Chemotaxis Study Using Optical Tweezers to Observe the Strength and Directionality of Forces of Leishmania amazonensis

Liliana de Ysasa Pozzo[1], Adriana Fontes[1], André A. de Thomaz[1], Luiz Carlos Barbosa[1], Diana Copi Ayres[2], Selma Giorgio[2] and Carlos Lenz Cesar[1]

[1] Instituto de Física Gleb Wataghin, UNICAMP,Campinas,SP,Brazil
[2] Departamento de Parasitologia, Instituto de Biologia, UNICAMP, Campinas, SP, Brazil

ABSTRACT

The displacements of a dielectric microspheres trapped by an optical tweezers (OT) can be used as a force transducer for mechanical measurements in life sciences. This system can measure forces on the 50 femto Newtons to 200 pico Newtons range, of the same order of magnitude of a typical forces induced by flagellar motion. The process in which living microorganisms search for food and run away from poison chemicals is known is chemotaxy. Optical tweezers can be used to obtain a better understanding of chemotaxy by observing the force response of the microorganism when placed in a gradient of attractors and or repelling chemicals. This report shows such observations for the protozoa *Leishmania amazomenzis*, responsible for the leishmaniasis, a serious tropical disease. We used a quadrant detector to monitor the movement of the protozoa for different chemicals gradient. This way we have been able to observe both the force strength and its directionality. The characterization of the chemotaxis of these parasites can help to understand the infection mechanics and improve the diagnosis and the treatments employed for this disease.

Keywords: optical tweezers, chemotaxis, gradient

1. INTRODUCTION

Unicellular microorganism must actively search for the right chemical environment to survive. To do that it must senses the chemical gradient around it and direct its movement in the right direction, towards the gradient of attractive chemical substances, and away of repellent toxic substance ones. This kind of response is called chemotaxis. Chemotaxis is not the only microorganism taxis, because it also senses and respond to temperature, osmotic pressure, pressure, light and other parameter gradients involved in its survival. Microorganism sense the chemical environment with external membrane biochemical receptors. When the microorganism is very small it becomes a point sensor and the chemical gradient must be sensed by keeping the memory of local concentration while it moves around in a sequence of swimming and tumbling movements driven by the flagells or cilia. Chemotaxis has been extensively studied from two point of views: a black box point of view where the response is observed as a function of the stimulus, and a biochemical point of view where the biochemical reactions triggered by the receptors are observed[1,2,3]. Only nowadays, with Optical Tweezers setup, the direct measurement of the vector force under a chemical gradient became possible. The objective of

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this work, therefore, is to study a microorganism impulse force under several chemical gradient conditions with Optical Tweezers Photonic Force setup. For that we measured the propulsion forces of flagellum of the protozoa *Leishmania amazonensis* under a one dimension stationary chemical gradient.

The protozoa *Leishmania amazonensis* is an eukaryote of the Trypanossomatidae family and Kinetoplastida order responsible for leishmaniasis, a serious tropical disease that affects about 30 million people in Africa, Mid Orient and Central and South America[4]. The parasite has two forms: promastigotes and amastigotes. The parasite life cycle involve two hosts. The first is an insect, a mosquito of the Phlebotominae subfamily that splits in two generous, the Phlebotomus prevailing in Africa, Europe and Asia, and the Lutzomyia, prevailing in Americas[5,6]. The second host is a vertebrate that includes men, monkeys, dogs and others. Inside the mosquito guts the parasite takes the flagellar promastigote form capable to move in response to external gradients. It is transmitted to the second host when the female mosquito sucks the blood it needs for reproduction. In the second host bloodstream the parasite, still in the promastigote form, infect the macrophage cells, where it change for the non flagelled amastigote form. Inside the macrophages the amastigote form reproduce and are further released to the blood stream by breaking up the macrophage membrane, reinfecting other macrophages. This process marks the onset of the disease in the host. The temperature and chemical environments of the promastigote and amastigote forms are quite different and it is possible to force the change to the more motile promastigote form in culture just by a poor food and lower temperature environment.

The gradient was obtained by connecting two large chambers with a tiny duct capable to keep the chemical gradient constant for more than ten hours. The propulsion forces of the flagellum of the microorganism was measured with a photonic force setup. For that a 9 μm diameter microsphere attached to the parasite was trapped with a Nd:YAG laser and the scattered light was monitored with a quadrant detector. The direct trapping of the microorganism was avoided by the use of a large microsphere, which also helps to keep the parasite outside of the trapping beam region. We observed the behavior of the protozoa *Leishmania amazonensis* (eukaryote) under several glucose gradients. We observed that the free protozoa sensed the chemical gradient by swimming in circles for three to five times following by tumbling. We also observed a higher force strength clearly directed towards the glucose gradient suggesting that force direction and strength are also used to control its movement.

2. MATERIALS AND METHODS

Figure 1 shows the device we develop to create a stationary concentration gradient. This device has two large reservoirs with a tiny duct connecting them. The gradient for a stationary diffusion is given by $\vec{\nabla}\boldsymbol{C} = (\boldsymbol{C}_2 - \boldsymbol{C}_1)/\boldsymbol{L}$, and can be controlled by the concentrations in the two reservoirs. There are, however, conditions on the relations between the duct and reservoirs parameters to maintain the stationary behavior.

