Measurement of macrophage adhesion by optical tweezers with backward-scattered detection

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ABSTRACT

Macrophages are members of the leukocyte family. Tissue damage causes inflammation and release of vasoactive and chemotactic factors, which trigger a local increase in blood flow and capillary permeability. Then, leukocytes accumulate quickly to the infection site. The leukocyte extravasation process takes place according to a sequence of events that involve tethering, activation by a chemoattractant stimulus, adhesion by integrin binding, and migrating to the infection site. The leukocyte extravasation process reveals that adhesion is an important part of the immune system. Optical tweezers have become a useful tool with broad applications in biology and physics. In force measurement, the trapped bead as a probe usually uses a polystyrene bead of 1 μm diameter to measure adhesive force between the trapped beads and cell by optical tweezers. In this paper, using the ray-optics model calculated trapping stiffness and defined the linear displacement ranges. By the theoretical values of stiffness and linear displacement ranges, this study attempted to obtain a proper trapped particle size in measuring adhesive force. Finally, this work investigates real-time adhesion force measurements between human macrophages and trapped beads coated with lipopolysaccharides using optical tweezers with backscattered detection.

Keywords: optical tweezers, backscattered detection, force measurements, macrophage, lipopolysaccharides.

1. INTRODUCTION

Macrophages are members of the leukocyte family. Leukocytes normally circulate in the blood stream by deforming passively to minimize disturbances to the environment. Tissue damages cause inflammation and release of vasoactive and chemotactic factors, which trigger a local increase in blood flow and capillary permeability. Then, leukocytes accumulate quickly to the infection site. The leukocyte extravasation process takes place according to a sequence of events that involve tethering, activation by a chemoattractant stimulus, adhesion by integrin binding, and migrating to the infection site. To accomplish this, leukocytes must recognize the inflamed endothelium and adhere strongly enough so that they won't be swept away by flowing blood. Macrophages are activated by a variety of stimuli in the course of an immune response, like lipopolysaccharides (LPS). LPS, a major component of the outer membrane of bacteria, acts an endotoxin and induces a strong response from normal animal immune systems. Macrophages excel at phagocytosis that plays an important role in host defense against invading pathogens. The phagocytosis process can be divided into four discrete steps: adhesion, ingestion, fusion with lysosomes, and degradation. The leukocyte extravasation process and macrophage phagocytosis reveal that adhesion is an important part of the immune system.

Optical tweezers have become an important tool to measure forces in biology. The near infrared light is typically used as a laser source to reduce the damage to a cell or cellular organelles and the biological objects can be held and moved by exerting piconewton (pN) forces. The system has many applications such as force measurements of molecular motors or macro-molecules, imaging 3D cavity structures, and studying small variations in local diffusion or viscosity. Optical tweezers perform a wider range of experiments through the integration of a quadrant photodiode (QPD) for position detection. The trapped particle, driven by Brownian motion, is in a harmonic potential built by the optical tweezers. These trapped particle signals from the position detection system are applied to calibrate stiffness of the optical tweezers using a power spectrum method[1, 2]. In the force measurement, adhesive force strength is calculated by multiplying trapping stiffness and trapped bead displacement.

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This paper only considers the force in the lateral direction because force measurement in one dimension is sufficient for most single molecule detaching experiments[3]. Using the ray-optics model[4, 5], this work first calculated optical stiffness of optical tweezers and defined the linear displacement ranges which show the optical tweezers as an optical spring. By the theoretical values of optical stiffness and linear displacement ranges, this study attempted to obtain a proper trapped particle size in measuring adhesive force. Finally, this work investigates real-time adhesion force measurements between human macrophages and micro-sized beads coated with LPS by optical tweezers with backward-scattered detection.

2. EXPERIMENTAL SETUP AND MATERIALS

2.1 Experimental setup

Figure 1 describes the experimental setup of optical tweezers with backward-scattered detection. A fiber laser (1064 nm, YLR-10-1064, IPG Photonics) was used as a light source for the trapping laser. A half-wave plate and a polarizing beam splitter cube were integrated to control the laser power. Changing the telescope (L1, L2) spacing also changes the laser divergence that enters the objective, and the axial location of the laser focus. The experiment steered the laser beam into a modified inverted microscope (Nikon TE2000-U) and the high numerical aperture (NA) oil-immersion objective (100X, N.A. = 1.4, Plan Apo VC, Nikon) yielded a tight focus to trap micro-sized beads. The maximum power of the trapping laser at the back aperture of the objective was 800 mW.

