Chinese Science Bulletin 2003 Vol. 48 No. 5 503—508

Measurements of leucocyte membrane elasticity based on the optical tweezers

GUO Honglian[1*], CAO Qinhong[3*], REN Dongtao[3], LIU Guoqing[3], DUAN Jianfa[2], LI Zhaolin[1], ZHANG Daozhong[1] & HAN Xuehai[2]

1. Optical Physics Laboratory, Institute of Physics and Center for Condensed Matter Physics, Chinese Academy of Sciences, Beijing 100080, China;
2. State Key Laboratory of Biomacromolecles, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China;
3. State Key Loboratory of Plant Physiology and Biochemistry, College of Biology Sciences, China Agricultural University, Beijing 100094, China

Correspondence should be addressed to Guo Honglian (e-mail: redlotus@aphy.iphy.ac.cn) or Cao Qinhong (e-mail: caoqinhong74@sina.com)

* The first two authors have the same contributions to this paper.

Abstract **A 1-μm in diameter polystyrene bead coated with lectin is trapped by optical tweezers which is formed by a focused laser beam (740 nm). A leucocyte adhered to the bottom of sample cell is chosen to close to the trapped bead. When their stable combination is confirmed, the leucocyte is displaced by moving the sample stage. A "tether" is then formed between the trapped bead and the membrane. The force acting on the tether is measured by a detector equipped on the optical tweezers system. The deformation of the membrane is diverse in different contact conditions. When the contact area is small, the tether is very thin and the force on it is 7.4 pN (10^{-12} N), while the tether is much thicker under a larger contact area and its corresponding force is about 14 pN. It is presumed that the latter is related to the deformation of cytoskeleton.**

Keywords: optical tweezers, elasticity of membrane, tension of membrane, cytoskeleton.

The membrane is a material barrier that isolates the cell from the exterior environment. It also functions as a modulator of cell membrane permeation. Almost all the activity of cell is related to the cell membrane. The mechanics of biological membrane is a very important property distinguished from the chemical one. It represents a characteristic of membrane from the view of its component and assembly. More and more attention has focused on how cell regulates its migration, deformation, exocytosis, endocytosis and respondence to the shear force exerted by blood flow and how the change of the cell membrane components affects the mechanical property. Due to the limitation of method and means, however, the mechanism of the cell membrane has not been well known so far.

The study of mechanical property of leucocyte is not only of basic value to cell rheology but also of clinical significance. The amount of the leucocyte is the least in the blood cell. The study on the leucocyte rheology lags far behind that of the red blood cell and its rheology property has a great effect on micro-cycle. The thrombosis, phagocytosis and immunity are closely related to the state of leucocyte and all these functions are based on the morphological changes of the leucocyte. Compared with the red blood cell, its size is larger and the rigidity is greater, so its large deformability is probably related to the membrane and its cytoskeleton.

The cell membrane is brought about a deformation by an external force. According to the difference of contact area, there are two forms of deformation, one is large area deformation and the other is "point extraction". In the previous work, the large area deformation is widely adopted such as compression of cell with two parallel plates and micropipet aspiration technique[1]. The method of micropipet is still in use owing to its simplicity. The so-called "point extraction" is by vertical motion of the micro-needle connected to the membrane, the glass micro-needle is coated with membrane associated molecules. When the needlepoint is combined with the cell membrane, the membrane is deformed by vertically moving the micro-needle. The bend of micro-needle is correspondent to the deformation extent of cell membrane[2]. Comparably, the former is much general and easy to operate. However, due to its large area contact, it is difficult to know the tension inhomogeneity and the details of membrane deformation. In contrast, using the latter technigue the tension in different regions can be measured and problems caused by membrane plasticity can be overcome in some extent. However, the practical operation and measurement precision limit its application. Optical tweezer is a very useful tool for measurements of membrane tension. It can exert piconewtons to tens of piconewtons force on dielectric object[3,4] and its measurement precision is better than 0.1 pN.

