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DYNAMICAL PROPERTIES OF PLASMID DNA MOLECULE IN PULSE REGIME BY OPTICAL TWEEZERS

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ABSTRACT

The dynamical properties of the plasmid DNA molecule in the optical tweezers using pulsed Gaussian laser beam is investigated. The finite difference Langevin equation (FDLE) describing the dynamics of polystyrene microsphere linking to plasmid DNA molecule is presented. This FDLE is numerically solved to discuss the influence of parameters of pulsed Gaussian laser beam as peak intensity, duration on the dynamical properties of polystyrene microsphere. From the variances of position, velocity or all forces in pulsing time, the conditions to stretch a plasmid DNA molecule into stretching state, where the maximum stretched length is about its contour length are discussed and found out.

Indexing terms/Keywords

Laser trapping; Optical devices; Medical and Biotechnology.

Academic Discipline And Sub-Disciplines

Physics and Medical and Biotecnology

SUBJECT CLASSIFICATION

Dynamics of molecule

TYPE (METHOD/APPROACH)

Theory, Simulation, Computation physics

1. INTRODUCTION

There are many works interesting on using optical tweezers to trap the biological molecules, especially the DNA proteins [1-8]. In almost of previous works, the optical tweezers is using to investigate the extension force of all phage of DNA [1-14], [17-20], only. Lately, the equation of extension force of DNA molecule is modified [21] and the dynamics of polystyrene microsphere linking to λ-phage DNA molecule in optical tweezers are investigated [22]. In the last work, we have investigated the dynamic of the polystyrene microsphere linking to plasmid DNA molecule in the CW regime, i.e. using a CW Gaussian laser beam. The influence of all parameters as the peak intensity, beam waist of the laser Gaussian beam well as the initial position of polystyrene microsphere on the stretching process of the plasmid DNA molecule is discussed [23]. Right now, the interest is focused on the dynamic of polystyrene microsphere in the pulse regime, i.e., the motion of it in the optical tweezers using the pulsed Gaussian laser beam.

In this paper using FDLE the dynamics of the polystyrene microsphere, consequently, stretching process of plasmid DNA molecule in pulse regime are investigated. The influence of all parameters of pulsed Gaussian laser beam on variance of all forces, consequently, position, velocity of polystyrene microsphere, which is considered in the near stretching state will be discussed.

2. FINITE DIFFERENT LAGEVIN EQUATION

Consider plasmid DNA molecule is embedded in a suitable fluid, i.e., the refractive indexes ratio $m = n_b / n_f > 1$,where n_b is refractive index of trapped bead and n_f is refractive index of fluid [24,25]. One end of DNA molecule is linked to a glass cover slip through a surface-anchored RNA (ribonucleic acid) polymerase, the opposite end is attached to polystyrene microsphere (trapped bead), which is captured or held under tension with optical tweezers, and its position is monitored by a pulsed Gaussian laser beam, whose intensity distribution is given by:

$$I(r,t) = I_0 \exp\left(-\ln 2\frac{r^2}{W_0^2}\right)\exp\left(-\ln 2\frac{t^2}{T_0^2}\right) \quad (1)$$

where $I_0 = I(0,0)$ is the peak intensity at $r = 0$, i.e., at center of optical tweezers, and $t = 0$ $t = 0$, W_0 is the beam waist, and T_0 is the ½ duration of the pulse. The often-used experimental geometry of DNA molecule with optical tweezers is shown in Fig. 1a [11,13].

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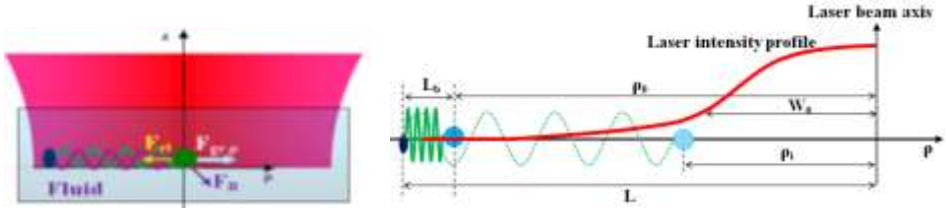

Fig.1. a) Cartoon of an often-used experimental geometry; b) The beginning set-up of trapped bead in tweezers.

