ISSN 2347-6893
Volume 9 Number 2
Journalof Advances in Biology

DYNAMICS OF POLYSTYRENE MICROSPHERE LINKING TO λ-PHAGE DNA MOLECULE IN OPTICAL TWEEZERS USING PULSED GAUSSIAN LASER BEAM

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ABSTRACT

The trapping process of the biological micro-particle in the tweezers using CW laser beam has been investigated in series of our works. In the CW regime, the dynamics of polystyrene microsphere linking to λ-phage DNA molecule depending on the optical force, Brown force and its elastic force are simulated and discussed. In this paper we present the finite difference Langevin equation (FDLE) describing the dynamics of trapped bead linking to DNA molecules in an optical tweezers. Using FDLE the dynamics, i.e., the position-time characteristics of polystyrene microsphere linking to λ-phage DNA molecule are simulated in the pulse regime. The obtained results show that all principle parameters as the peak intensity, beam waist radius, and duration of pulsed Gaussian laser beam, well as the setting-up position of DNA molecule influence on the dynamics, especially on the "pulling" and "stability" times of trapped bead. The conditions for λ-phage DNA molecule in stretching state are discussed for experimence.

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 interests focused on optically trapping the biological molecules, especially the DNA proteins [1-8]. In almost of previous works, the optical tweezers is using to measure the extension force of all phage of DNA [1-14], [17-20]. Using experimental elastic parameters, Mack [13], Sharma [14], Hamdi [16], Bustamante]17], and Bauman [18] have derived an approximation equation of the extension force, describing the dependence of the elastic force on the extension of DNA molecules. Although those equations describe not at all the nature of DNA molecules, so then it has been modified and used for dynamical equation [37, 39]. At the same time, there are investigations the dynamic of the free particle embedded in fluid with Brownian force and optical force in pulse regime [21, 22], but of bead linking to DNA molecule in CW regime [39, 40], only. Up to now, the dynamics of the bead, which influence on the stretching process of DNA molecule in the optical tweezers using pulsed Gaussian laser beam are interesting questions. In this paper we present analyzing of the FDLE to describe the dynamic of trapped bead linking to DNA molecules in optical tweezers. With the aim to investigate the stretching process of λ-phage DNA molecules relating to motion of polystyrene microsphre in fluid in the pulse regime, the role of all principle parameters as the peak intensity, duration and beam waist radius of the pulsed Gaussian laser beam, well as the setting-up position of λ-phage DNA molecule linking to the polystyrenre microsphere, and conditions to keep it in stretching state are discussed.

2. Finite different Langevin equation

2.1 The principle model of tweezers for DNA molecules

Consider DNA molecule is embedded in a suitable fluid, i.e., the refractive indexes ratio m>1 [29,30]. 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, which is captured or held under tension with optical tweezers, and its position is monitored by a pulsed laser beam. The often-used experimental geometry of the DNA molecule linking to trapped bead in the optical tweezers is shown in Figure 1 [11, 13].

DOI : 10.24297/jab.v9i2.4005

ISSN 2347-6893
Volume 9 Number 2
Journalof Advances in Biology

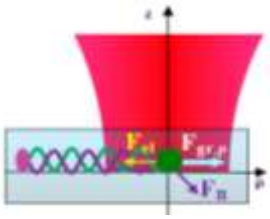

Fig. 1. Cartoon of an often-used experimental geometry.

The aim of optical tweezers is to keep the DNA molecules in stretching state. The polystyrene microsphere plays the role to link DNA molecules to center of optical tweezers and glass cover slip. As shown in Figure 1, the polystyrene microsphere is under acting of three forces: the elastic force of DNA molecules, Brownian force of embedding fluid and optical force.

2.2. Extension force in DNA molecule

Chemically, deoxyribonucleic acid (DNA) molecule is a long polymer made up of a linear series of subunits known as nucleotides and typical found in a highly compact "supercoiled" configuration. This is a result of the higher entropy, and therefore lower Gibbs free energy, of the compacted state [23]. To stretch DNA molecule thus requires an input of energy, which implies the presence of spring-like properties. The entropic spring-like force of DNA molecule can be understood by a worm-like chain (WLC). This approximates the λ-phage DNA strand as a continuously bendable thin chain. As an example, the model WLC of fibrillin molecule is presented in Figure 2.

