Noise force spectroscopy with Optical Tweezers

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Abstract: In this work we will present a study of noise force spectroscopy using optical tweezers. In particular, we will study the fluctuations of two kinds of systems, a passive one, polystyrene spheres of known radius (bead), and a biological system like the red blood cells. Experimentally, we will directly optically trap the systems at rest and measure the force signal during 30s. Once we get the force signal, we will compute the power spectra which present a Lorentzian shape. By fitting the power spectrum to a Lorentzian, we will obtain the friction coefficient and the stiffness of both systems. The results are compatible with the theoretical predictions and the experimental values obtained by different techniques.

I. INTRODUCTION

The fluctuation-dissipation theorem is a powerful tool to predict the behaviour of systems in equilibrium. The study and measurement of fluctuations is crucial for understanding the evolution of a wide variety of systems, including biological ones such as the red blood cells.

Red blood cells (RBCs) are the most common blood component. Their cytoplasm is rich in haemoglobin, a biomolecule that binds with oxygen allowing the RBCs to carry it from the lungs to the body tissues. RBCs have a diameter of $6-8\mu m$, are disc-shaped and present a high deformability which allows them to flow through tiny blood vessels [1].

Flickering of RBCs is a well-known phenomenon that has been studied for years and can be directly observed under optical microscopy. Initially, it was described as an equilibrium process due to the thermal fluctuations of the cell membrane, however, more recent studies using optical tweezers suggest a non-equilibrium explanation [2].

Optical tweezers (OTs) are instruments that use highly focused laser beams to hold and move sub-microscopic objects. Therefore, they are ideal to perform experiments in the fields of biology, nanoengineering and physics, among others [3] [4]. Specifically, OTs can work precisely at the nanoscale and, because of that, they are ideal for measuring fluctuations in biological systems such as RBC flickering.

The present work will focus on the physical quantities that we can extract from flickering. Specifically, we study two different kinds of systems: a passive one, a 3μm diameter polystyrene bead, and a system of biological relevance such as RBCs.

By directly trapping the systems in the optical trap, measuring the force signal and performing a power spectra analysis, we are able to compute the stiffness and the friction coefficient of both systems.

II. OPTICAL TWEEZERS

Figure 1a depicts the scattering of the laser beams when they interact with objects like beads (upper panel) and RBCs (lower). The scattering process changes the light momentum, implying that a force is exerted upon the trapped object. This phenomenon is known as radiation pressure, and it is the fundamental principal behind optical tweezers (OTs).

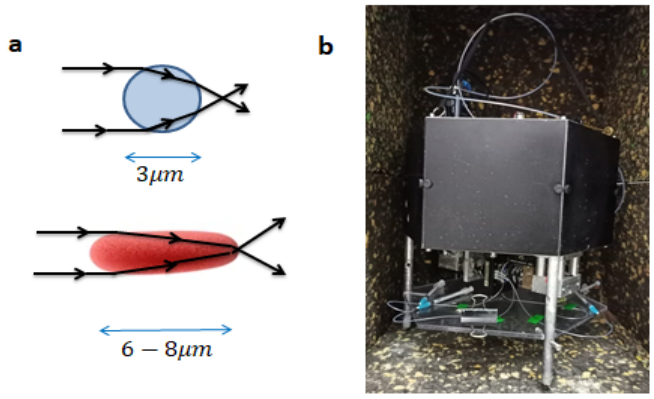

FIG. 1: (a) Scheme of the scattering of the lasers beams when they interact with the bead (upper panel) and the RBC (lower panel). (b) Image of the mini-Optical Tweezers setup.

Arthur Ashkin is considered the father of the optical tweezers. His work on microparticles manipulation with laser beams started in the late 1960s and was culminated in 1986 with the invention of the optical tweezers. For his contribution on this field, he was awarded with the 2018 Nobel Prize in Physics.

Optical Tweezers use high numerical aperture objectives to focus two counterpropagating lasers beams creating an optical trap. Furthermore, OTs are able to measure and exert forces in the order of picoNewtons and distances in the order of nanometers. Their resolution and the possibility to measure at high frequency, makes

them ideal to perform single-molecule and flickering experiments.

A concern of OTs is the possibility that the highly focused lasers may heat up the trapped object. As a result of this effect, samples such as RBCs, could be damaged [5]. In order to prevent this effect, OTs tipically work with infrared light ($800nm - 1064nm$) which minimizes the optical absorption by water. The heating is also mitigated by the liquid medium surrounding the trapped object, so we can study their fluctuations and mechanical properties without much concern.