In order to further study the mechanical property of biological membranes, experiments based on optical tweezers system have been carried out since the mid 1990s and the membrane tensions in different kinds of cells have been measured[5], from which the direction of lipid flow in neuron cell and relations between membrane and cytoskeleton were obtained. To develop this technique, we design and set up an optical tweezers system equipped with high-resolution displacement and force detector[6,7] and introduce a method on measurements of membrane elasticity. In this report, taking the process of the membrane tether formation and its force measurements as an example, we present the method in detail and some primary results. It can be seen that the tethers are very different with the different contact areas and times. Consequently, the forces on them are different too. This indi-

© 1994-2012 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

cates that the contacted area is a very important factor and the cytoskeleton may play a great role in the membrane deformation.

1 Materials and methods

(ⅰ) Materials. Lectin, poly-l-lysine, penicillin, streptomycin and BSA are purchased from Sigma, Corp.; ATP (SERVA); fluorescence probe (Pharmacia, Corp.); 1-μm-diameter polystyrene bead (Duke Scientific, Corp.); bovine serum, RPM1-1640 (Hyclone, Corp.); other reagents are of or much higher than analytical purity.

(ⅱ) Compenents of optical tweezers system. The optical tweezers system consists of inverted optical microscope (Leica DMIRB, Germany) with high numerical aperture objective (HCXAPO 100×/NA 1.30), Ti-sapphire laser (3900S, American Spectra Physics Inc.), laser power supply, water-cooled system and three-dimensional piezoelectric driven stage (NIS-70, Israel) with the maximal range of 70 μm and sub-nanometer resolution. All these apparatuses are put on an air cushion vibration-isolated table (Shanghai Mechanical Academy). The image of sample is projected onto the surface of CCD (Charge Coupled Device) camera (CoolSNAP-fx, America), then acquired by an image acquisition board interfaced on a computer. A method of fluid dynamics is adopted in the force calibration[8,9].

(ⅲ) Polystyrene beads coating. Take 50 μL suspension of beads with concentration of 5% and sonicate them for 10 min; take 10 μL (0.1 mg/mL) lectin and mix it with beads; incubate for 2 h at room temperature and oscillate it during this process; wash the bead three times with PBS; resuspend in 200 μL PBS buffer.

(ⅳ) Incubation of leucocytes and wall-adhered process. Leukemia cell lines-Jurkat is brought from Initial Medicine Institute, the Chinese Academy of Medical Sciences. The culture medium is made up of 10% bovine serum, 2 g/L $NaHCO_3$, 100 u/mL penicillin and RPMI-1640 of 100 u/mL streptomycin. The CO_2 incubator is set to be 37℃, 5% CO_2 and saturated humidity. Synchronization of cells must be done before experiments. After generation, cells are incubated overnight then displaced to 4℃ refrigerator for 8 h and then incubated for 16—17 h in 37℃ incubator. The leucocyte is attached to the bottom of sample cell with poly-l-lysine. The process of poly-l-lysine coating of the sample cell is as follows: dip the coverslips into 100% ethanol then air them; Take 20 μL with 5 mg/mL concentration poly-l-lysine onto the surface of coverslip which is put on parafilm; After 2 h, wash them for 20 s with continuously distilled water, it should be noted that the time must be controlled strictly; tilt them on filter paper and dry naturally then put them into 50℃—70℃ oven for 2 h.

(ⅴ) Measurements of elasticity of leucocyte membrane. Take the logarithm prophase leucocytes and coated polystyrene beads with the amount ratio of 2 to 1 to the sample cell and mix them. Put another coverslip onto it and seal it with resin. Place it on the sub-stage of microscope. The coarse adjustment of stage in the x, y direction is done by hand and z direction by the unit of electronic control of microscope, while the nanometer precision adjustment of stage is controlled by a computer. Trap a bead then choose a cell and close to the bead. Wait for a few minutes to ensure a stable combination. The way to determine whether the combination is firm or not is to shut off laser. If the bead escapes from the original position, they are not well combined. Extraction of tether is performed by moving the stage. The displacement of the trapped bead is monitored by a CCD camera.

2 Results and discussion

(ⅰ) Set-up of optical tweezers system and force calibration. Taking the output efficiency of laser and spectrum property of optical components into consideration, we set the operation wavelength of laser at 740 nm. In doing so, we keep the biological objects away from damage-sensitive wavelengths, at the same time, the trap is visible. The schematic diagram of optical tweezers system is shown in Fig. 1. The light from the Ti-sapphire laser is expanded by lenses L1 and L2 and becomes parallel then is reflected into the bottom port of the inverted microscope by mirror M2. Lens L3 and Tube lens are confocal to ensure the parallel beam before entering the objective. The expanded laser beam must be filled with the back aperture of objective to increase the intensity gradient and trapping efficiency.