This study also used the probing laser (632.8 nm, He-Ne laser, LGK 7628, LASOS Lasertechnik GmbH) to detect the position of a trapped micro-sized bead. A telescope (L3, L4) with micrometer position adjustment steered the focal spot of the detection beam. The laser passed through a beam splitter and then moved into the microscope via the side port. The high NA objective simultaneously collected the backward-scattered light of the probing laser from the trapped bead, and the light was imaged on a quadrant photodiode (QPD, G6849, Hamamatsu) through the beam splitter. A bright-field illumination was achieved using a halogen lamp through a condenser. The images were taken using a CCD camera with a hot mirror blocking the unwanted laser light. In this experiment, the x-y position of the sample was controlled precisely based on the piezo-electrical controlled sample holder.

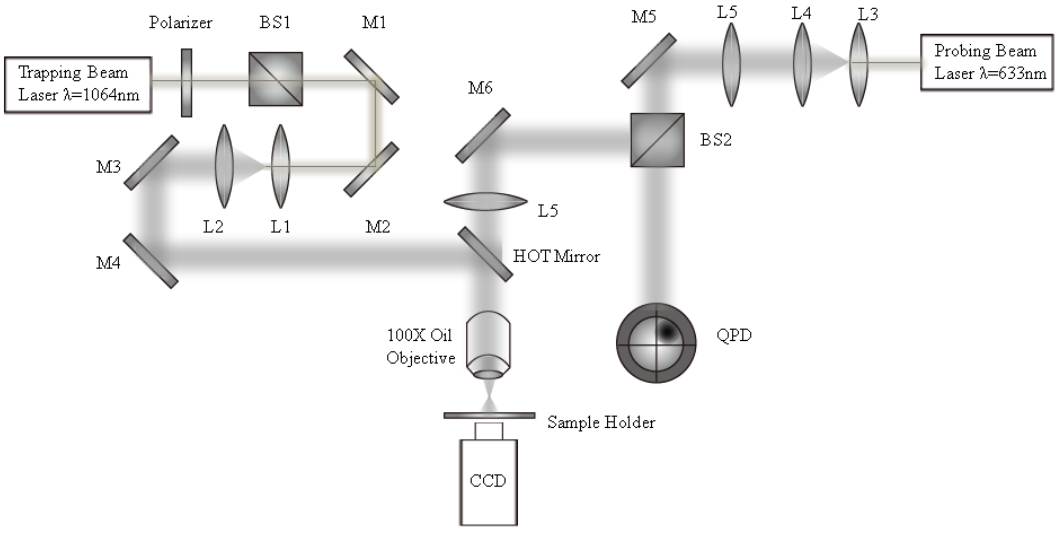

Figure 1. Schematic diagram of the optical tweezers system with backward-scattered detection. Experimental setup: L: lens, M:Mirror, BS: Beam Splitter, PBS: polarization beam splitter cube, QPD: quadrant photodiode, and CCD: charge coupled device camera.

2.2 Force calibration

The experiment used the power spectrum density (PSD) method, based on thermal fluctuations of the trapped bead, to estimate optical stiffness. The Brownian motion of a trapped bead near the center of the optical tweezers in one dimension is well described by the Langevin equation[2]:

$$\gamma \frac{dx(t)}{dt} + k_{OT} x(t) = F(t) \tag{1}$$

where F(t) is the random thermal force, k_{OT} is the optical stiffness, and $\gamma = 6\pi\eta a$ denotes the Stokes drag coefficient for a micro-sized bead of radius, a, moving in a fluid with viscosity η. The PSD of the trapped bead is described by a Lorentzian function:

$$S_x(f) = \frac{k_B T}{\gamma \pi^2 (f_c^2 + f^2)} \tag{2}$$

where k_B is Boltzmann's constant, T is the absolute temperature, and

$$f_c = \frac{k_{OT}}{2\pi\gamma} \tag{3}$$

is the corner frequency. The PSD of the thermal force, *F(t)*, is given by $S_F(f) = 4\gamma k_B T$. The corner frequency divides the Brownian motion into two regions. When $f \gg f_c$, the PSD drops as $1/f^2$, indicating free diffusion. When $f \ll f_c$, the PSD is approximately constant, given by:

$$S_x(0) \approx \frac{k_B T}{\gamma \pi^2 f_c^2} = \frac{4\gamma k_B T}{k_{OT}^2}. \tag{4}$$