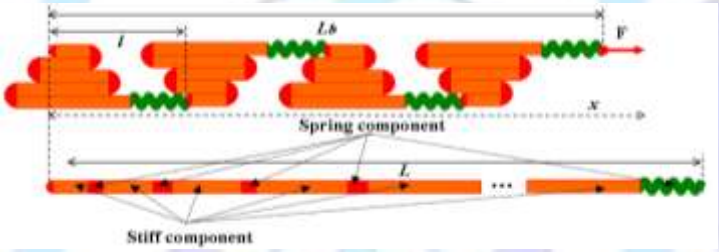

Fig.2. Model WLC of the DNA molecule [37, 40].

One DNA molecule consists of n segments with length of l . Every segment consists of spring and stiff components. The length of all segments is $L_b = nl$ called stable chain length, at which directional correlation between chain segments decay. If an applied force F attaching to free end (opposite end is attached to an anchor), the chain is stretched with extension x . With increasing of applied force, the extension increases longer and more longer to the maximum value, which is the contour length L . The force-extension relation is approximately given by follows [37]:

$$F_{el}(x) = \frac{k_B T}{L_b}\left[\frac{x - L_b}{L} + \frac{1}{4}\frac{1}{\left(1 - (x - L_b)/L\right)^2} - \frac{1}{4}\right] \quad (1)$$

where $k_B = 1{,}38 \times 10^{-23} J/K$ is the Boltzmann's constant, T is absolute temperature (K).

2.3. Brownian force

The Brownian motion of the trapped bead is controlled by the Brownian force, which is the fluctuating force due to random impulses from the many neighboring fluid molecules and given by

$$F_B = \sqrt{2k_B T \gamma}\, W(t) \quad (2)$$

where $\gamma = 6\pi\eta a$ is the friction coefficient, η is the viscosity of fluid, a is the radius of bead, $W(t)$ is the white noise, which is characterized by the following properties [27]: 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 $t_1 \neq t_2$.

2.4. Optical force

The position of trapped bead is controlled by the laser beam with wavelength λ , focused by high-numerical aperture (NA) microscope objective [15] to waist of radius, W_0 with peak intensity, I_0 in its center. This beam is seem to be as a Gaussian beam given by follows:

$$I(z,r) = I_0 \left(\frac{W_0}{W(z)}\right)^2 exp\left(-2\left(\frac{r}{W_0}\right)^2\right) exp\left(-2\left(\frac{t}{T_0}\right)^2\right) \quad , \tag{3}$$

where $I_0 = I(0,0)$ (W/cm^2) is the peak intensity at tweezers center, $W(z) = W_0\sqrt{1+\left(z/z_0\right)^2}$ is the beam radius at z, $z_0 = pW_0^2 / l$ is the Rayleigh range [33], t is the time and T_0 is the pulse duration. Irradiated by this laser beam, the polystyrene bead with refractive index n_b embedded in fluid with refractive index n_f on a plane will be acted by transverse optical force, which are given by follows:

$$\begin{aligned} \vec{F}_{gr,r}(z,r) &= \frac{s}{2}\nabla_r I(z,r) = \\ &- \vec{r}\, 2n_f r a^3 I_0 \left(\frac{m^2-1}{m^2+2}\right)\left(\frac{W_0}{W^2(z)}\right)^2 exp\left(-2\left(\frac{r}{W(z)}\right)^2\right) \end{aligned} \tag{4}$$

where $s = n_f a^3\left(\frac{m^2-1}{m^2+2}\right)$ is the polarizability in Rayleigh regime [34], n_f is the refractive of fluid, $m = n_b / n_f$ is the ratio of refractive indexes, n_b is the refractive index of bead.