Force calibration is a precondition for force measurements with optical tweezers and the method of fluid flow is widely adopted in the calibration. In our experiments, the 1-μm polystyrene bead is taken as a standard object for force calibration. When the stage runs at a constant velocity, the viscous force can be calculated from the Stokes' law: $F = 6\pi\eta r v$. Here r is the radius of bead, η the fluid viscosity and v the flow velocity. The displacement of the bead from the center of the trap is measured by standard weighted centroid calculation[7]. Thus, the relationship between the displacements and forces on bead can be obtained, as shown in Fig. 2. The slope of the linear fit gives the trap stiffness k = 0.14 pN/nm. Its linear range is about 200 nm. The output power of the laser is 460 mW and its corresponding escaping force is 30 pN, which represents the maximal force that can be exerted on bead.

(ⅱ) Deformation of leucocyte and force measurements. The suspended leucocytes cannot be deformed with a single laser trap, so they must be attached to the sample cell. In our experiments, the coverslips coated

© 1994-2012 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

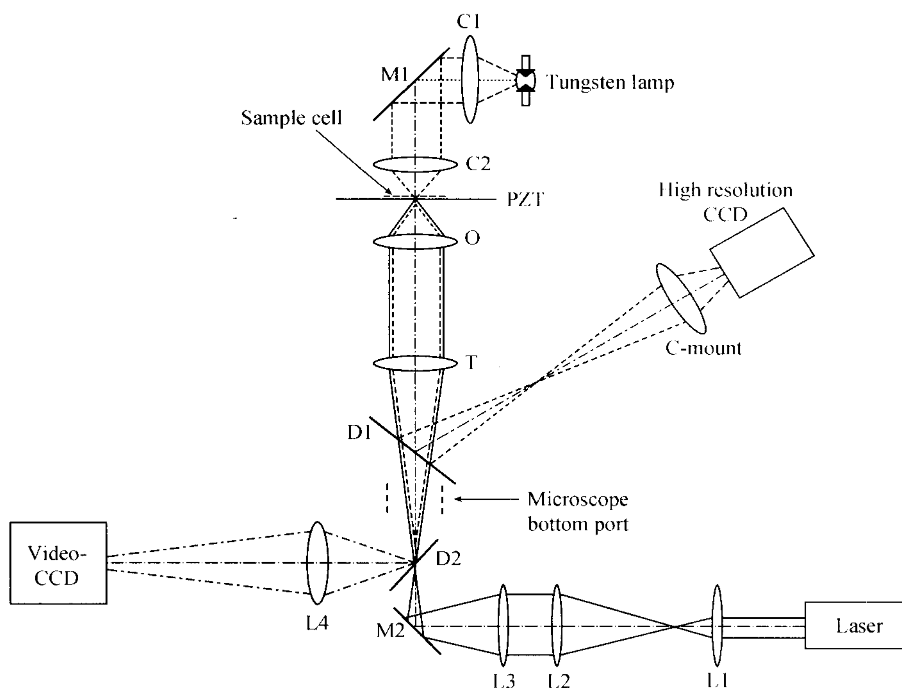

Fig. 1. Schematic diagram of the optical tweezers system. The solid lines represent the laser paths and the dashed lines the illuminating paths. L1, L2: lenses for beam expanding; M1, M2: mirrors for beam steering; L3 and tube lens are confocal; O: microscopy oil-immersion objective (HCXAPO 100 × /1.30); C1, C2: lenses for illumination light condensing; D1 (R: 400—650 nm; $T > 650$ nm), D2(R: 750—1100 nm; $T < 750$ nm): dicroic filter; C-mount: the interface between microscope and CCD camera, its magnification is 0.5; L4: auxiliary lens for imaging; PZT: piezo-electric-transducers used for micro-operation of sample cell and force calibration.