The inset of Figure 2 describes the relations between the detector responses and the trapped particle displacements. This inset provides a detector sensitivity of 0.394 V/μm and a linear detection range of the trapped bead of 1.97μm. Figure 2 shows the PSD of a 3μm bead, as shown by the black line. The PSD was fitted to a Lorentzian function, as shown by the gray dashed line. This measurement yielded a corner frequency of 1070 Hz and an optical stiffness of 190 pN/μm.

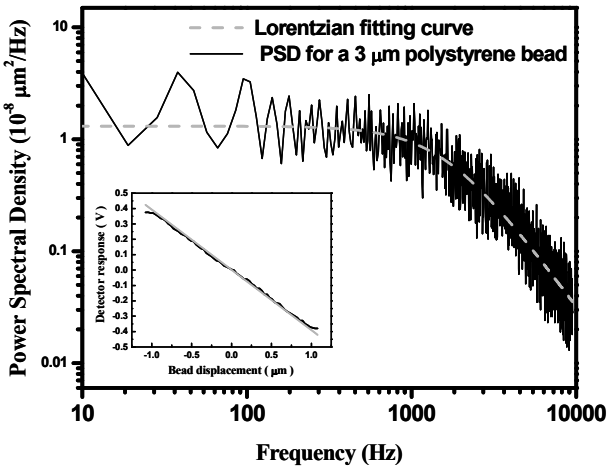

Figure 2. Power spectrum density of the thermal position fluctuations of a 3 μm bead in the laser trap.

2.3 Cell culture

Macrophages (cell line Thp-1, ATCC No. TIB-202, American Type Culture Collection, Rockville, MD) were cultured under standard procedures for use in the adhesion experiments. The cells were maintained in RPMI 1640 medium (Gibco) supplemented with 10% FBS, L-glutamine, and penicillin at 37℃ in 5% CO_2-humidified incubators. The cells were suspended in the cell culture medium at a final concentration of 5×10^5 cells/ ml. Differentiation and adherence were obtained by incubation with phorbol 12-myristate 13-acetate (PMA) (100-160nM, Sigma Aldrich) for 1-3 days[6]. Before experiments, the cells were washed off the culture dish with phosphate-buffered saline (PBS) and maintained in RPMI 1640 medium containing 10% FBS for 30 minutes.

2.4 Preparation of LPS-coated beads

Adsorption of LPS onto polystyrene micro-sized beads was based on an early paper of Ofek et al[7]. Equal volumes of polystyrene micro-sized beads (3 μm diameter, Molecular Probes) were mixed with LPS (200 mg/ml) in PBS. The mixture was incubated for 4 h at room temperature and the micro-sized beads were then blocked with LPS. After blocking, the micro-sized beads were washed and centrifuged at 7,000 rpm for 5 min in a microcentrifuge. The procedure was repeated twice to remove nonadsorbed LPS.

3. THEORETICAL ANALYSIS

According to ray-optics model[4, 5], each incident ray that passes through a micro-sized bead provides two main optical forces $\vec{F}_i$: one is a scattering force, which acts parallel to the incident ray, and the other is a gradient force, which acts perpendicular to the incident ray, as shown in Figure 3(a).

$$\vec{F}_i = \frac{n_s P_i}{c}\{[R_i \sin 2\theta_i - (1-R_i^2)\frac{\sin(2\theta_i - 2\varphi_i) + R_i \sin 2\theta_i}{1+R_i^2+2R_i\cos 2\varphi_i}]\,\hat{y}' + [1+R_i\cos 2\theta_i - (1-R_i^2)\frac{\cos(2\theta_i - 2\varphi_i) + R_i\cos 2\theta_i}{1+R_i^2+2R\cos 2\varphi_i}]\,\hat{z}'\} \quad (5)$$

where c is the velocity of light in vacuum, P_i is the power of ray i, R_i is the reflectance at the interface of ray i, and θ_i and φ_i are the angle of incidence and refraction of ray i. Based on Eq. (5) which is the optical force induced by one ray, we can obtain the total optical force caused by a focusing laser beam using the summing effects of the whole bundle of rays.

