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# Studying taxis in real time using optical tweezers: Applications for *Leishmania amazonensis* parasites

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ABSTRACT

Beads trapped by an optical tweezers can be used as a force transducer for measuring forces of the same order of magnitude as typical forces induced by flagellar motion. We used an optical tweezers to study chemotaxis by observing the force response of a flagellated microorganism when placed in a gradient of attractive chemical substances. This report shows such observations for *Leishmania amazonensis*, responsible for leishmaniasis, a serious disease. We quantified the movement of this protozoan for different gradients of glucose. We were able to observe both the strength and the directionality of the force. The characterization of the chemotaxis of these parasites can help to understand the mechanics of infection and improve the treatments employed for this disease. This methodology can be used to quantitatively study the taxis of any kind of flagellated microorganisms under concentration gradients of different chemical substances, or even other types of variable gradients such as temperature and pressure.



## 1. Introduction

Unicellular microorganisms must actively search for the right chemical environment in order to survive. For performing this task they must sense the chemical gradient around them and direct their movement towards the gradient of attractive chemical substances, and away from repellent toxic substance. This kind of response is called chemotaxis. Chemotaxis is not the only kind of taxis of microorganisms. They also sense and respond to temperature, osmotic pressure, pressure, light and other parameter gradients involved in their survival. Microorganisms 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 the local concentration while it moves around in a sequence of swimming and tumbling movements driven by the flagella or cilia. The investigation of chemotaxis is essential for understanding an infection process. Parasites recognize and are attracted by cells about to be infected.

There are many studies in the literature on chemotaxis, and other kind of taxis, such as osmotaxis, performed by cells and microorganisms. Chemotaxis, for example, has been extensively studied from two points of view: (1) a black box point of view where the response is observed as a function of the stimulus and (2) a biochemical point of view where the biochemical reactions triggered by the receptors are observed [Law and Aitken, 2005; Khan et al., 2004; Neuman et al., 1999]. The majority of the work on chemotaxis is on leukocytes, which have a kind of slow taxis based on crawling, unlike the taxis performed by bacteria and protozoa. Bleul et al. (1996), Nagasawa et al. (1996) and Nelson et al. (1975) are among the most cited reports in this area. For parasites there are more studies on the chemotaxis of bacteria than that of protozoa [Blair, 1999; Rao et al., 2008; Barros et al., 2006]. Some methods are commonly used to study microorganism chemotaxis. The capillary assay, first created by Pfeffer (1888) and later improved by Adler (1973) is the most commonly used method, especially for quantitative analysis. The methodology developed by Adler was adapted by Oliveira and applied to study the chemotaxis of promastigote forms of leishmanias. This kind of methodology is quantitative, but the measurements are based only on the number of cells found near the end of the higher concentration gradient capillary. Law improved the methodology, and mathematical treatment became easier to carry out than in

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previous works, but the parameter measured was the same as in Adler's work. Another methodology, introduced by Barros et al. (2006) to study the chemotaxis of leishmania, was based on measurement of the mean time of straight line movement. Movement in a straight line is defined here as the absence of abrupt changes of direction.

The development of the optical tweezers [Ashkin et al., 1986; Bustamante et al., 2003] allowed real time measurement of the force vectors, intensity and direction, of living parasites under chemical or other kinds of gradients. This would be the ideal tool to perform black box observations of the taxis response of cells and microorganisms with high sensitivity to capture instantaneous responses to a given stimulus. Forces involved in the movement of unicellular parasites are very small, about the same order of magnitude as the forces generated in an optical tweezers. Optical tweezers rely only on light radiation pressure to trap and manipulate microscopic particles. It is a fast, contactless, non-destructive and highly sensitive force measurement tool capable of measuring forces ranging from tens of femto-Newtons to hundreds of pico-Newtons.

In this work, we propose a methodology to study microorganism chemotaxis in real time using an optical tweezers. We applied this methodology in order to investigate the *Leishmania amazonensis* protozoan, in promastigote form, by measuring the flagellum impulse force under glucose one-dimension stationary chemical gradients.