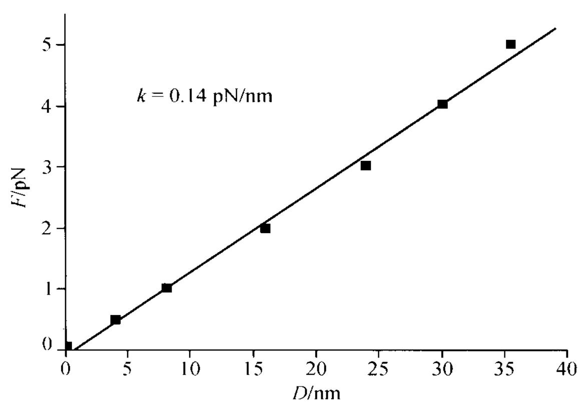

Fig. 2. The dependence of forces on the displacements of the bead.

with poly-l-lysine are used for fixing the leucocyte. The adhesion of bead and the leucocyte is performed as follows. Trap a bead then move the stage to make a leucocyte close to it. Adjust the focal plane of objective to ensure the bead and leucocyte is parfocal. Wait for a few minutes. After the stable combination, drag the cell away from the bead. When the bead is displaced from the cell membrane, a tether is formed between the bead and membrane. Its diameter is very small and close to the resolution limit of microscope. It can be faintly seen in microscope. The force exerted on it can be obtained by measuring the displacement of bead from the trap center. In our system, a video CCD camera is used for tracking the bead movement and another high-resolution cooled CCD camera is for monitoring and acquiring the whole image of the bead and cell.

The image of tether by deformation of cell membrane under an external force and the displacement of the bead are shown in Fig. 3(a) and (b), respectively. In Fig. 3(a), the diameter of the tether is about 0.3 μm and the length is 5.8 μm. In Fig. 3(b), the zero position represents the center of the trap and the fluctuation around zero is the Brownian motion of the trapped bead, SD represents the standard deviation of the fluctuation. When the tether is formed, the force exerted on it can be calculated by the product of bead displacement and the trapping stiffness. Its value is 7.4 pN in Fig. 3, which represents the membrane tension of leucocyte. This value is a little lower than

© 1994-2012 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

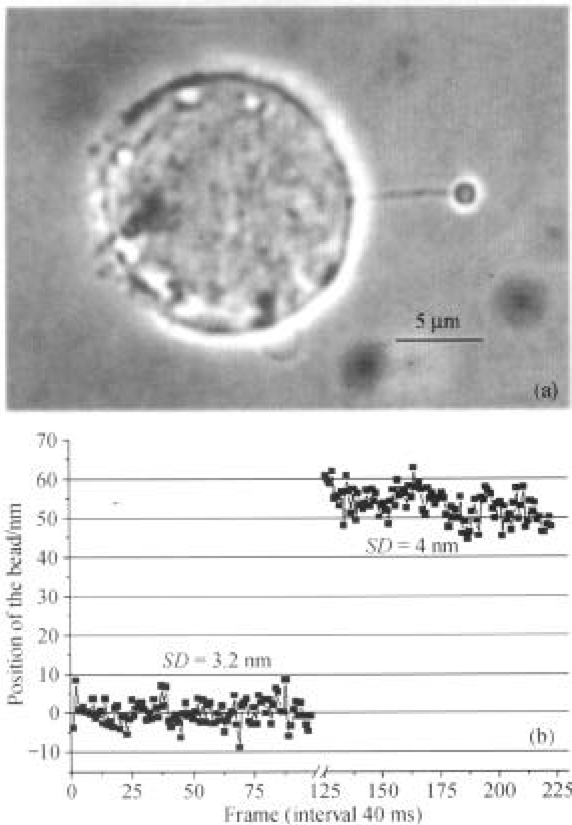

Fig. 3. (a) Picture of the tether formed by membrane deformation; (b) displacement of the bead under the drag of the tether.