2.5. Finite difference Langevin equation

Using Eqs. (1), (2) and (4), we obtain the general Langevin equation (GLE) describing the dynamic of bead linking to DNA molecules in optical tweezers, which is given by follows [21, 38]:

$$m\ddot{r}(t) = -g\dot{r}(t) + F_{gr,r}(r(t)) - F_{el}(r(t)) + \sqrt{2k_B T g}W_r(t) \tag{5}$$

where m is the bead mass. The term in the left of Eq.5 is inertial. In the right of Eq.5, the first term is friction, second is restoring, third is stretching and the last is white noise.

We consider a polystyrene microsphere plays a role of trapped bead with radius $a \approx (0.15,\ 0.25)mm$ [15, 34], average density of 1.35g/cm^3 [24, 25], and its mass of $m \approx 1.5\times 10^{-18}kg$. The bead is embedded in water with viscosity $h = 0.001Ns/m$ at temperature T of 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 experiment [32], consequently, it is often possible to drop the inertial term (i.e. set $m = 0$) in the left of Eq.5. 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 $t_i = i\Delta t$. If Δt is sufficiently small, $r_i \approx r(t_i)$. A FDE is obtained from the GLE as given following [21, 22, 38, 39]:

$$r_i = r_{i-1} + \frac{-F_{gr,r}(r_{i-1}) + F_{el}(r_{i-1})}{g}\Delta t + \sqrt{2k_B T / g}\left(\sqrt{W_1 W_2} - \sqrt{W_3 W_4}\right)\Delta t \tag{6}$$

which is called as a FDLE, where $W_i, i = 1,2,3,4$ are random values of white noise at t_i. 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.

3.The stretching process of λ-phagr DNA molecules

For experience in the future, we consider a single l -phage DNA molecule with ionic condition of 1.86mM Na^+[18] is attached to a polystyrene microsphere with radius of $a = 0.25mm$, refractive index of $n_b = 1.57$ [34, 35] which is embedded water with refractive index of $n_f = 1.326$ [34,36] viscosity $h = 0.001Ns/m$ at temperature T of 300K[15]. The pulsed laser beam with wavelength of $l = 1.06mm$ can be focused to waist of $W_0 = (2,\ 4)mm$ radius, its peak intensity can be changed in the interval $I_0 = \left(3\times 10^5,\ 100\times 10^5\right)W/cm^2$, and its duration can be modulated in the range of $T_0 = (0.5,\ 4)ms$. Using all of parameters given above and considering the trapped bead liking to λ-phase DNA molecule, which has stable length of $L_b = 89nm$ and contour length of $L = 16mm$ [18] is placed in setting-up position on

ISSN 2347-6893
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the left from the center of tweezers with a distance of $L_{set} \gg L - L_b$ (μm) as shown in Figure 3. For simulation the stretching process of λ-phase DNA molecule in trapping region (the laser irradiating region), we consider by certain ways the bead has initial simulation position, $r_0(mm)$.

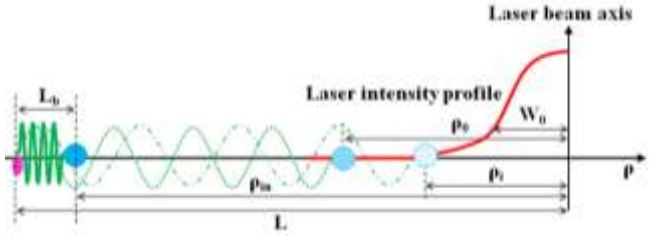

Fig.3. The beginning set-up of λ-phase DNA molecule linking to polystyrene microsphre in optical tweezers.

Firstly, the dynamics of polystyrene microsphere in optical tweezers are simulated by Eq.(6) with time step of 1μs ($\Delta t=10^{-6}$s [15]) for the case of CW laser beam with intensity of $I = 3' 10^5 W / cm^2$ and $W_0 = 2mm$ [4] for different initial position, ρ_0, and illustrated in Figure 4. From Figure 4, we can see that with given parameters the polystyrene microsphere be held in the center of tweezers when the distance from its position to the tweezers center is shorter than 4μm (3-blue line in Figure 4). If the bead is located in distance to the tweezers center longer than 4μm (1-cyan and 2-green lines in Figure 4), the trapped bead never moves to the tweezers centers, that means it never be catch by tweezers.