The *L. amazonensis* protozoan is an eukaryote of the Trypanossomatidae family and Kinetoplastida order responsible for leishmaniasis, a serious tropical disease that affects approximately 30 million people in Africa, the Middle East and Central and South America [WHO, 2001–2002]. The parasite has two forms: promastigotes (flagellated) and amastigotes (non-flagellated). The parasite life cycle involve two hosts. The first is an insect, a mosquito of the Phlebotominae subfamily that subdivides into two genera, the Phlebotomus prevailing in Africa, Europe and Asia, and the Lutzomyia, prevailing in the Americas [Gontijo and Carvalho, 2003; Handman, 2000]. The second host is a vertebrate, including man, monkeys, dogs and others. Inside the mosquito's gut the parasite takes the flagellar promastigote form capable of moving in response to external gradients. It is transmitted to the second host when the female mosquito sucks the blood that it needs for reproduction. In the blood stream of the second host, the parasite, still in the promastigote form, infects the macrophage cells, where it changes into the non-flagellated amastigote form. Inside the macrophages the amastigote form reproduces 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 induce the change from amastigote to promastigote form in the culture medium.

Using this real time chemotaxy investigation with optical tweezers, we observed that *L. amazonensis* changes not only the direction of its movement and its flagellum force, but it also moves in a completely different manner in the presence of glucose. The free protozoan sensed the chemical gradient by swimming in circles from three to five times following by tumbling, in contrast with an erratic, non-directional movement under zero chemical gradient. We believe that this methodology can be used to quantitatively study not only chemotaxis, but also taxis of any kind of flagellated microorganisms under concentration gradient of different chemical substances, or other types of variable gradients such as temperature, pressure, and osmotic pressure. This study can help to better understand the cellular biology of the parasite and to investigate the chemotaxis of microorganisms by the cells that they usually infect. The study of cellular biology is important for an understanding of leishmaniasis and can help to develop new kind of treatments for this serious disease.

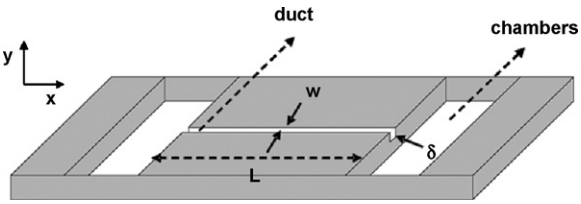


**Fig. 1.** Schematic view of device for creating the concentration gradient.

## 2. Materials and methods

The gradient was obtained by connecting two large chambers with a tiny duct capable of keeping the chemical gradient constant for more than 10 h. Fig. 1 shows the device we developed in order to create a stationary concentration gradient. The gradient for a stationary diffusion is given by $\vec{\nabla}C = (C_2 - C_1)/L$ and can be controlled by the concentrations in the two reservoirs. There are, however, conditions affecting the relations between the duct and reservoir parameters for maintaining the stationary behavior.

Assuming that the condition of stationary diffusion is instantaneously valid, the concentration between the two reservoirs tends to be equal in time due to a diffusive flux. If time becomes very large the condition of stationary diffusion is achieved. Taking $C_2$ and $C_1$ as the concentration in each reservoir and t as an arbitrary time, the number of particles flowing throughout the two chambers 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 these differential equations:

$$\begin{cases} \dfrac{dC_2}{dt} = -D\left(\dfrac{\delta W}{LV_2}\right)(C_2 - C_1) \\ \dfrac{dC_1}{dt} = D\left(\dfrac{\delta W}{LV_1}\right)(C_2 - C_1) \end{cases} \Rightarrow \begin{cases} C_2(t) = C_2(0)e^{-pt} + C_{eq}[1 - e^{-pt}] \\ C_1(t) = C_1(0)e^{-pt} + C_{eq}[1 - e^{-pt}] \end{cases} \tag{1}$$

Solving the system above, we get:

$$C_{eq} = \frac{V_1C_1(0) + V_2C_2(0)}{V_1 + V_2} \quad \text{and} \quad p = D(\delta W/L)\left[\frac{V_1 + V_2}{V_1V_2}\right] \tag{2}$$

Thus, where $V_1 = V_2 = V$, we can see that the system reaches equilibrium in a time of the order of:

$$\tau = \frac{1}{p} = \left(\frac{L}{D\delta W}\right)\left[\frac{V_1V_2}{V_1 + V_2}\right] \text{ or } \tau = \frac{LV}{2D\delta W} \tag{3}$$

This time is 4815 h for the parameters used, $\delta = 100\ \mu m$, $L = 2.6$ cm, $w = 0.3$ cm, $V = 0.02\ cm^3$ and the glucose diffusion coefficient $D = 5 \times 10^{-7}\ cm^2/s$. This fact guarantees the condition of stationary diffusion.