III. LANGEVIN EQUATION AND POWER SPECTRUM

For a particle moving inside a one-dimensional harmonic potential, a good model for an optical trap, the Langevin equation is

$$m\frac{d^2x}{dt^2} + \gamma\frac{dx}{dt} + kx = f(t) + \eta(t), \tag{1}$$

where m is the particle's mass, γ is the friction coefficient, k is the trap stiffness, $f(t)$ is the external force and $\eta(t)$ is the noise associated to fluctuations. Notice that $< \eta(t) >= 0$ and $< \eta(t)\eta(s) >= 2k_BT\gamma\delta(t-s)$ where k_B is the Boltzmann constant, T is the temperature, and $\delta(t-s)$ is Dirac's delta function .

Considering $f(t) = 0$, the equation can be solved in the overdamped limit ($m = 0$),

$$x(t) = x(0)e^{-\frac{kt}{\gamma}} + \frac{1}{\gamma}\int_0^t \eta(s)e^{-\frac{k(t-s)}{\gamma}}ds \tag{2}$$

The auto-correlation function is defined as

$$R_{xx}(t) = E[x(0)x(t)] =< x(0)x(t) > \tag{3}$$

Introducing Eq.(2) in Eq.(3) and knowing from the equipartition theorem that $< x^2(0) >= \frac{k_BT}{k}$, the auto-correlation function is expressed as,

$$R_{xx}(t) =< x^2(0) > e^{-\frac{kt}{\gamma}} = \frac{k_BT}{k}e^{-\frac{kt}{\gamma}} \tag{4}$$

The power spectrum is defined as the Fourier transform of the auto-correlation function of a signal:

$$S_{xx}(\nu) = F_t[R_{xx}(t)] \tag{5}$$

Then, we perform the Fourier transform in Eq. (4),

$$\hat{R}_{xx}(\omega) = \int_{-\infty}^{\infty}\frac{k_BT}{k}e^{-\frac{k\Delta t}{\gamma}}e^{-i\omega t}dt = \\ = \frac{2k_BT\gamma}{\gamma^2\omega^2 + k^2} = \frac{2k_BT}{\gamma(\omega^2 + \omega_c^2)}, \tag{6}$$

where $\omega_c = k/\gamma$ is the angular corner frequency.

Finally, the force power spectrum in terms of the frequency ν ($\omega = 2\pi\nu$) is obtained considering its relation with the position one,

$$S_{ff}(\nu) = k^2S_{xx}(\nu) = \frac{k_BTk^2}{2\pi^2\gamma}\frac{1}{(\nu^2 + \nu_c^2)} \tag{7}$$

IV. SPECTRAL ANALYSIS

In the previous section we derived the power spectrum of a bead diffusing inside an optical trap from its Langevin equation. The result, Eq.(7), follows a Lorentzian shape:

$$S_{ff}(\nu) = \frac{A}{\nu^2 + \nu_c^2} \tag{8}$$

where A is the amplitude and $\nu_c = k/2\pi\gamma$ is the corner frequency. Comparing Eq.(7) and Eq.(8) we find the relation between parameters A and ν_c and the ones with physical meaning, k and γ:

$$k = \frac{\pi A}{k_BT\nu_c} \tag{9}$$

$$\gamma = \frac{\pi A}{2k_BT\nu_c^2} \tag{10}$$

Therefore, by fitting the power spectra to Eq.(8), we obtain the parameters A and ν_c. Then, from Eq.(9) and Eq.(10), we extract k and γ.

To obtain the power spectra, we trap the systems on the optical trap and measure during $30s$ the force signal at a high sampling frequency. It is necessary to record at a sampling frequency at least five times larger than the characteristic corner frequency and in our case we took data at $100kHz$. It is important to mention that, in order not to damage the RBCs, we perform the experiments at low laser power (a few mW), corresponding to a trap stiffness of $k = (2.20 \pm 0.08) \times 10^{-2}pN/nm$. This value is obtained from the slope of the force-displacement curve of a bead fixed on the tip of a micropipette (Figure 2, this procedure is explained in detail in [6]).

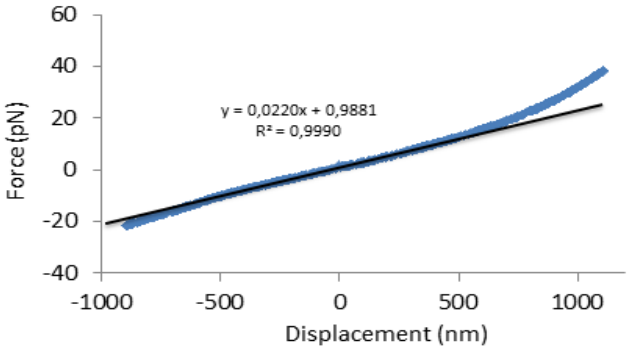

FIG. 2: Force-displacement curve of a bead fixed on the tip of a micropipette together with a linear fit between 10 pN and -10 pN.

As shown in Figure 3a, the force signal oscillates around zero since we want to study the fluctuations of the systems at zero external force. Once we get the force signal, the power spectra is computed by Fourier transforming its square modulus. After that, a boxcar average (BCA) filter in logarithmic scale is applied, as shown in Figure 3b. We impose a similar statistical weight to the BCA points, as we are fitting along different orders of magnitude.