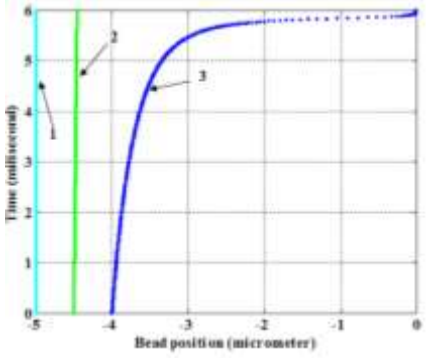

Fig. 4. The dynamics of polystyrene microsphere located at different positions from tweezers centers: ρ_0=-5μm (1-cyan); ρ_0=-4.5μm (2-green) and ρ_0=-4μm (3-blue) simulated for case of CW laser beam with $I = 3' 10^5 W / cm^2$ and $W_0 = 2mm$.

It can be explained by that the polystyrene microsphere is located in place too far from the laser beam axis, where the laser intensity is too small. Thus the optical force acts not on the polystyrene microsphere, which moves randomly under action of Brownian force, only. In the case of $r_0 = 4mm$, the polystyrene microsphere moves to the tweezers center ($r = 0mm$) after the called "pulling" time, $T_p = 6ms$, and due to CW regime it will be stably held here (1-blue line in Figure 5), where the intensity of laser beam is a peak and unchanged (2-red line in Figure 5) [40]. There are the questions, what is the dynamic of the polystyrene microsphere when the CW laser beam replaced by the pulsed laser beam, and what is the dynamics of polystyrene microsphere, i.e., the position-time characteristic on the actions of all forces in the pulsing time of pulses. To investigate the dynamic of the polystyrene microsphere in the pulse regime, now we consider, the laser beam is a Gaussian pulsed beam which has intensity distribution given as Eq.(3) with peak of $I_0 = 3' 10^5 W / cm^2$, duration of $T_0 = 1ms$ (that means the pulsing time is $6T_0 = 6ms$ about the time that the bead needs to move from position $r_0 = 4mm$ to the tweezers center in the CW regime) and the beam waist radius of $W_0 = 2mm$.

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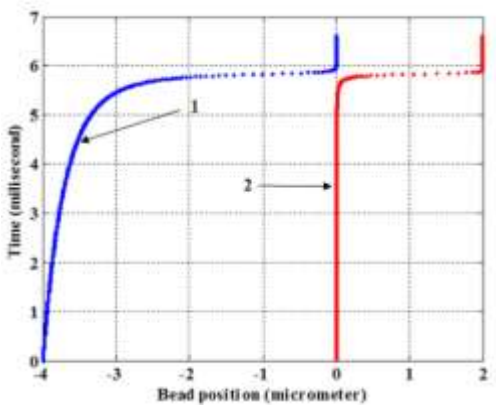
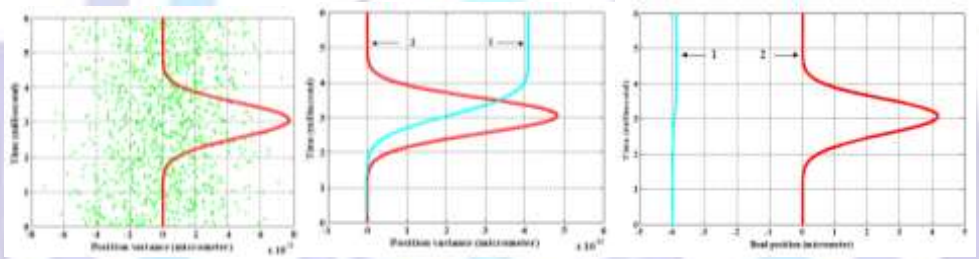


Fig.5.Bead position (ρ) in trapping time (1-cyan) simulated for the case of CW laser beam with $I = 3' 10^5 W / cm^2$ and $W_0 = 2mm$, and profile of laser intensity (au) irradiating on trapped bead (2-red).