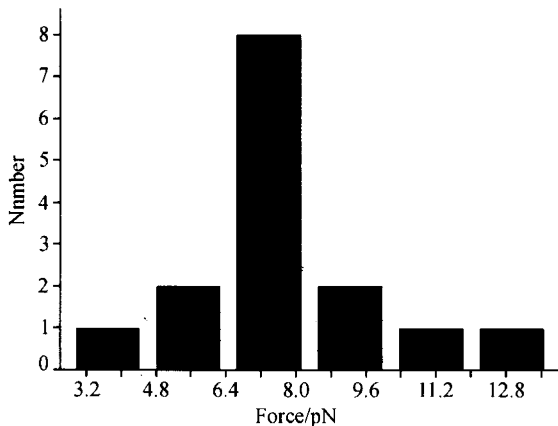

Fig. 4. Force distributions in 15 measurements.

that of MP Sheetz who measured the membrane tension of neural cell and fibroblast cell with the same method as ours, which implies a high deformability of phagocytosis-possessed leucocyte[11]. Because of the large fluctuation of the tether force, we choose tethers similar to those in Fig. 3(a) in diameter and gray scale and analyze the forces statistically. The histogram of the forces is shown in Fig. 4, its range is from 3 to 13 pN, 50% of which is consistent with the result of Fig. 3(b). The lengths of the tethers vary from 5 to 20 μm and the range of diameters is 0.2—0.5 μm. The average force is 7.4 pN in eight measurements and its standard deviation is ± 0.8 pN. In the measurements, we have not found a direct dependence of forces on tether lengths. Due to the synchronization of the cell before experiments, most cells are in state of M, so the results reflect the representative deformation of the leucocyte dragged by the optical trap. However, with the increase of contact area and time and different contact regions, the tethers are diverse and the forces on them are very different. When the tether is thin and transparent, the force on it is small, while the tether is thicker and denser, the force is much larger. However, the force is not so large on such tether as a transparent thick tube. The example is shown in Fig. 5. Fig. 5(a) is a thinner tether than that shown in Fig. 3(a). The diameter increases gradually from the bead to the membrane end. In the bead end the tether is very thin while in the membrane end the tether gets thick and dense, which indicates that the tether in the membrane end contains the solid matters. The force on it is 13 pN. Fig. 5(b) is a thicker and denser tether. Its contact area is about 0.7 μm^2. The tether is more like a tube than a filament. In the end of bead, the diameter of the tether is about 0.6 μm while in the membrane end it is about 1 μm and the tether contains organelles and transport vesicles. The force on it is about 14 pN. When the contact area gets larger or time gets longer, the diameter of tether gets much larger as shown in Fig. 5(c). The inclusion in the tether increases and the force on it is 19 pN. In Fig. 5(d), the contact area is similar to that in Fig. 5(c). However, the tether appears transparent which indicates that high density materials and vesicles do not exist in the tether. Its corresponding force is 3.8 pN, which proves that there is no cytoskeleton in this tether. Obviously, the deformation of the membrane is diverse which provides a very good experiment model for study on the mechanical property of the membrane.

The up-to-date study on the membrane structure indicates that fluid-mosaic model put forward in 1972 has to be modified. The two dimensional homogeneous membrane model can not give reasons for the motion of membrane protein in a local area, fusion of cells, cell deformation and directional movement, and so on. What is the material foundation and regulation of the tether formation under the point extraction? Hochmuch et al. extracted tethers on cytoskeleton-removed red blood cell and liposome made of pure phospholipid using micro-needle[12], which proved that the tether is a phospholipid bilayer tube derived from the deformation of phospholipid layer. It can be formed by extracting the phospholipid bilayer or a

