Progress in phototaxis mechanism research and micromanipulation techniques of algae cells*

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Abstract Phototactic movement is a characteristic of some microorganisms' response to light environment. Most of the algae have dramatically phototactic responses, underlying the complicated biological, physical and photochemical mechanisms are involved. With the development of the micro/nano and sensor techniques, great progress has been made in the research of the algae phototaxis. This review article summarizes the progress made in the research on the functional phototactic structures, the mechanisms of photo-response process and photodynamics of phototaxis in algae, and describes the latest developed micro-tracking technique and micromanipulation technique. Moreover, based on our own research results, the potential correlation between the phototaxis and photosynthesis is discussed, and the directions for future research of the phototactic mechanism are proposed.

Keywords: **phototaxis, algae, photosynthesis, tracking, micromanipulation.**

Living organisms utilize light in two ways: one is as the source of energy; the other is as a way to obtain the environment information[1]. Photosynthetic microorganisms get both abilities: obtaining light energy as well as using light for the sensory function. This is well incarnated in microorganisms' phototactic movement. Phototaxis is used to characterize the organisms' oriented movement to light, which could be toward (positive phototaxis) or away (negative phototaxis) from light. It has been accepted that phototaxis makes photosynthetic microorganisms seek the best light environment for growth and metabolism under specific physical and chemical conditions[2—4].

Recently, some progress has been made in research of photochemistry mechanism of the phototaxis, but a final conclusion has not been drawn yet due to the limitation of research methods and tools. Due to the light signals control the microorganisms' phototactic movement, the energy and signal transduction process is a research focus. This review article summarizes the progress made in the research on the functional phototactic structures, the mechanisms of photo-response process and photodynamics of phototaxis in algae, and describes the latest developed micro-tracking technique and micromanipulation technique. And, the directions for future research of the phototactic mechanism and its application prospects are proposed.

1 Functional structure for the phototaxis

Most algae have phototactic behaviors, and they have photoreceptor to receive light and actuator to execute the phototactic movement. Taking the unicellular biflagellate *Chlamydomonas* as an example, they have a light sensing organism—eyespot located on one foreside of the cell. The eyespot takes up about one percent of the cell surface and is about 0.65 μm cross[5]. Two flagella are distributed asymmetrically to the eyespot at alga's anterior end. The flagellar motility in *Chlamydomonas* is subject to three types of regulation: phototaxis, photoshock, and quiescence (Fig. 1). However, instead of swimming on straight lines, the alga rotates counterclockwise around its longitudinal axis and thus pursues a helical path[7—9], so that the photoreceptor in the eyespot could receive light continually and the cell could stay right orientation to light by adjusting the heading direction simultaneously.

2 Molecular mechanism for the phototaxis

2.1 Related functional molecules

The photoreceptors are embedded in the plasma membrane of the algae[10]. In the unicellular biflagel-

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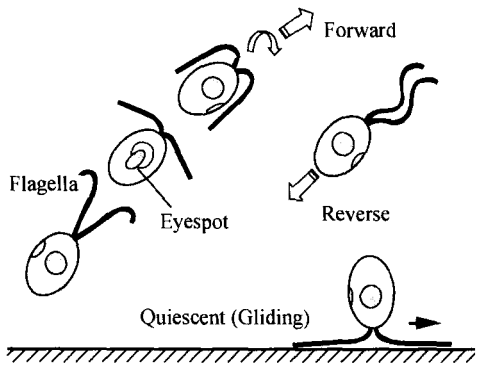

Fig. 1. Three types of *Chlamydomonas* behaviors are corresponded to different physiologically regulated flagellar motilities[6].

late *Chlamydomonas*, the photoreceptors in the eyespot are rhodopsins[11], which are light-sense organisms in phototactic movement. The rhodopsins trigger ion current across the membrane in several milliseconds when the light is absorbed by the cell[12]. Then, how does the eyespot work as a light receptor and affect the phototactic movement? In 1991, Harz et al. presented that the rhodopsins proteins in the eyespot mediate the Ca^{2+} concentration in cellular membrane when the alga cell is in phototactic movement[13]. The plasma membrane of flagella depolarizes by changing the Ca^{2+} concentration in cellular membrane of the flagella, then the flagella beat and produce impetus. Meanwhile, the Ca^{2+} concentration mediates the flagella behavioral styles[14,15]. It could be supposed that the communication within these parts is likely realized by diffusing of the chemical messengers such as Ca^{2+} and cAMP[16]. In Holland's experiment, the change of the photocurrents induced by the rhodopsins was recorded to find the direct link between the two; moreover a simple model for the light transmission process in green alga was established[17]. Whereas, it was not the symbol of signal transduction and transmission at the molecular level but only the macroscopic measurement of the current.