Then, we consider a bundle of parallel incident rays propagating through a focusing lens and focused to a point in Figure 3(a). The direction of a single ray I can be described as $(\sin\Theta_i\cos\Phi_i, \sin\Theta_i\sin\Phi_i, \cos\Theta_i)$. Assuming that the center of a bead locates at coordinates (x, y, z), $(x, y, z) = (0,0,0)$ is the focus point of ray i, the distance vector from the center of the bead to the incident ray i, $\vec{d}_i$, can hence be shown as follows:

$$\begin{aligned}\vec{d}_i = {} & [-x(\cos^2\Theta_i + \sin^2\Theta_i + \sin^2\Phi_i) + y(\sin^2\Theta_i\cos\Phi_i\sin\Phi_i) + z(\cos\Theta_i\sin\Theta_i\cos\Phi_i)]\,\hat{x} \\ & + [x(\sin^2\Theta_i\cos\Phi_i\sin\Phi_i) - y(\cos^2\Theta_i\sin^2\Theta_i\cos^2\Phi_i) + z(\cos\Theta_i\sin\Theta_i\sin\Phi_i)]\,\hat{y} \\ & + [x(\cos\Theta_i\sin\Theta_i\cos\Phi_i) + y(\cos\Theta_i\sin\Theta_i\sin\Phi_i) - z\sin^2\Theta_i]\hat{z}.\end{aligned} \quad (6)$$

For every ray, as shown in Figure 3(a), the direction of the ray and the distance vector $\vec{d}$ is along $\hat{x}$, $\hat{y}$ and $\hat{z}$, respectively. Consequently, the total force induced by the focused rays is given by:

$$\vec{F}(x, y, z) = \int_0^{2\pi}\int_0^{\Theta_{Max}} \vec{F}_i[P_i(\Theta_i,\Phi_i), \hat{y}'(\Theta_i,\Phi_i), \hat{z}'(\Theta_i,\Phi_i), \vec{d}_i(\Theta_i,\Phi_i,x,y,z)]\,d\Theta_i\,d\Phi_i \quad (7)$$

where Θ_{Max} is the maximum value of Θ_i, which is limited by the numerical aperture of the focusing lens. Furthermore, optical tweezers are conventionally like an optical spring. According to Eq. (8), the transverse stiffness of the optical spring is given by the first-order approximation of (x, y, z):

$$k_x = k_y = \left.\frac{\partial F(x,y,z)}{\partial x}\right|_{(0,0,0)}. \quad (8)$$