The aim of optical tweezers is to keep the DNA molecules in stretching state. The attached bead plays the role linking DNA molecules to center of optical tweezers and glass cover slip. As shown in Fig.1a, the bead is under acting of three forces: the elastic force of DNA molecules, Brownian force of fluid and optical force. The general Langevin equation (GLE) describing the dynamic of bead linking to DNA molecules in optical tweezers [25].

$$m\ddot{r}(t) = -g\dot{r}(t) + F_{gr,r}(r(t)) - F_{el}(r(t)) + \sqrt{2k_BT\,g}W_r(t) \tag{2}$$

where m is the bead mass, $g = 6pha$ is the friction coefficient, h is the viscosity of fluid, a is the radius of bead, $W_r(t)$ is the white noise at position $r(t)$, $F_{gr,r}(r(t))$ is the transverse gradient optical force, which depends on the intensity distribution of laser beam, radius of trapped bead, and polarizability of the bead in the fluid, and given following [21,26,32]:

$$F_{gr,\rho}(\rho(t)) = -2n_f\rho(t)a^3I_0\left(\frac{m^2-1}{m^2+2}\right)^2 exp\left[-\ln 2\times\left(\frac{\rho(t)}{W_0}\right)^2\right]exp\left[-\ln 2\times\left(\frac{t}{T_0}\right)^2\right] \tag{3}$$

where $\sigma = n_f a^3\left(\frac{m^2-1}{m^2+2}\right)$ is the polarizability in Rayleigh regime [27], $F_{el}(r(t))$ is the elastic force, which depends on the extension of DNA molecule $r(t) - r_0$ (see Fig.1b), and given as following:

$$F_{el}(\rho(t)) = \frac{k_BT}{L_b}\left[\frac{\rho(t)-\rho_0-L_b}{L} + \frac{1}{4}\frac{1}{\left[1-(\rho(t)-\rho_0-L_b)/L\right]^2} - \frac{1}{4}\right] \tag{4}$$

where $k_B = 1{,}38\times 10^{-23}J/K$ is the Boltzmann's constant, and T is absolute temperature (K) [21], and the last term in the right of Eq.2 describes the Brownian motion of the polystyrene microsphere controlled by the Brownian force:

$$F_B = \sqrt{2k_BT\gamma}W(t) \tag{5}$$

where $W(t)$ is the white noise, which is characterized by the following properties [34]: the mean $\langle W(t)\rangle = 0$ for at all t; $\langle W^2(t)\rangle = 1$ for each value t; and $W(t_1)$, $W(t_2)$ are independent of each other for $t_1 \neq t_2$. Finite difference simulation of GLE are straight-forward: the continuous-time solution $r(t)$ of an GLE is approximated by a discrete-time sequence r_i, which is the solution of corresponding finite difference equation (FDE) evaluated at regular time steps $t_i = i\Delta t$. Eq.2 will used for a polystyrene microsphere with $a \approx 0.05mm$ [15,27], average density of $1.35g/cm^3$ [25,28], and its mass of $m \approx 1.5\times 10^{-18}kg$, which is embedded in water with viscosity $h = 0.001Ns/m$ at temperature $T = 300K$ [15], so the friction coefficient is $g = 6pha \approx 94.3\times 10^{-10}kg/s$. Thus, the momentum relaxation time $t = m/g \approx 0.17\times 10^{-8}s$ is much smaller than the time scales of typical experiments [29], consequently, it is often possible to drop the inertial term (i.e. set $m = 0$). If Δt is sufficiently small, $r_i \approx r(t_i)$, a FDE is obtained from the GLE as follows [21,22,26,27,]:

$$r_i = r_{i-1} + \frac{-F_{gr,r}(r_{i-1}) + F_{el}(r_{i-1})}{g}\Delta t + \sqrt{2k_BT/g}\left(\sqrt{W_1W_2} - \sqrt{W_3W_4}\right)\Delta t \ , \tag{6}$$

which is called as a finite different Langevin equation (FDLE), where $W_i, i = 1,2,3,4$ are random values of white noise at t_i [15,34]. The solution is obtained by solving the resulting FDLE recursively for r_i, using the values r_{i-1} and r_{i-2} from previous iterations. In the next section , Eq.6 will be used to investigate the stretching process of plasmid DNA linking to polystyrene microsphere under action of all forces in the optical tweezers.

A single plasmid DNA molecule with ionic condition of 10mM Na+ having a stable length of $L_b = 47nm$ and contour length of $L = 1.33mm$ [18] is attached to a polystyrene microsphere with refractive index of $n_b = 1.57$,[25,30] which is embedded water with refractive index of $n_f = 1.326$ [27,31]. The optical tweezers is using a pulsed Gaussian laser beam with wavelength of $l = 1.06mm$, whose waist, peak intensity and duration can be changed. The polystyrene microsphere is placed at the beginning position, $r_0(mm)$ with a distance of $L_{set} » L - L_b$ (μm) on the left from the center of tweezers. That means $r_0 = -L_{set}$ as shown in Fig. 1b.

