.2 Deformed shape

Typical deformed shapes of a non-axially compressed microtubule are shown in Fig. 2. In this figure, the microtubule length, L , bead radius, r , and bending stiffness of microtubule, EI , are fixed at $15.7 \mu\text{m}$, $0.45 \mu\text{m}$ and $2 \times 10^{-24} \text{Nm}^2$, respectively, and the microtubule shapes are shown for four compressive forces $P = 0.06, 0.08, 0.10$ and 0.12 pN . It can be seen from Fig. 2 that the tangential angle of the microtubule at the bottom end, ψ_1 , approaches to zero and then becomes negative.

The effect of increasing the bending stiffness on the microtubule's deformed shape is also investigated. The microtubule deformed shapes are shown in Fig. 3. In this figure, the microtubule length, L , radius of beads, r , and compressive force, P , are fixed at $15.7 \mu\text{m}$, $0.45 \mu\text{m}$ and 0.08 pN , respectively, and the microtubule shapes are given for four bending stiffnesses $EI = 1.8, 2.0, 2.2$ and $2.5 \times 10^{-24} \text{Nm}^2$. As expected, it can be seen from this figure that by increasing the bending stiffness, the deflection of the microtubule decreases.

Fig. 2 The effect of the force P on the microtubule-deformed shapes ($L = 15.7 \mu\text{m}$, $r = 0.45 \mu\text{m}$, $EI = 2 \times 10^{-24} \text{Nm}^2$ and $P = 0.06, 0.08, 0.10$ and 0.12 pN)

Fig. 3 Influence of microtubule bending stiffness on the microtubule's deformed shape

5 Conclusion

The bending stiffness of the microtubule is one of the main parameters needed in quantitative analyses of microtubule mechanics. Despite some achievement in analyzing microtubule mechanics, to the authors' knowledge, no closed form solution for determining the deformed shape and bending stiffness of a non-axially compressed microtubule has been presented so far. In this paper, a semi-analytical solution for predicting the deformed shape and bending stiffness of a non-axially compressed microtubule is presented. It may be considered as a valuable task because the most remarkable solutions in determining microtubule rigidity by using buckling force measurement were based on different numerical and/or iterative techniques when their extension to different cases was difficult.

The present solution allows us to determine the bending stiffness of the microtubule, given the original length, buckling force and deflected length. The variation of the deformed shape with the buckling force and bending stiffness is also examined. The proposed method is validated by comparing the obtained results with available data in the literature. The comparison shows that the present analytical formulation provides the same accuracy with reduced numerical effort.

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