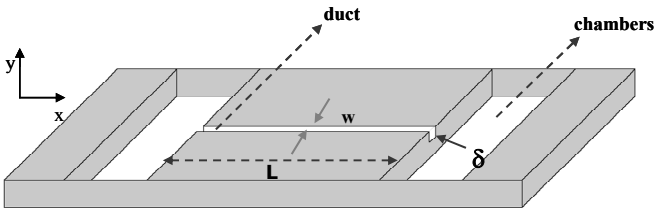

Figure 1. Esquematic view of device.

Assuming that the condition of stationary diffusion is valid instantaneously, the concentrations between the two reservoirs tend to be the same with the time due to the diffusive flow. If this time is big enough, the condition of stationary diffusion will be guaranteed. Taking the reservoir's concentrations C_1 and C_2 in the arbitrary time t, the quantity of particles flowing through the duct will be $(\Delta Q/\Delta t) = -\delta w D(C_2 - C_1)/L$. In this case the concentrations vary with time in agreement with those differential equations:

$$\begin{cases} \dfrac{dC_2}{dt} = -D\dfrac{\delta w}{LV_2}\left[C_2 - C_1\right] \\ \\ \dfrac{dC_1}{dt} = D\dfrac{\delta w}{LV_1}\left[C_2 - C_1\right] \end{cases},$$

where V_1 and V_2 are reservoir's volume. Replacing the

$$\begin{cases} C_2(t) = C_2(0)e^{-pt} + C_{eq}\left[1 - e^{-pt}\right] \\ \\ C_1(t) = C_1(0)e^{-pt} + C_{eq}\left[1 - e^{-pt}\right] \end{cases}$$

solution in the system above a we get $C_{eq} = [V_1 C_1(0) + V_2 C_2(0)] \,/\, [V_1 + V_2]$ and $p = D(\delta w/L)[(V_1 + V_2)/V_1 V_2]$. From this, we can see that system reach the equilibrium in a time of the order $\tau = 1/p = [L/D\delta w][V_1 V_2/(V_1 + V_2)]$, or $\tau = (L\,V)/(2D\delta w)$ for the case $V_1 = V_2 = V$. This time is 4815h for the parameters used, δ = 100 μm, L = 2,6 cm, w = 0,3 cm, V = 0,02 cm^3 and the glucose diffusion coefficient D = $5x10^{-7}$ cm^2/s. This fact guarantee the stationary diffusion condition

The optical force was measured by the displacement of a microsphere from its equilibrium position. The optical geometric model was used to calculate the force. The movement was monitored by the trap Nd:YAG light scattered with a quadrant photodetector (Model: QP506SD2 Pacific Sensor Incorporated) in the usual Photonic Force setup[7-10], which is a very fast (MHz) method.

3. EXPERIMENT

Experimental setup used in this work is shown in figure 2. Two dielectric mirrors on the back focal plane of the microscope send the scattered light to the quadrant detector mounted on a XY translation stage. A 5 cm long focal

distance lens control the beam size on the detector and the signals were sent to an oscilloscope (Tektronix, model TDS 1012).

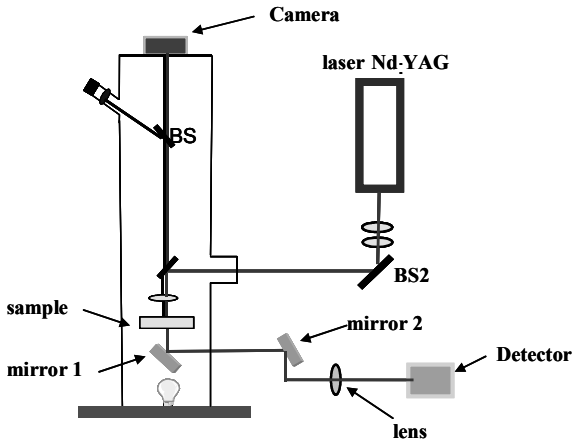

Figura 2. Experimental setup.

By moving a fixed polystyrene bead with 9 μm diameter in the x and y directions we obtained the quadrant detector calibration shown in figure 3. We used the geometrical optics model to extract the force from the calibrated signals. The use of large diameter microsphere was necessary to keep the parasite out of the optical trap light beam.