The experimental setup used in this work is shown in Fig. 2. The propulsion force of the flagellum of the protozoan was measured with an optical tweezers by using a polystyrene bead, connected to the parasite, as a force transducer. After calibration, assuming a geometrical optics model, by measuring the displacement of the bead from the equilibrium position, it was possible to determine the numerical values for the optical force and consequently for the flagellum force of the parasite. Previous calibration of this procedure against hydrodynamic force showed good results [Fontes et al., 2005]. The displacements of the bead from the equilibrium position were obtained by measuring the scattered light of the laser used in the optical tweezers with a quadrant detector (QP506SD2 - Pacific Sensor Incorporated) [Rice et al., 2003; Rohrbach and Stelzer, 2002; Gittes and Schmidt, 1998;

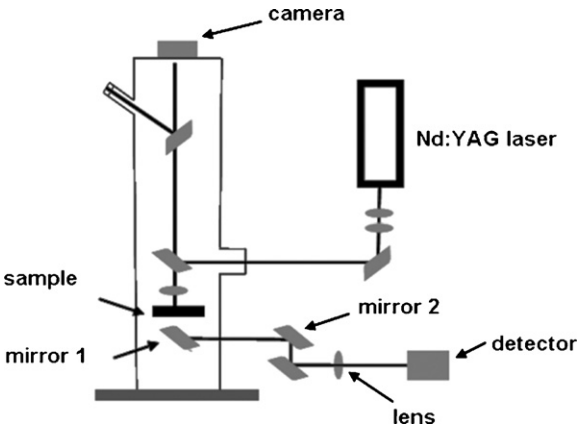


**Fig. 2.** Experimental optical tweezers setup used for taxis studies.

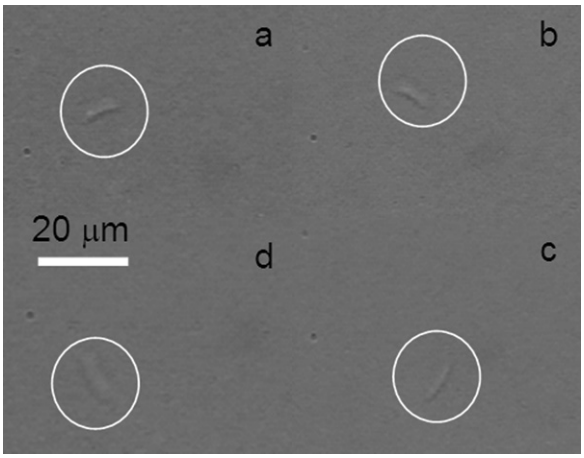


**Fig. 4.** Parasite swims in circles under glucose gradients. The figure shows four frozen frames of the video taken in a time sequence during this circular motion.

Allersma et al., 1998]. A 9 μm diameter polystyrene bead attached to the parasite was trapped with an Nd:YAG laser using an Olympus high numerical aperture objective (100× – numerical aperture NA = 1.25). The direct trapping of the microorganism was avoided by using a large bead, which also helps to keep the parasite outside of the trapping beam region. Beads with large sizes are necessary, because we observe that the parasite stays in front of the beam when it is trapped with small beads, causing scattered light by the parasite interfering on the bead displacement measurements. In beads with large diameters these situations never happen.

Two dielectric mirrors (mirrors 1 and 2) on the back focal plane of the microscope sent the scattered light to the quadrant detector mounted on an XY translation stage. A 5 cm long focal distance lens controls the beam size on the detector and the signals were sent to an oscilloscope (Tektronix, model TDS 1012). By moving a fixed polystyrene bead with 9 μm diameter in the $x$ and $y$ directions using a microscope translation stage we obtained the quadrant detector calibration shown in Fig. 3. This calibration links the bead displacements with the scattered light.

We observed the behavior of the protozoan *L. amazonensis* in real time under two glucose gradients (0.2 and 0.5%). For the measurements we add 20 μl of the culture medium solution with the parasites to the reservoir 1 and 20 μl of culture medium with glucose only to the other reservoir (reservoir 2). The measurements were taken in different concentrations for the same parasite.