2.2 Genetic research on the phototaxis

Molecular biologists always focus many attentions upon the *Chlamydomonas*. Now, many laboratories in the world have developed the *Chlamydomonas* experimental system[18]. Being isolated easily, the *Chlamydomonas* mutants are favorable in genetic research. For example, the molecular map of the *Chlamydomonas reinhardtii* nuclear genome has been developed successfully[19].

Experiments on eyespot-defective algae mutants indicated that there are three phenotypes: eyeless strains, strains with little eyespots and strains with multi-eyed mutant strains. The phototactic responses of the eyeless strains are obviously decreased, but not disappeared[5]. Furthermore, the *Chlamydomonas* mutant displaying phototaxis but not photophobic has been isolated[20].

With more attempts to research, identification of the rhodopsins genes encoding for photoreceptor pigments of algae is required imminently to obtain the photoreceptor proteins by cloning these genes and their expression. Recently, the genes encoding two distinct rhodopsins CSRA and CSRB have been identified in the *Chlamydomonas* genome. CSRA senses stronger light and has a maximal absorption peak around 510 nm. CSRA senses weaker light and has a maximal absorption peak near 470 nm. Rhodopsin CSRA generates a faster photocurrent than rhodopsin CSRB, and just these currents mediate the phototactic behaviors[21,22]. The findings of the complete structures of the two rhodopsins push forward the research on work mechanism of the photoreceptor. Furthermore, the research of Govorunova et al.[23] indicated that rhodopsins proteins CSRA and CSRB exhibit distinct light saturations and spectrum properties, and give different contributions to the phototaxis.

2.3 Structures of the functional proteins

The structure of the *Chlamydomonas* flagella is composed of nine cylindric doublet microtubules and two single microtubules—the central pair. The bending motion of flagella depends on localized sliding between pairs of doublet microtubules generated by multiple dynein ATPase isoforms attached to each doublet. Up to know, the *Chlamydomonas* flagellar dyneins are the most well characterized proteins from the structural and functional aspects[6]. More than 40 genes related to the assembly and function of the flagella have been cloned and sequenced successfully[24]. Research indicated that the activation of the flagella microtubules has a direct link to the Ca^{2+}-binding protein Fa1p. The signal transduction between the basal body and flagella can not be completed, if the mutant inactivate to the Ca^{2+}[25].

The molecular research on the alga cells has got many outstanding achievements, such as confirmation of the crystal structure for the functional proteins, molecular manipulation from genetic standpoint, re-

search on gene defective mutants, signal transduction channels and each relative intermediates in transfer sequence, and molecular mechanism of motion drive. Whereas research on the molecular mechanism of phototaxis is far from enough to answer such questions as how the light signals transduce to the intercellular current, and how the current transmits step by step and ultimately lead to the flagella motility. The free radical transduction processes are always accompanied by the current transduction in biological system. So, the transient paramagnetic resonance spectrometry for the free radical intermediate may be an effective and direct way to research the current transduction.

3 Phototactic mechanisms of the algae

The mechanism research of the algae's phototactic movement can be traced back to the early 20th Century. The problem how the cells respond to the light stimuli has not been figured out completely for the past century. Taking *Chlamydomonas* as an example, it is known that the alga could orientate to the light by its flagella beating. However, there still remains many problems unresolved, one of which is how the cell receives the light energy and transfers them to the signals that used to mediate the flagellar movement.