The dynamic of polystyrene microsphere under action of Brownian force only, and the time profile of laser pulse (au) are simulated and illustrated in Figure 6(a). It is clear that the Brownian force makes the bead oscillate around the initial position with a variance smaller than 8×10^{-12} μm. Those variances are shorter less than the stable length of λ-phase DNA molecule of $L_b = 89nm$. It means that the Brownian force contributes not on stretching of λ-phase DNA molecules.



(a) **(b)** **(c)**

Fig.6. a) The position variance of polystyrene microsphere in pulsing time under action of Brownian force (dots-green); (b) The position variance of polystyrene microsphere in pulsing time under action of elastic force (1-cyan); (c) Dynamic of polystyrene microsphere (1-cyan) inder action of three forces; and time profile of laser pulse (red); simulated for the case of pulsed laser beam with peak intensity of $I_0 = 3' 10^5 W / cm^2$ and duration of $T_0 = 1ms$, and beam waist of $W_0 = 2mm$.

The position variance of the polystyrene microsphere under the action of the elastic force increases (1-cyan line in Figure 6(b)) with the increasing of laser intensity (2-red line in Figure 6(b)), and reaches a maximum of 4×10^{-13} μm when the laser intensity reaches peak, but it is smaller than that of Brownian.

The dynamic of polystyrene microsphere in the distance from tweezers center of $4mm$ under three forces in pulsing time $6T_0 = 6ms$ is illustrated in Figure 6(c) (1-cyan line). It can see that, the pulsed laser beam with peak intensity of $I_0 = 3' 10^5 W / cm^2$, waist radius of $W_0 = 2mm$ and duration of $T_0 = 1ms$ (2-red line in Figure 5) has an action on the polystyrene microsphere, but the optical force is not large enough to pull the polystyrene microsphere to the tweezers center. In the tail of the pulse, where the pulse intensity reduces to zero, i.e., the optical force decreases, but the position of the bead is seen not changing due to that the elastic force is smaller than that of Brownian.

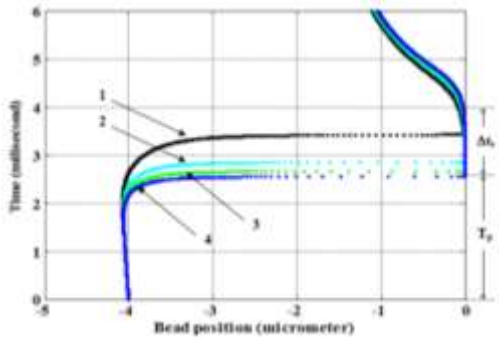
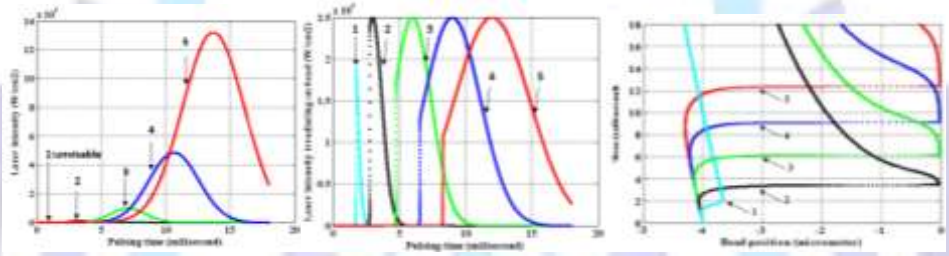


Fig.7. Dynamic of polystyrene microsphere in pulsing time simulated for the case of pulsed laser beam with duration of $T_0 = 1ms$, beam waist of $W_0 = 2mm$, and different peak intensities: $I_0 = 22.5' 10^5 W/cm^2$ (1-black), $I_0 = 50' 10^5 W/cm^2$ (2-cyan), $I_0 = 75' 10^5 W/cm^2$ (3-green), $I_0 = 100' 10^5 W/cm^2$ (4-blue).