 © 1994-2012 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

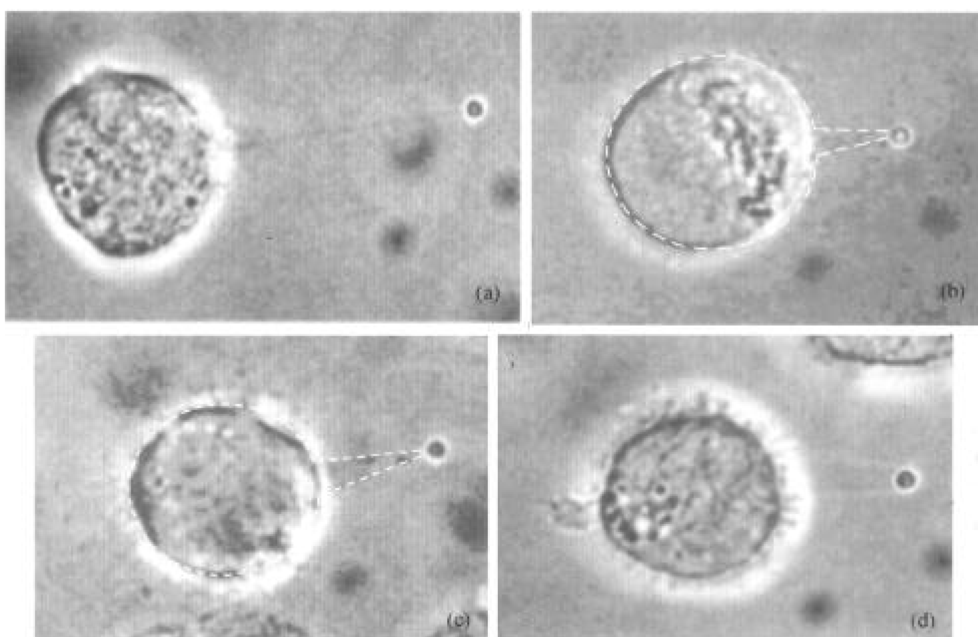

Fig. 5. (a) Image of a thin and long tether; (b), (c) images of thicker and denser tethers. In order to distinguish them, the dashed lines are used to draw the outlines of the membranes and the tethers; (d) image of a thick but transparent tether.

membrane protein. The result in Fig. 3 is similar to this. However, this model cannot give a reasonable explanation for a cytoskeleton-involved tether. The discovery of the cytoskeleton is the material basement behind that phenomenon. In our results, it can be seen that the cytoskeleton may be involved in the membrane deformation, whenever diameter, density, inclusion of tether or force changes. So the possibility of the membrane deformation is shown in Fig. 6. Kusumi's research shows that the motion of the membrane proteins are localized according to single molecular tracking and suggests that the local area motion is related to the cytoskeleton anchored on the membrane. Many proteins anchored on the membrane have been found (such as band 3). So if the extraction is from such proteins the tether will contain the cytoskeleton. Dai et al. proved it by comparing the forces on tether created from normal membranes and swollen ones[13], they found that the endocytosis and exocytosis are inversely proportional to the tether force which indicates that the membrane fusion resulting from membrane deformation needs weak membrane tension and elasticity. This fact shows that the membrane functions are closely related to the mechanical property of the membrane.

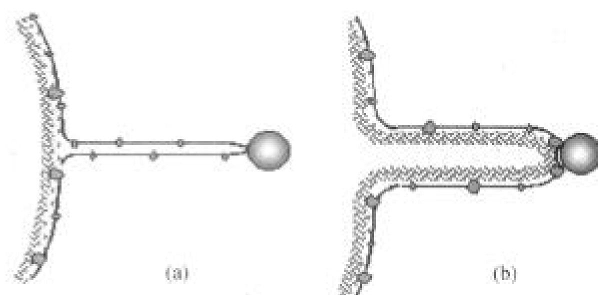

Fig. 6. Schematic diagram of tether with and without cytoskeleton. (a) Without cytoskeleton; (b) with cytoskeleton.

Due to the advantage of non-contact and non-invasion of the optical tweezer and its nanometer and piconewton's resolution, the tweezer becomes a very powerful tool that can never be substituted by other techniques. By adjusting the output power of the laser, the range of trapping forces changes according to different requirements. In the application of biological membrane, the inhomogeneity of membrane and effect of the cytoskeleton can be studied by changing the contact area and time. The preliminary results in this report indicate that the cell membrane elasticity is closely related to the membrane proteins and cytoskeletons. This implies that we can take

© 1994-2012 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

the mechanical property of membrane as a criterion for identification of normal and pathological cells and pathological cells before and after treatment, which is of great significance for disease diagnosis and drug screening.

Acknowledgements The authors would like to thank Prof. Fuyu Yang for his suggestion and comments, and Profs. Ming Yuan and Bingying Cheng for their advice and help. The help of Zhuncai Chen is also very valuable. This work was supported by the National Natural Science Foundation of China (Grant Nos. 1989380 and 10174097).