3.1 Phototactic photo-response properties of the algae

Past research showed that the algae only response to a certain range of light due to the eyespot's reflection and interference effects to the light[10]. Different algae give different photo-responses under the light stimuli with different types and wavelengths[26]. By analyzing the experimental data of some research groups, it has been found that the unicellular green algae's phototactic responses are strongest when the action spectrum is between 460 nm and 560 nm[27]. Moreover, the positive phototactic movement of a cyanobacterium, *Phormidium uncinatum*, is maximally sensitive at 390, 480 and 560 nm, and the positive phototactic movement of another cyanobacterium, *Cylindrospermum alatosporum*, has peak spectral sensitivity at 450 nm and 640 nm[28]. To explain these phenomena, Wing-On Ng et al. analyzed the effects of intensity and wavelength of the light stimuli on the phototactic behavior, and their study suggested that there were at least two light input pathways regulating the phototaxis. Moreover the action spectrum and fluent rate-response properties were described[29].

Algae need light to complete photosynthesis process, what relationships between the photosynthesis and the light-receiving and signal transmission in phototaxis are still being researched. Grossman et al.[30] found that changing wavelengths of light would take effects on the biosynthesis of the phycobilisomes (PBS), which dominant light harvesting complexes of Cyanobacteria. Furthermore, Cyanobacteria tune light harvesting and photosynthetic function to both light intensity and nutrient availability, and integrate two responses together.

It is advantageous to analyze the kinetic properties of phototactic algae by establishing the relationship between the exciting light and the flagella's beating rule using macroscopic kinetic parameters. In order to obtain the relation between the exciting light and the flagella's moving rules, some researchers[31] held the alga cell to stay at the end of the micropipette with long-term near-infrared light monitoring the cell, and got the light-flagella beating curves. By long-term observing the flagellum beating of the *Chlamydomonas reinhardtii* cell, flagella responses including the beating frequency, stroke velocity, and stroke duration of each flagellum were measured, and the changes in the pattern of the effective and recovery strokes of each cilium associated with negative phototaxis were demonstrated[32]. Now, relative studies are still developed under the given light pattern and wavelength, and the general equation between the light and the response has not been confirmed.

3.2 Photodynamic characteristics of the algae

Phototaxis mechanisms involve complicated biological, physical and photochemical processes. Corresponding to the phototactic orientation process of the unicellular algae, Schaller's model[33] showed the alga could keep tracking the light once it reaches right phototactic orientation by regulating the internal Ca^{2+} concentration gradually to approach its normal resting level. Furthermore, the conclusion that the variety of the Ca^{2+} concentration in flagella mediates the switches between positive and negative phototaxis was proved in his model. Probability and statistical methodologies are often used to process the great quantities of experiments data in dynamics research. For example, Hill et al.[34] developed a "Biased Random Walk Model" by analyzing the trajectories of the

Chlamydomonas nivalis influenced by the gravitaxis and phototaxis. The model leads to Fokker-Planck Equation for the probability distribution function of the orientation of the cells, and uses macroscopic parameters to describe the microorganisms' movement. Moreover, Hill et al.[35] developed another model for light absorption by simulating the process that the photoreceptor was shaded by cells self-rotating. This model described how the photoreceptor molecules in *Euglena gracilis* cells receive the light signals when exposed to polarized light, and proved the control strategies for negative phototaxis. With the development of the computer science, computer simulation methodology is added to data analysis and modeling[36].

4 Tracking and micromanipulation for motile cells

It is necessary to use micro-vision technique for observing and tracking microorganisms in the study of phototactic movement. Due to the development of tracking technique, continuous observation can be performed while the natural cells swimming freely, without fixing them or slowing them down. For instance, Thar et al.[37] developed a system which allowed simultaneous 3-D tracking of several free-swimming microorganisms with diameters of >10 μm using an infrared diode laser and two CCD cameras. The relationship between the field depth and illumination efficiency to system resolution were also analyzed. Debeir et al.[38] proposed a combination of mean-shift-based tracking processes to establish moving cell trajectories through phase-contrast video microscopy. The influence of cell morphologies, dynamical changes and image resolution on tracking was also discussed. Action et al.[39] detected and tracked rolling leukocytes using gradient and a radial active model, and the accuracy rate reached 87%. The continuous observation, simple positioning, locating and recording of cells were achieved in these studies, but the cells can not be pursued and kept in the center of vision field. Moreover, there have not enough workspaces and high magnification in the systems. Ogawa et al.[40] established a novel automatic real-time tracking system for continuous evaluation of freely swimming *Paramecium* cells. The stage where the chamber put on was controlled by a high-speed vision system so as to keep the cell in the center of view field and assure enough magnification and good tracking performance. But the tracking system was only at the single-cell level.