Consequently, the re-stabilizing process of λ-phase DNA molecules is very slow. If the peak intensity is increased to a threshold value of $I_0 = 22.5' 10^5 W/cm^2$, the polystyrene microsphere will be in the tweezers center after the "pulling" time of $T_p = 3.5ms$. But it has a tendency to return immediately to the initial position due to elastic force (1-black line in Figure 7). Due to the dependence of elastic force on the extension [17, 37], the returning velocity at tweezers center is larger than that at initial position (see Figure 6 (c)). In this case, the stability time of bead is very short. Simulating with different larger peak intensities of laser pulse (Figure 7), we can see that the stability time, Δt_s increases with the increasing of peak intensity. Moreover, the peak intensity is larger, the polystyrene microsphere reaches the tweezers center earlier that means "pulling" time, T_p shorter. Considering the duration of laser pulse can be changed, then the dynamics of polystyrene microsphere is simulated and illustrated in Figure 8(c). We can see that the pulse duration, T_0 is longer, the stability time, Δt_s is longer, but the "pulling" time, T_p is longer. This phenomenon can be explained by that the temporal distribution of laser intensity (see 5-red line in Figure 8(a)) of the longer pulse is more equilateral in the middie and lower in the head and tail, moreover, the intensity irradiating on the polystyrene microsphere keeps near the threshold value in a cetain time (Figure 8(b)).



(a) **(b)** **(c)**

Fig.8. The temporal distribution of intensity without polystyrene microsphre (a), with pulled polystyrene microsphere (b) and relating dynamics of polystyrene microsphre (c) in tweezers using the pulsed laser beam with peak intensity of $I_0 = 25' 10^5 W/cm^2$, beam waist of $W_0 = 2mm$, and different durations: $T_0 = 0.5ms$ (1-cyan), $T_0 = 1ms$ (2-black), $T_0 = 2ms$ (3-green), $T_0 = 3ms$ (4-blue), and $T_0 = 4ms$ (5-red).

However, if the pulse duration is too short (in our interested case, the pulsing time $T_0 = 0.5ms < 1ms$ - invisible line in Figure 8(a)), the bead reaches not to tweezers center, never (1-cyan line in Figure 8(c)). This process is similar to that shown in Figure 6(c). It is clear that the temporal distribution of laser intensity influences on the "pulling" time and stability time of polystyrene microsphere. This situation is similar to that of the spatial distribution of laser intensity (Figure 9(a)). We can see the different dynamics of polystyrene microsphere simulated for the case of pulsed laser beam with different beam waist illustrated in Figure 9(c). In the case of beam waist radius is about $W_0 = 1mm$ (1-red in Figure 9(c)), due to the polystyrene microsphere is not irradiated by laser beam (1-red point in Figure 9(b)) then it reaches not to center of the tweezers. In other cases, the "pulling" time, T_p is shorter and stability time, Δt_s is longer if the radius of beam waist is longer (2-blue, 3-green, and 4-cyan linses in Figure 9(c)). Thus, to stretch the λ-phage DNA molecule into stretching state, i.e., the contour length is of $L \gg 16mm$, it means to keep stability the linking polystyrene microsphere with radius of $a = 250nm$ embedded in the water at the tweezers center, it is need to use the pulsed laser Gaussian beam with threshold values as: the peak intensity $I_0 > 25' 10^5 W/cm^2$, waist radius $W_0 > 2mm$, and duration $T_0 > 1ms$. That means to trap the polystyrene microsphere into tweezers center, it must use the laser energy of $E = 44' 10^{-4} J$ at least. The "pulling" time and stability time depend not only on the peak intensity but also on the pulse duration and the beam waist radius. To reduce the pulling time and increase the stability time, it must to reduce the duration together to increase the peak intensity and beam waist. But in interested case above, the initial position of polystyrene microsphere is considered to be $r_0 = 4mm$ by external force, so the obtained threshold values together with laser energy are too small in comparison to that when the initial position is the setting-up one, i.e., $r_0 \circ r_{in}$.