References

1. Evan, E. A., New membrane concept applied to the analysis of fluid shear- and micropipette-deformed red blood cells, Biophys. J., 1973, 13: 941—954.
2. Yasuda, K., Shindo, Y., Ishiwata, S., Synchronous behavior of spontaneous oscillations of sarcomeres in skeletal myofibrils under isotonic conditions, Biophys. J., 1996, 70: 1823—1829.
3. Ashkin, A., Dziedzic, J. M., Optical trapping and manipulation of viruses and bacteria, Science, 1987, 235: 1517—1520.
4. Ashkin, A., Dziedzic, J. M., Yamana, T., Optical trapping and manipulation of single cells using infrared laser beams, Nature, 1987, 330: 769—771.
5. Sheetz, M. P., Laser Tweezers in Cell Biology, Introduction, Methods Cell Biology, 1998, 55: xi—xii.
6. Yao, X. C., Li, Z. L., Chen, B. Y. et al., Effects of spherical aberration introduction by water solution on trapping force, Chinese Physics, 2000, 9: 824—826.
7. Guo, H. L., Yao, X. C., Li, Z. L. et al., Measurements of the displacement and trapping force on micron-sized particles in optical tweezers system, 2002, 45: 919—925.
8. Sleep, J., Wilson, D., Simmons, R. et al., Elasticity of the red cell membrane and its relation to hemolytic disorders: An optical tweezers study, Biophys. J., 1999, 77: 3085—3095.
9. Simmons, R. M., Finer, J. T., Chu, S. et al., Quantitative measurements of force and displacement using an optical trap, Biophys. J., 1996, 70: 1813—1822.
10. Zhang, J. B., Applied Method and Technology in Cell Biology, Beijing: Beijing Medical University and Peking Union Medical College United Press, 1995, 273—275.
11. Schmid Schonbein, G. W., Leukocyte biophysics, An invited review, Cell Biophys., 1990, 17: 107—135.
12. Hochmuth, R. M., Shao, J., Dai, J. et al., Deformation and flow of membrane into tethers extracted from neuronal growth cones, Biophys. J., 1996, 70: 358—369.
13. Dai, J., Sheetz, M. P., Mechanical properties of neuronal growth cone membrane by tether formation with laser optical tweezers, Biophys. J., 1995, 68: 988—996.

(Received October 15, 2002)

Chinese Science Bulletin 2003 Vol. 48 No.5 508—510

Chaotic system for the detection of periodic signals under the background of strong noise

LI Yue[1] & YANG Baojun[2]

1. Department of Information Engineering, Jilin University, Changchun 130012, China;
2. Department of Geophysics, Jilin University, Changchun 130026, China

Correspondence should be addressed to Li Yue (e-mail: liyue84@21cn.com)

Abstract **We propose a method to study the chaotic system for the detection of periodic signals in the presence of strong background noise. The numerical experiments indicate that the chaotic system constructed from the modified Duffing-Holmes equation is sensitive to the weak periodic signal mixed with noise, and it has certain immunity to noise. The signal to noise ratio for the system can reach to about –91 dB.**

Keywords: chaotic system, weak periodic signal, detection, signal to noise ratio (SNR).

The periodic signal detection under the strong background noise is one of the basic issues in the fields of signal detection and signal processing, and it is extensively utilized in the fields, such as the information inception in communication engineering, radar information detection, the electronic antagonistic technology, the biomedicine signal processing, the long-distance detection of earthquake signals, and the industrial broken-down diagnosis. The study of the weak signal detection began in the 1950s, and the techniques have been developed both in time domain and frequency domain[1]. As for the methods of time domain, Birx proposed to apply the chaotic theory to the weak signal detection in 1992[2], but the author just showed the experimental results and did not investigate the principle behind. Over the past ten years, there have been very few reports about detection of weak signals using the chaotic system. It was Abarbanel et al. who pointed out that the nature of the system should be considered in which the signal to be detected is to calculate Lyapunov exponents[3], and the effects of the weak signal detection is relative to the amplitudes of the noise[4]. Researchers in China have achieved some results in the chaotic system detection of harmonic and square waves under the background of strong noise[5—7], and the SNR reached to about –60 dB. In this study we report the successful examples using the chaotic system, which is constructed from the modified Duffing-Holmes equation to detect the periodic signal under the background of strong noise.

 © 1994-2012 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net