Micromanipulation for cells is closely associated with tracking techniques. A common closed-loop control system composed of them should give attention to complexity, sensitivity, precision and efficiency of the interaction between controller and objects. The research on non-contact control and manipulation of microorganism is one of the hotspots in micro/nano manipulation techniques and is an inevitable trend. The manipulation methods for micro particles or cells based on electromagnetic effect have great advantages in various non-contact control types. For instance, Hosu et al.[41] took the convenient intracellular manipulation using a versatile magnetic tweezer and an inverted microscope. Hakho et al.[42] constructed a biological micromanipulation system with microelectromagnets, a ring trap, and a matrix. A ring trap was a circular conducting wire topped with an insulating layer, whereas a matrix consists of two arrays of wires, aligned perpendicular to each other that are separated and covered with insulating layers. The motion of magnetic particles and biological systems in a fluid could be controlled by local magnetic field profiles on micrometer length scales.

As a new way for non-contact control, optical micromanipulation can offer positional precision down to the angstrom level. Using this method, cells tracking and manipulation for DNA, biological macromolecules and molecular motors can be performed. These gave us insight into colloidal dynamics, Brownian motion, atomic systems, and even superconductivity. For example, in a mimic environment of the tissues, cells were tracked and manipulated by means of diffractive optical elements. Meanwhile, the position and intensity of laser beam were also controlled[43]. Bing et al.[44] developed a microscope integrated vertical cavity surface emitting laser (VCSEL) array trapping system, which was capable of independent control, rotation, and batch processing of biological cells. In this tracking system, multiple cells could be processed parallel, and the limitation of complex and uncertain objects in conventional optical tweezers was broken through. Because the laser micromanipulation may burn the cells, the control methods which are undamaged to biological organ based on its physiological properties become the focus now. In Darnton's research[45], bacteria swarming to liquid beads generated both translation and rotation. In that way, "auto-mobile chips" were assem-

bled. Weibel et al.[46] presented a method for harnessing the power produced by intracellular biological motors. This method used surface chemistry to attach polystyrene beads to algae cells, and the swimming cells were steered by phototaxis. These motile microorganisms can transport microscale loads (3-μm-diameter beads) at velocities about 100—200 μm/sec and over distances as large as 20 cm. Galvanotaxis also can be used to control microorganisms[40]. These methods provide innovative ideas for research on micromanipulation.

We recently constructed a high-speed micro vision tracking system and phototactic control system (Fig.2) for this kind of research. The unicellular biflagellated alga, *Chlamydomonas reinhardtii*, is the observing sample for its sensitive phototaxis. Cells' swimming in a chamber (50 mm × 50 mm, 100 μm depth) is approximately considered as moving in a 2-D plane. The images of swimming cells are captured by CCD cameras and image capture board. The shape, motion and control parameters of cells are calculated by the image processing system. When the cell is swimming away from the center of vision field of microscope, the computer sends instructions to DSP controller immediately. Then, the X-Y precise stage together with the chamber moves in real time under the control of DSP. Thus, the cell is always near the center of vision field. When the swimming cells need to be controlled by phototaxis, the computer sends instructions to DSP to modulate exterior light stimuli circuits. Moreover the cells are guided by regulating the position and intensity of light source. To analyze the relationship between phototaxis and photosynthesis in algae cells, the light pattern and intensity can be changed. When the photosynthesis pigments and phototaxis receptors are stimulated separately, their effects on phototactic movement are displayed.

We have succeeded in photo functions matching and structure motilities of cyanobacterium photosynthetic machine, and found that the light-induced transition between the state 1 and state 2 depends completely on a directional movement of PBSs towards photosystem I (PSI) or photosystem II (PSII). The "Parallel Connection, Limited Movement" model for structure and energy matching between the PBSs and photosystems has been developed by us, indicated that the energy distribution between the two photosystems in balance is regulated by dissociation of PSI trimers into the monomers under a case from light to dark, while re-aggregation of PSI monomers into the trimers will occur under a case from dark to light. This explains the state transition model of cyanobacterium clearly, which has been argued for many years.