ISSN 2347-6893
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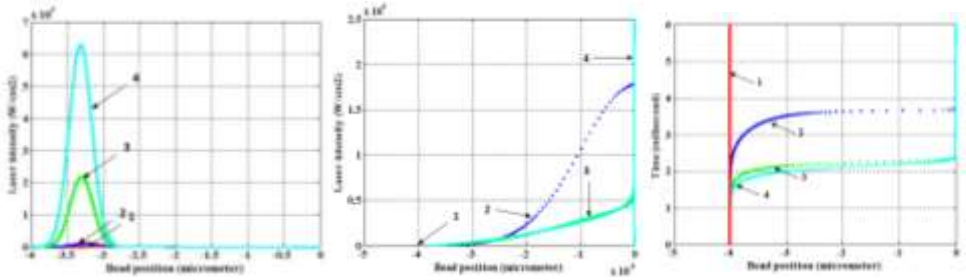

(a) **(b)** **(c)**

Fig.9. The spatial distribution of laser intensity without (a) and with pulled polystyrene microsphere (b) and relating dynamics of polystyrene microsphere (c) in tweezers using the pulsed laser beam with peak intensity of $I_0 = 25' 10^5 W/cm^2$, durations of $T_0 = 1ms$ and different beam waist: $W_0 = 1mm$ (1-red), $W_0 = 2mm$ (2-blue), $W_0 = 3mm$ (3-green), and $W_0 = 4mm$ (4-cyan).

It is clear for example using a CW laser beam with waist of $W_0 = 14mm$, peak intensity of $I_0 = 1' 10^6 W/cm^2$ to pull the polystyrene microsphere linking to λ-phage DNA molecule from position of $r_0 = -14mm$ (» 90% of contour length) to tweezers center $r_0 = 0$. The dynamic of polystyrene microsphere is simulated and illustrated in Figure 10.

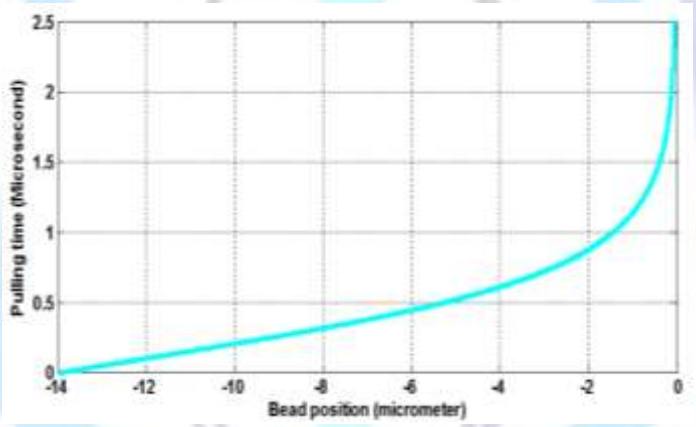

Fig.10. Dynamic of bead placed at $r_0 = -14(» 90\%L)mm$ trapped by CW laser Gaussian beam with beam waist of $W_0 = 14mm$, and peak intensities: $I_0 = 1' 10^6 W/cm^2$.

We can see to pull the polystyrene microsphere to tweezers, i.e., to stretch the λ-phage DNA molecule to stretching state it is need to use a laser energy of $E = 2' 10^{-2} J$. This is too larger for the conditional laser used for optical tweezers. So to reduce the threshold values of pulsed laser beam, the λ-phase DNA molecule with linking polystyrene microsphere should be placed nearier the tweezers center.

4. Conclusion and outlook

Based on the finite different Langevin equation, the dynamics of polystyrene microsphere linking to single l -phage DNA molecule in the optical tweezers using the pulsed laser beam are investigated. The simulated results show that the trapping process charactering by the "pulling" and stability times depends on the initial position of the trapped bead, peak intensity, duration time and beam waist of the pulsed laser beam. For all interested above parameters, there are the threshold values as initial position of $r_0 = -4mm$, peak intensity of $I_0 = 25' 10^5 W/cm^2$, duration time of $T_0 = 1ms$, and beam waist radius of $W_0 = 2mm$ for which the bead is trapped.

This threshold value will be reduced if the optical tweezers is used for other DNA molecules with contour length shorter. However, the threshold values of parameters are relating one to another. To have a collection of parameters for that the bead always is trapped and stability in the tweezers center it must more to investigate the dynamic of the bead with different collection of parameters. Moreover, the certain conditions to trap other DNA molecules with shorter contour length should be investigate in detail. Those questions will be investigated more clearly in the future.

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DOI : 10.24297/jab.v9i2.4005