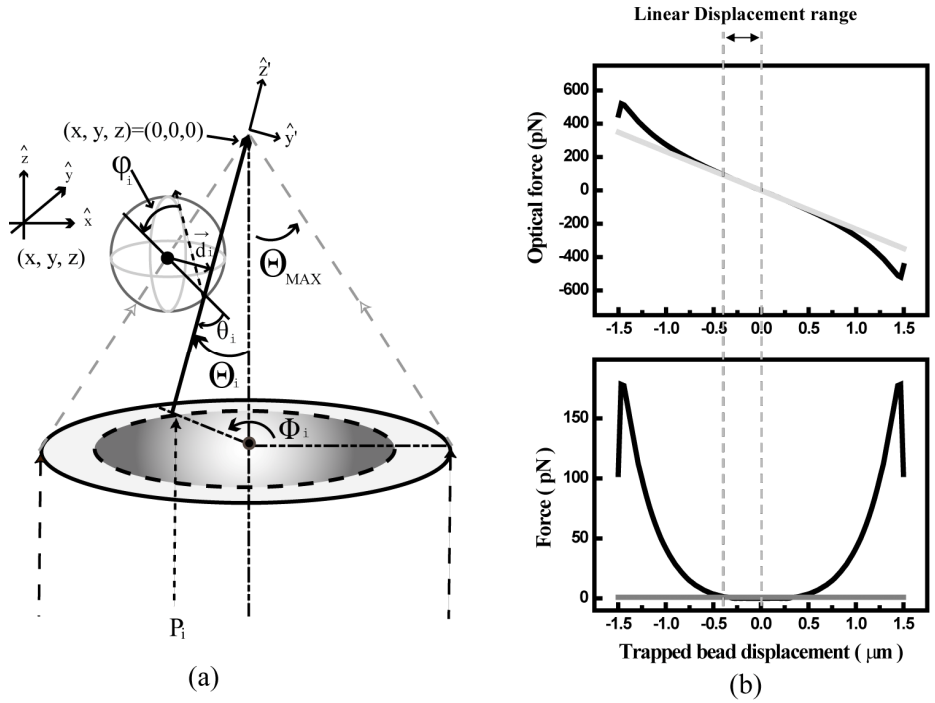

Figure 3. (a) Schematic diagram of a bundle of rays focused at a point. (b) As shown in the top of figure, the x component of the optical force upon the 3 μm bead with a relative displacement in the black line. The gray line describes the fitting line of the optical force. As shown in the bottom of figure, the black curve describes the difference between the optical force and the fitting line, and the dark gray line is one pN.

According to this model, it is valid to calculate the optical force upon a bead around the focus of the focusing laser. We assume that a bead is trapped in water, and that the refractive index of the trapped bead is 1.57. The optical tweezers system is assumed to be composed of a 400 mW laser and an objective of 1.4 N.A., and the intensity of the focusing laser rays in different directions is also assumed to be uniform. Under the same hypothetical condition discussed above, the top of Figure 3(b) presents the x component of the optical force upon the 3 μm bead with a displacement between the focus of the optical tweezers and the center of the bead in the black line. Additionally, the gray line describes the fitting line of the optical force. To acquire trapping force, it is necessary to define a linear displacement range where the optical force is shown as an optical spring and this linear displacement range also equals the maximum displacement of a trapped bead. In the bottom of Figure 3(b), the black curve describes the difference between the optical force and the fitting line and the dark gray line is one pN. Then, we determine the linear displacement range where the difference is smaller than one pN. Hence, the linear displacement range is 0.3 μm. Force measurement calculates trapping force of the optical tweezers by multiplying optical stiffness and trapped bead displacement. Therefore, we obtain the maximum trapping force by the product of the optical stiffness and the linear displacement range of a trapped bead to be 69.7 pN

4. RESULTS AND DISCUSSION

Under the same method discussed above, the maximum trapping forces by multiplying the optical stiffness and the linear displacement range and these forces are almost a constant, 69.7 pN, as shown in figure 4. Therefore, the maximum trapping force does not depend on the trapped bead diameter. In measuring adhesive force, the adhesive force is decided by the product of stiffness and trapped bead displacement. The displacement is described by the ruptured signal that the trapped bead is detached from the sample and returned to its equilibrium position in the trap when the trapping force is larger than the adhesive force. Hence, the trapped bead displacements decrease with bead diameters when an adhesive force is measured by a fixed trapping force due to different trapped beads. The bigger bead as a probe of optical tweezers

can be used to enhance the ruptured signal of the adhesive force measurement when the binding force is weaker. But the bigger bead causes increased adhesive binding numbers because it increases the contact area between the trapped bead and the sample. For increasing the ruptured signal and decreasing the adhesive binding number, this work chose a 3 μm bead to be an optical tweezers probe.

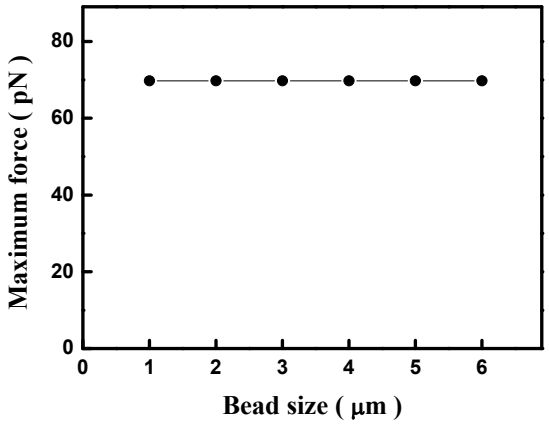

Figure 4. The relationships of the diameters of trapped beads to the maximum trapping forces.