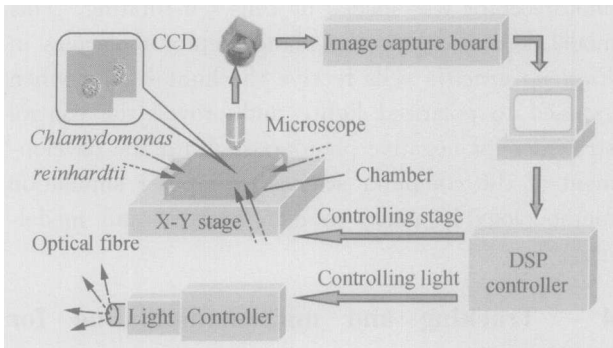

Fig. 2. System configuration of alga cell tracking and phototaxis controlling.

5 Conclusion and research prospects

So far, the research on cell phototactic mechanisms has focused on exploring the connection between the light stimuli and macroscopic movement characteristics of the algae[47,48]. Till now, none quantitative light-response model has been built, only qualitative analyses were made. Initial conclusion drawn from past research is that the movements of motors in flagella are regulated by rhodopsins photoreceptors when the cell is in phototactic movement, and some relationships have been found[49,50], which add more theoretical supports to the research.

Future research prospects are:

(1) Phototactic mechanisms of the algae

The phototactic behavior is due to alga's physical need for light, and as a photosynthetic organism, this need may have a direct connection with the need for photosynthesis. To improve the photosynthetic efficiency, the first thing is getting enough light intensity, and then the precondition is a balanced distribution of the excitation energy from the light-harvesting systems to PSI and PSII. Seeking the connections between the phototactic parameters and photosynthetic parameters (the fluorescence fluctuation characteristics of different photosynthetic machine) under different state (dark or leaning to certain photosystem imbalanced light), is an innovative approach to explore the phototactic mechanism.

(2) Developing phototactic dynamic model

As the intact biological motor, the flagella of unicellular alga, whose diameter is only several hundreds nanometers, produces energy and propels for the cell. Research on cell's dynamic characteristics needs to be expanded, including moving velocity, rotating angle, instantaneous velocity, inertia, and viscoce friction coefficient. Furthermore, the flagellar behavior, including beating frequency, stroke velocity, stroke duration of each flagellum, and relative phase of the two flagella need to be studied. Taking the luminous flux, light intensity and angle of the light beam as inputs, a phototactic dynamic model for the inputs and the cells' motility need to be developed. The objective of researching and seeking the relationship between the energy of the biological motor and the light parameters based on the simulation and modeling methodologies is trajectory control and energy release for the biological cells. All these are the fundamental supports to the loading and micro transportation by the intact motors of the cells.

(3) Cell tracking and micromanipulation techniques

To achieve automatic and accurate cell control, the following requirements need to be satisfied, including continuous observation for unfixed and fast-moving cells, big enough space for movement control, and particular watching for the exact activities of special cells with high resolution. But, traditional microscopes are limited in: discontinuous observation of fast-moving cells; fixing the work space in the visual field; observing with low resolution for not to lose the cell, but lose detailed characteristics. These problems need to be solved for accurate control of the cells.

With the development of the computer vision technique, to capture with a thousand frames rate is possible, while to process real-time images with a high frame rate is still difficult. We need to research how to obtain and measure the kinetic parameters. Researches on recognizing the characteristics of the cells, identifying the relative positional variety of the motile cells and the feedback of the vision information should be extended. These key technologies are the pushes to realize the microorganism-carrier transportation mechanism and manipulation for micro objects. Moreover, these will likely lead to magnitude applications in MEMS, biology, medical and so on.