3. FINITE DIFFERENT LAGEVIN EQUATION

Firstly, we check the dynamic of polystyrene microsphere placed at $r_0 = 1.2mm » L = 1.33mm$ in stretching state in optical tweezers using CW Gaussian laser beam with $I_0 = 5´10^5 W/cm^2$ and $W_0 = 1mm$. Using Eqs.1 and 3÷6 with simulated time step of 1μs ($Dt = 1´10^{-9}s$), the position-time characteristic and intensity distribution are illustrated in Fig.2.

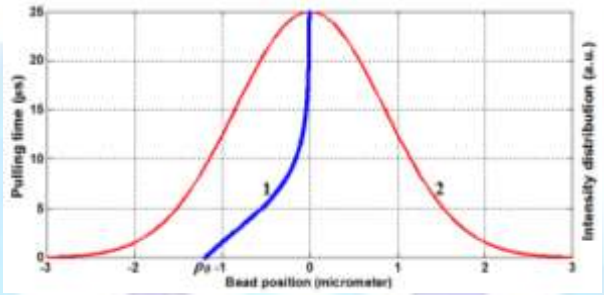

Fig.2 Position-time characteristic (line 1) and intensity distribution (line 2) of polystyrene microsphere linking to plasmid DNA molecule in optical tweezers using CW laser beam with $I_0 = 5\times10^5 W/cm^2$, $W_0 = 1\mu m$.

As is shown in Fig.2, after a pulling time about $T_p » 20ms$ the polystyrene microsphere moved into center of tweezers. Since of CW laser beam, i.e., intensity is constant at center of tweezers, the polystyrene microsphere is kept in stable state when $t > T_p$. Thus, the given values of CW laser beam are seen to be the conditions to make a plasmid DNA molecule to be in the stretching state, where the maximum stretched length is about its contour length, $L_{str} = |r_0| » L$. The question is what will happen when the CW laser beam is replaced by a pulsed one. To understand that, we consider a pulsed Gaussian laser beam with controllable parameters, but the ½ duration is about the pulling time, i.e. $T_0 » T_p = 20ms$. Considering a pulsed Gaussian beam with the ½ duration, $T_0 = 10ms$, beam waist $W_0 = 1mm$ and peak intensity changes from $I_0 = 5\times10^6 W/cm^2$ down to $I_0 = 5\times10^4 W/cm^2$, the dynamics of polystyrene microsphere in the pulse regime are illustrated in Fig.3. The results show that in the pulse regime, the considered pulsed Gaussian laser beam with peak intensity smaller than $I_0 = 5\times10^5 W/cm^2$ can not to pull polystyrene microsphere into center of tweezers; With peak intensity of $I_0 \geq 1\times10^6 W/cm^2$ polystyrene mircrosphere reaches the center of tweezers after the pulling time of $T_p » 30ms$, which is longer than that in CW regime, $T_p » 20ms$ as shown in Fig.2. The pulling time will be shorter with increasing of peak intensity (line 5 in Fig.3). It is different to that in CW regime where polystyrene microsphere is in stable state, in the pulse regime, after reaching the center of tweezers, the polystyrene microsphere is pulled back, immediately. This situation can be explained by the changing of total force acting on it.

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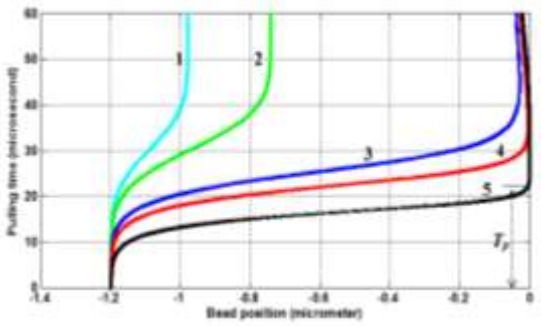
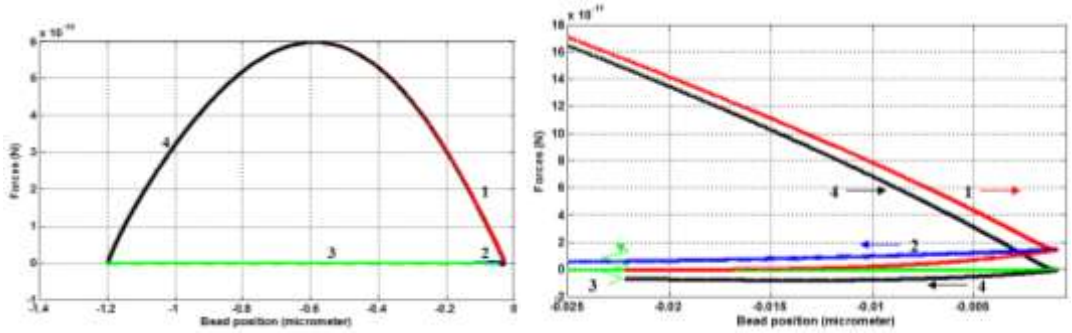