## 3. Results and discussion

We first observed the behavior of the parasite in the presence of the glucose gradient. It began to swim in circles for three to five times followed by tumbling (see Fig. 4). Fig. 4 shows four frozen frames of the video taken in a time sequence during this circular motion. The protozoan apparently feels the gradient around it by this circular and tumbling motion, unlike a bacterium, which feels the gradient by a straight swimming and tumbling motion. Without the gradient the parasites show only erratic movement. Fig. 5 shows the plot of the vector force (for 0.5% and 0.2%) with a clear directionality towards the gradient. These results suggest that both the force strength and direction can be used by the microorganism to perform its chemotaxis. The sensitivity for gradient concentration depends on the microorganisms and the chemical substance, while the force sensitivity of the optical tweezers system can go down to forces as small as 100 fN, much smaller than the noise force variations of the parasites. We have been able to detect fast changes in the parasite movement to gradients as small as 0.2% with roughly a factor of 5 for the forces up and down with respect to the gradient. If we assume a linear model for this difference, the two forces would be the same for a 0.04% gradient. However, if one needs more sensitivity it is possible to average the time over long periods in order to detect small changes in the parasite behavior, but at the cost of losing real time observations. We should also bear in mind that standard chemotaxis capillar studies wait for about 1 h [Barros et al., 2006].

Bray (1983) was the first to propose that leishmania could perform chemotaxis. Leslie et al. (2002) proposed that leishmania could also response to osmotic gradients. According to Barros, the response to osmotic gradients happens only for sugar concentrations above 2%. From Fig. 5, we can observe that when the glucose concentration is higher, the directionality of the movement is more defined and the force response is also higher. Compared to other

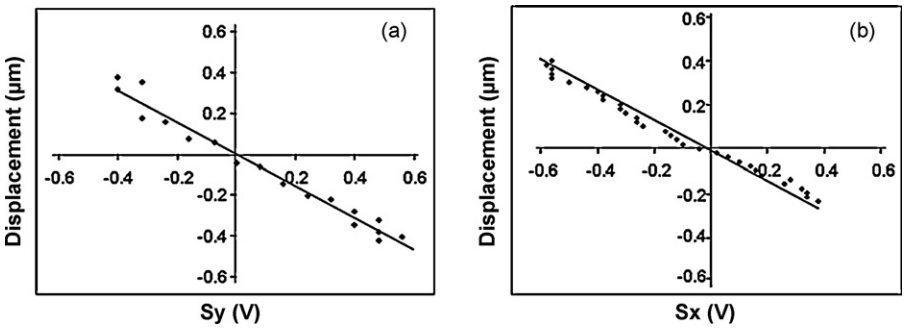


**Fig. 3.** The $x$ (b) and $y$ (a) signal calibration plots using the quadrant detector.

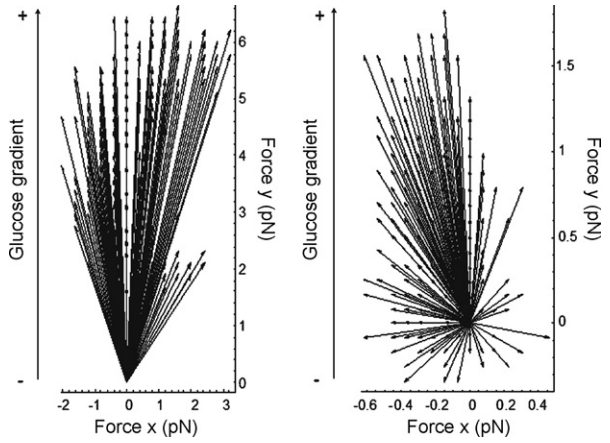


**Fig. 5.** Bidimensional vector force plot for 0.5% (left) and 0.2% (right) glucose gradients.

methods cited in the introduction, we believe that the advantages of the optical tweezers method are: the capability of observing the directionality and strength of the force in real time; capability of observing the same single microorganism behavior under different gradients instead of taking averages over a large number of parasites.

In conclusion, we have developed a methodology to measure in real time the bidimensional vector forces ($x$ and $y$) of parasites under stationary one-dimensional gradients of concentrations of any kind of chemical substance. We observed that *L. amazonensis* changes the force of the flagellum and not only the direction but also the nature of its movement in the presence of glucose. This system can be used to quantitatively study the taxis of any kind of microorganism under concentration gradients of different chemical substances, or other types of gradients such as temperature, pressure, or osmotic pressure. The investigation of chemotaxis is an essential part for an understanding of an infection process. Parasites recognize and are attracted by the cells that will be infected. The study of this cellular biology can help to understand diseases such as leishmaniasis and this comprehension can help to develop new kind of treatments for this serious disease.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.micron.2009.02.008.

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