References

1 Presti P. and Delbruck M. Photoreceptors for biosynthesis energy storage and vision. Plant Cell Environment, 1978, 1: 81—100.
2 Gest H. Phototaxis and other sensory phenomena in purple photosynthetic bacteria. FEMS Microbiology Review, 1995, 16: 287—294.
3 Mayer A. M. *Chlamydomonas*: adaption phenomenain phototaxis. Nature, 1968, 217: 875—876.
4 Takahashi T. and Watanabe M. Photosynthesis modulates the sign of phototaxis of wide-type *Chlamydomonas reinhardtii*. FEBS Letter, 1993, 336: 516—520.
5 Dieckmann C. L. Eyespot placement and assembly in the green alga *Chlamydomonas*. BioEssays, 2003, 25: 410—416.
6 Mitchell D. R. *Chlamydomonas* flagella. Journal of Phycology, 2000, 36: 261—273.
7 Buder J. Zur Kenntnis der phototaktischen Richtungsbewegungen. Jahrbuch der wissenschatlichen Botanik, 1917, 58: 105—220.
8 Rtiffer U. and Nultsch W. High speed cinematographic analysis of the movement of *Chlamydomonas*. Cell Motility, 1985, 5: 251—263.
9 Witman G. B. *Chlamydomonas* phototaxis. Trends in Cell Biology, 1993, 11: 403—408.
10 Foster K. W. and Smyth R. D. Light antennas in phototactic algae. Microbiology Review, 1980, 44: 572—630.
11 Foster K. W., Saranak J., Nakanishi K. et al. A rhodopsin is the functional photoreceptor for phototaxis in the unicellular eukaryote *Chlamydomonas*. Nature, 1984, 311: 756—759.
12 Ehlenbeck S., Gradmann D., Braun F. J. et al. Evidence for a light induced H^+ conductance in the eye of the green alga *Chlamydomonas reinhardtii*. Biophysical Journal, 2002, 82: 740—751.
13 Harz H. and Hegemann P. Rhodopsin regulated calcium currents in *Chlamydomonas*. Nature, 1991, 351: 489—491.
14 Bessen M., Fay R. B. and Witman G. B. Calcium control of waveform in isolated flagellar axonemes of *Chlamydomonas*. Journal of Cell Biology, 1980, 86: 446—455.
15 Otomo C. K. and Brokaw C. J. Bending patterns of *Chlamydomonas* flagella Ⅱ. Calcium effects on reactivated *Chlamydomonas* flagella. Cell Motility, 1985, 5: 53—60.
16 King S. I. and Dutcher S. K. Phosphorylation of an inner dynein arm complex in *Chlamydomonas reinhardtii* is altered in phototactic mutant strains. Journal of Cell Biology, 1997, 136: 177—191.
17 Holland E. M., Harz H. and Hegemann P. Control of phobic behavior responses by rhodopsin-induced photocurrents in *Chlamydomonas*. Biophysical Journal, 1997, 73: 1395—1401.
18 Xie C. X., Han W. and Yu Z. L. Progress of *Chlamydomonas* as a modal organism. Hereditas (in Chinese), 2003, 25(3): 350—354.
19 Kathir P., LaVoie M., Silflow C. D. et al. Molecular map of the *Chlamydomonas reinhardtii* nuclear genome. Eukaryotic Cell, 2003, 2(2): 362—379.
20 Matsuda A. and Yoshimura K. Isolation and characterization of novel *Chlamydonomas* mutants that display phototaxis but not photophobic response. Cell Motility and the Cytoskeleton, 1998, 41: 353—362.
21 Nagel G., Oillig D., Hegemann P. et al. Channelrhodopsin-1: a light-gated proton channel in green algae. Science, 2002, 296: 2395—2398.
22 Suzuki T., Yamasaki S. and Asamizu E. Archacal-type rhodopsins in *Chlamydomondas*: model structure and intracellular localization. Biochemical Biophysical Research Communications, 2003, 301: 711—717.

23 Govorunova E. G., Jung K. W., Sineshchekov O. A. et al. *Chlamydomonas* sensory rhodopsins A and B: Cellular content and role in photophobic responses. Biophysical Journal, 2004, 86: 2342—2349.

24 Cole D. G. and Kinesin-II. Coming and going. Journal of Cell Biology, 1999, 147: 463—466.

25 Finst R. J., Kim P. J., Griffis E. R. et al. Fa1p is a 171 kDa protein essential for axonemal microtubulese vering in *Chlamydomonas*. Journal of Cell Science, 2000, 113: 1963—1971.

26 Nultsch W., Throm G. and Rimscha I. V. Phototaktische Untersuchungen an *Chlamydomonas reinhardtii* Dangeard in homokontinuierlicher Kultur. Archives of Microbiology, 1971, 80: 351—369.

27 Uhl R. and Hegemann P. Probing visual transduction in a plant from *Chlamydomonas reinhardtii*. Biophysical Journal, 1990, 58: 1295—1302.

28 Hader D. P. Photomovement. In: The Cyanobacteria. New York: Elsevier, 1987, 325—345.

29 Wing-On Ng., Grossman A. R., and Bhaya D. Multiple light inputs control phototaxis in *Synechocystis* sp. strain *PCC6803*. Journal of Bacteriology, 2003, 185(5): 1599—1607.