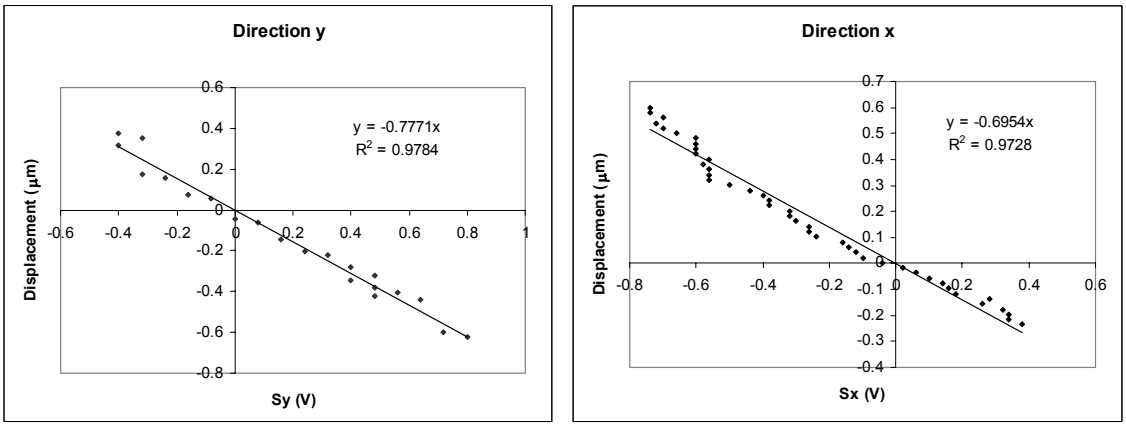

Figure 3. The x-y signal calibration plots using the quadrant detector.

4. RESULTS AND DISCUSSION

For the measurements we add 20 μl to the reservoir 1 of the culture medium solution with the parasites and 20 μl with culture medium with glucose only to the other reservoir. We first observed the behavior of the parasite in the presence of the glucose gradient. It began to swim in circles for 3 to 5 times followed by tumbling (see figure 4). Apparently, this protozoa feel the gradient around it by this circular motion and tumbling, instead of the bacteria straight swim and tumbling motion. Without the gradient they show only erratic movement.

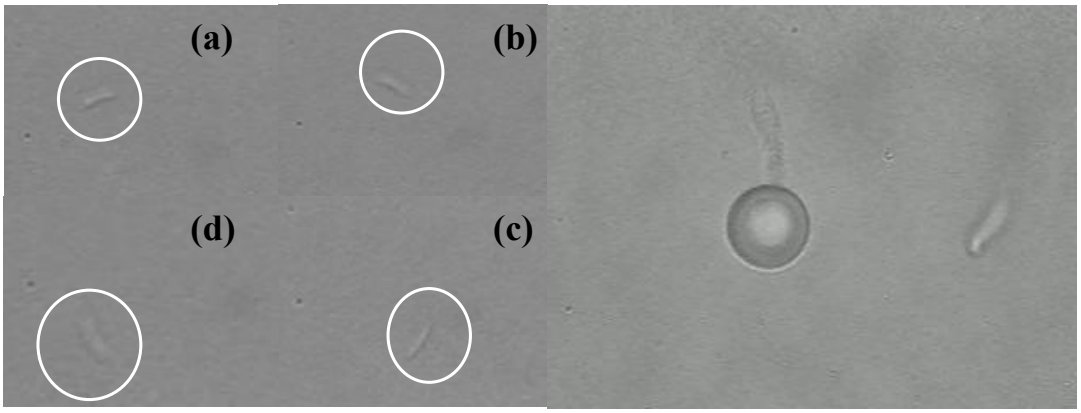

Figure 4 (a). Image of parasites in a duct with glucose. **(b)** Parasite coupled to the microsphere

The force strength was measured with the parasite attached to a microsphere for different concentrations of glucose. Figure 5 shows the plot of the vector force with a clear directionality towards the gradient. We also observed a change in the force strength for different concentrations. These results suggests that both the force strength and direction can be used by the microorganism to perform its chemotaxis.

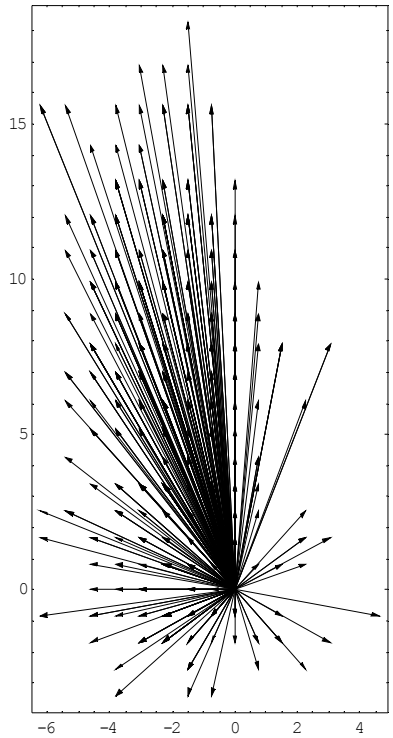

Figure 5. Force vector plot parallel and perpendicular to a 0.2% glucose gradient.

5. CONCLUSION

In conclusion, we developed a system to measure the bidimensional force vector (x and y) of parasites under a stationary unidimensional gradient of concentration of any kind of chemical substance. We observed that the *Leishmania amazonensis* changes the force of the flagella and not only the direction but also the nature of the movement under the presence of glucose. This kind of system can be used to study quantitatively the taxis of any kind of parasite under concentration gradient of different chemical substances, or even, temperature and other type of variable gradients.

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