Fig.3 Dynamics of polystyrene microsphere when use pulsed laser Gaussian beam with $W_0 = 1\mu m$, $T_0 = 10\mu s$, and $I_0 = 5\times10^4 W/cm^2$ (curve1), $I_0 = 1\times10^5 W/cm^2$ (curve2), $I_0 = 5\times10^5 W/cm^2$ (curve 3), $I_0 = 1\times10^6 W/cm^2$ (curve 4) and $I_0 = 5\times10^6 W/cm^2$ (curve 5) .

As an example, the changing of all forces acting on the polystyrene microsphere for the case of $I_0 = 5\times10^6 W/cm^2$ presented in Fig.4a. It is clear that, the polystyrene microsphere moves more and more nearer to the center of tweezers, if the $F_{total} = F_{gr,\rho} + F_{el} + F_B > 0$. In the region near the center of tweezers with dimension ($\approx 0,025\mu m$), which is shorter than microsphere radius ($= 0,05\mu m$), the polystyrene microsphere is seen to be in stable state till $F_{total} = 0$.



a **b**

Fig. 4 a) Forces acting on polystyrene microsphere at relating position and b) Forces near the center of tweezers and their orientations (arrow) when using pulsed Gaussian laser beam with $W_0 = 1\mu m$, $L = 1.33\mu m$ $\rho_0 = 1.2\mu m$ $T_0 = 10\mu s$, $I_0 = 1\times10^6 W/cm^2$; 1 (red): Optical force, $F_{gr,\rho}(\rho)$, 2 (blue): Elastic force, $F_{el}(\rho)$, 3 (green): Brownian force, $F_B(\rho)$ and 4 (Black): Total force $F_{total}(\rho)$.

Nearer the center of tweezers, the optical force reduces, meanwhile the plasmid DNA molecule come nearer the stretching state, consequently, the elastic force increases to its stretch modulus of $1\times10^{-9} N$ [16,33], which is larger than the maximum optical force of $6\times10^{-10} N$ (see Fig.4a). Since the Brownian force is too small can be neglected, when the elastic force is larger than optical force, i.e. $F_{total} < 0$, the polystyrene microsphere is pulled back. Moreover, from Fig.3 we can see that with increasing of peak intensity the pulling time, T_p reduces, that means the average velocity of polystyrene microsphere, V_a increases. For example, if $I_0 = 1\times10^6 W/cm^2$ then $V_a = 33mm/s$, and if $I_0 = 5\times10^6 W/cm^2$ then $V_a = 50mm/s$, which are more larger than its thermal velocity ($\approx 3mm/s$) in fluid [33]. It is more clearly shown in Fig.5a, which presents the change of temporal velocity of polystyrene microsphere in trapping process with different peak intensity. Combining with curve 1 (red line) in Fig.4a, it is clear, that the temporal velocity is proportional to optical force and increases with increasing of peak intensity. As well as the total force, in the region near the center of tweezers, the temporal velocity reduces to zero and changes its orientation (see Fig.5b). The results obtained above are simulated for duration of $T_0 = 10\mu s$, only. To have a stable condition for polystyrene microsphere, it must more investigate the dynamics with different pulse durations. Considering the CW Gaussian laser beam is modulated with durations $T_0 = 5, 10, 15, 20\mu s$, the dynamics of polystyrene microsphere in the pulsed regime are illustrated in Fig.6a.