30 Grossman A. R. and Bhaya D. Tracking the light environment by Cyanobacteria and the dynamic nature of light harvesting. Journal of Biological Chemistry, 2001, 276(15): 11449—11452.

31 Josef K., Saranak J. and Foster K. W. An electro-optic monitor of the behavior of *Chlamydomonas reinhardtii* cilia. Cell Motility and the Cytoskeleton, 2005, 61: 83—96.

32 Josef K., Saranak J. and Foster K. W. Ciliary behavior of a negatively phototactic *Chlamydomonas reinhardtii*. Cell Motility and the Cytoskeleton, 2005, 61: 97—111.

33 Schaller K., David R. and Uhl R. How *Chlamydomonas* keeps track of the light once it has reached the right phototactic orientation. Biophysical Journal, 1997, 73: 1562—1562.

34 Hill N. A. and Hader D. P. A biased random walk model for the trajectories of swimming microorganisms. Journal of Theoretical Biology, 1997, 186: 503—526.

35 Hill N. A. and Plumpton L. A. Control Strategies for the polarotactic orientation of the microorganism *Euglena gracilis*. Journal of Theoretical Biology, 2000, 203: 357—365.

36 Marangoni R., Preosti G. and Colombetti G. Phototactic orientation mechanism in the ciliate *Fabrea salina*, as inferred from numerical simulations. Journal of Photochemistry and Photobiology B: Biology, 2000, 54(2—3): 185—193.

37 Thar R., Blackburn N. and Kuhl M. A new system for three dimensional tracking of motile microorganisms. Applied and Environment Microbiology, 2000, 66(5): 2238—2242.

38 Debeir O., Van Ham P., Kiss R. et al. Tracking of migrating cells under phase-contrast video microscopy with combined mean-shift processes. IEEE Transaction on Medical Imaging, 2005, 24 (6): 697—711.

39 Acton S. T. and Ray N. Detection and tracking of rolling leukocytes from intravital microscopy. In: Proceedings of IEEE International Symposium on Biomedical Imaging. Arlington USA, April 15—18, 2004, 1235—1238.

40 Ogawa N., Hiromasa O., Ishikawa M. et al. Microrobotic visual control of motile cells using high-speed tracking system. IEEE Transaction on Robotics, 2005, 21(4): 704—712.

41 Hosu B. G., Jakab K., Banki P. et al. Magnetic tweezers for intracellular applications. Review of Scientific Instruments, 2003, 74 (9): 4158—4163.

42 Hakho L., Alfreda M. and Robert M. Micromanipulation of biological systems with microelectromagnets. IEEE Transaction on Magnetics, 2004, 40(4): 2991—2993.

43 Ferrari E., Emiliani V., Cojoc D. et al. Biological samples micromanipulation by means of optical tweezers. Microelectronic Engineering, 2005, 78—79: 575—581.

44 Bing S., Sanja Z., Mihri O. et al. Manipulation of microspheres and biological cells with multiple agile VCSEL traps. Sensors and Actuators, B, 2006, 113(2): 866—874.

45 Darnton N., Turner L., Breuery K. et al. Moving fluid with bacterial carpets. Biophysical Journal, 2004, 86: 1863—1870.

46 Weibel D. B., Garstecki P., Whitesides G. M. et al. Microoxen: Microorganisms to move microscale loads. Proceedings of the National Academy of Science of the United State of America, 2005, 102(34): 11963—11967.

47 Sineshchekov O. A., Jung K. H. and Spudich J. L. Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Science of the United State of America, 2002, 99: 8689—8694.

48 Foster K. W. Action spectroscopy of photomovement. In: Photomovement Comprehensive Series in the Photosciences. New York: Elsevier, 2001, 1: 51—115.

49 Nutsch T., Oesterhelt D., Marwan W. et al. A quantitative model of the seitch cycle of an archaeal flagellar motor and its sensory control. Biophysical Journal, 2005, 99: 2307—2323.

50 Smith E. F. and Yang P. The radial spokes and central apparatus: mechanochemical sensors that regulate flagellar motility. Cell Motility and the Cytoskeleton, 2004, 57: 8—17.