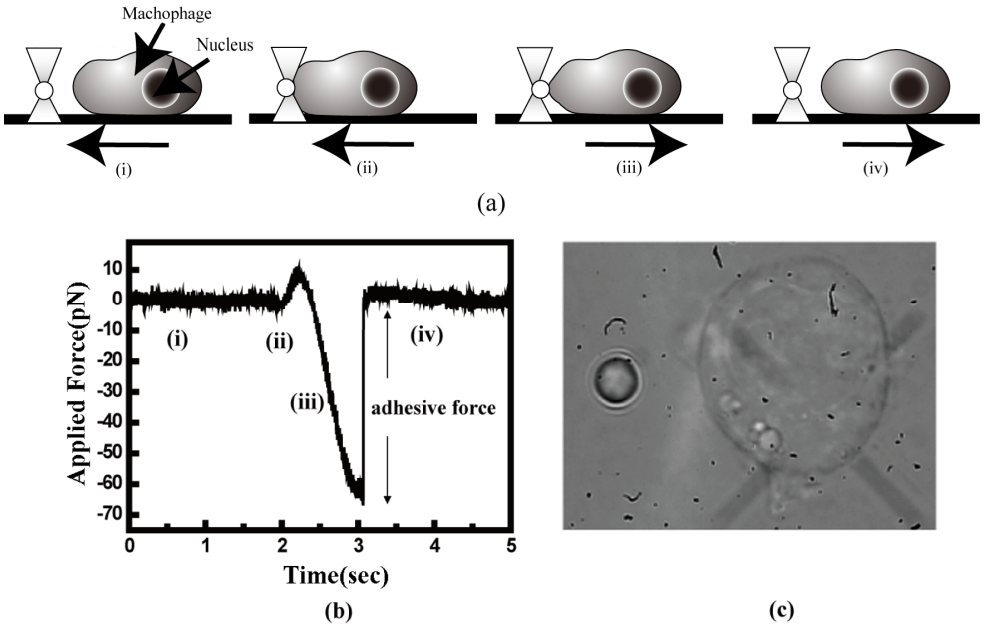

Figure 5. (a) A schematic presentation describing the adhesive force measurement. (b) Force trace of an actual adhesion experiment. (c) An image during an actual adhesion experiment.

Macrophage adhesion with LPS was measured by optical tweezers with backward-scattered detection and the probe of the optical tweezers was a 3 μm polystyrene bead. The adhesive force was calculated by multiplying trapping stiffness of

the optical tweezers and the trapped bead displacement when the bead was detached from the macrophage. As shown in figure 5(a), an immobilized macrophage is moved toward a trapped bead by the computer-controlled stage driven with piezoelectric actuator. Adhesive force is determined by attaching and detaching optically trapped beads to immobilized macrophages. Figure 5(b) describes a typical record of the displacement of the trapped bead during the measurement, and the position of the bead along with time exhibits a triangle-like shape. Therefore, the peak of the triangle indicates when the trapping force is sufficient to detach the bead from the macrophage. The adhesive force is obtained accordingly. Figure 7(c) shows the macrophage and the trapped bead coated with LPS during an actual adhesion experiment. To reduce the formation of multiple bonds, the retraction speed of the stage was adjusted to 1 μm/s and the contact time between attaching and detaching was kept at 0.4 s^3. Using optical tweezers with backward-scattered detection, the mean adhesive force between macrophages and LPS was 65.1 ± 5.3 pN and the mean adhesive force between macrophages and polystyrene beads were 26.0 ± 1.1 pN.

5. CONCLUSION

According to the RO model, optical stiffness decreases with bead diameters and linear displacement ranges are proportional to bead diameters in an optical tweezers system. The maximum trapping forces are almost a constant, 69.7 pN and therefore the maximum trapping force does not depend on the trapped bead diameter. In force measurement, the trapped bead displacements decrease with bead diameters when an adhesive force is measured by a fixed trapping force due to different trapped beads. But the contact area between the trapped bead and the sample may increase the adhesive binding number. For increasing the ruptured signal and decreasing the adhesive binding number, this work chose a 3 μm bead as a probe for optical tweezers. Finally, the adhesive forces between trapped beads coated with LPS and living macrophages were investigated by optical tweezers with backward-scattered detection and the mean adhesive force was 65.1 ± 5.3 pN.

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