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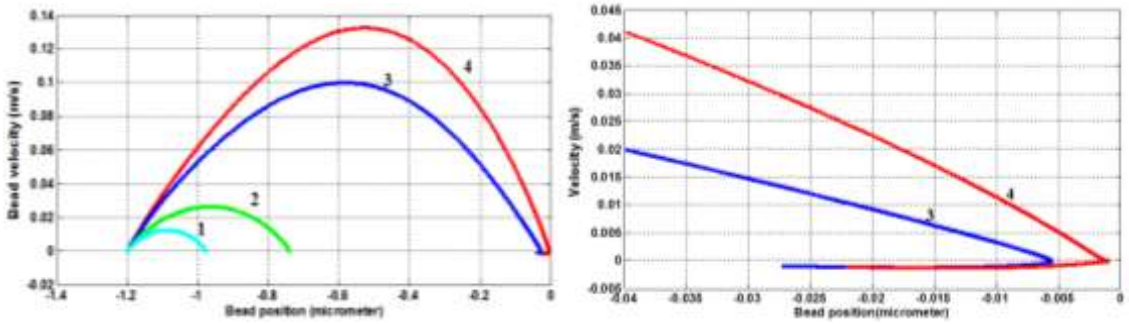
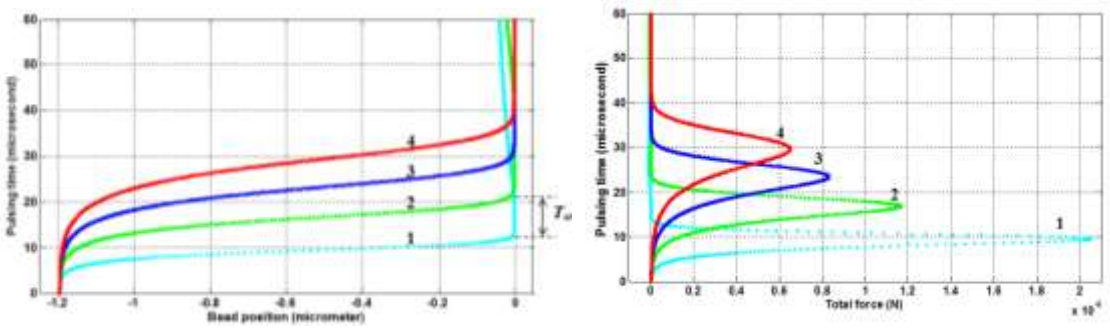


a b

Fig. 5 Temporal velocity of polystyrene microsphere at relating position when using pulsed Gaussian laser beam with $W_0 = 1\mu m$, $L = 1.33\mu m$, $\rho_0 = 1.2\mu m$, $T_0 = 10\mu s$, and different peak intensity of $I_0 = 5\times10^4 W/cm^2$ (1-cyan), $I_0 = 1\times10^5 W/cm^2$ (2-green), $I_0 = 5\times10^5 W/cm^2$ (3-blue), $I_0 = 1\times10^6 W/cm^2$ (4-red).

If the duration shorter then the total force rises up-to to peak more speedily (Fig.6b), consequently, the velocity increases and the pulling time, T_p reduces (Fig.6a). In other side, the total force falls down-to zero from peak more speedily (Fig.6b) leads the stable time, T_{st} reduces (Fig.6a). Thus, in the pulse regime, to pull the polystyrene microsphere into stable region near the center of tweezers, only, it must use the pulsed Gaussian laser beam with the beam waist of $W_0 = 1\mu m$, peak intensity of $I_0 = 5\times10^5 W/cm^2$ and duration of $T_0 \le 10\mu s$. But, when to increase the stable time of the polystyrene microsphere in region near center of tweezers or to keep the plasmid DNA molecule stable in stretching state, it must increase peak intensity $I_0 > 5\times10^5 W/cm^2$ and duration $T_0 > 10\mu s$.



a b

Fig.6 Dynamics of polystyrene microsphere (a) and Variance of total force in pulling time (b) when use pulsed Gaussian laser beam with $W_0 = 1\mu m$, $I_0 = 5\times10^6 W/cm^2$, and different durations $T_0 = 5\mu s$ (1-cyan), $T_0 = 10\mu s$ (2-green), $T_0 = 15\mu s$ (3-blue) and $T_0 = 20\mu s$ (4-red).

From above results, there are conclusion points: i) The dynamics of polystyrene microsphere as well as the stretching process of plasmid DNA molecule in optical tweezers using pulsed Gaussian laser beam are simulation investigated; ii) In the pulsed regime the stretching process, i.e., its pulling time, stable time and speed of the plasmid DNA molecule with ionic condition of 10mM Na+ linking to a polystyrene microsphere embedded in water depends on given parameters of the pulsed Gaussian laser beam, i.e., the pulling time and speed depend on the peak intensity, meanwile, the stable time depends on the duration; It can be kept in stretching state by optical tweezers with conditional collection of parameters as $W_0 = 1\mu m$, $I_0 > 5\times10^5 W/cm^2$, $T_0 > 10\mu s$ of the pulsed Gaussian laser beam. But the stable time is always shorter than that in CW regime. Moreover, the found out conditions with another given parameters gives us a collection of parameters for design experimental optical tweezers to stretch plasmid DNA molecule in the future.

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