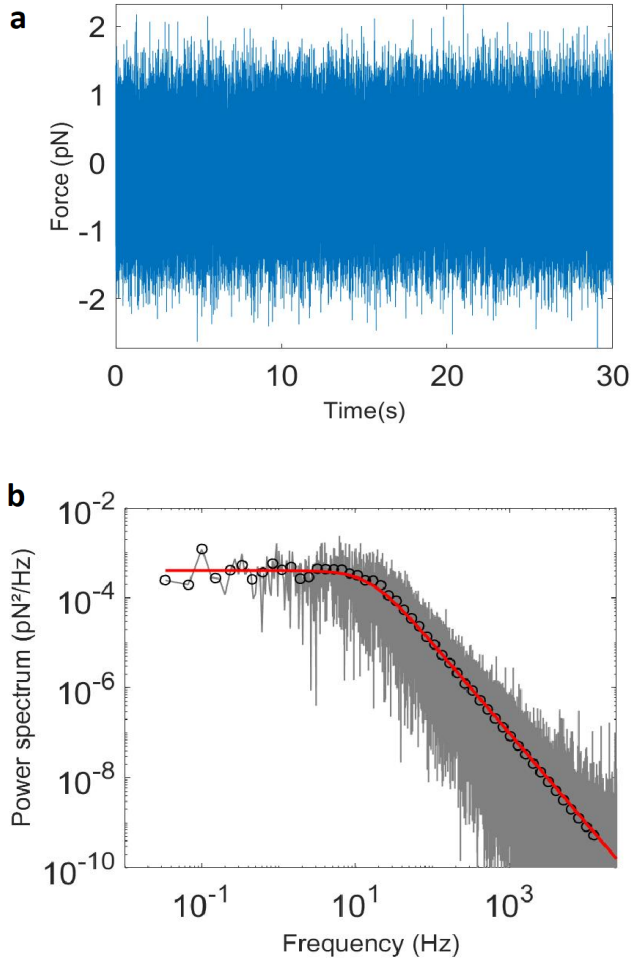

FIG. 3: (a) Force signal of a bead optically trapped. (b) Power spectrum of the force signal shown in (a). Raw power spectrum in grey, boxcar average filter in black circles and fit to Eq. (8) in red.

In Figure 4a are shown the BCAs and the fits to Eq.(8) for three different beads, each one represented in a different color and symbol. The three power spectra are compatible with each other, and their fits are hard to distinguish.

On the other hand, the measurements for the RBCs present more dispersion than the beads' ones. Notice that in Figure 4b, both BCAs and fits to Eq.(8) can be distinguished from one RBC to the other.

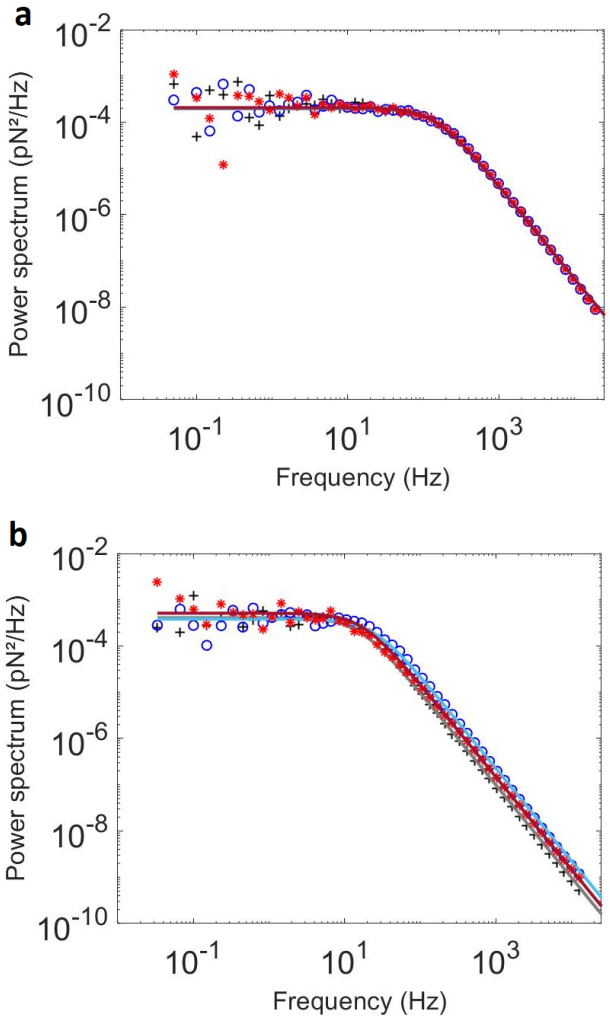

FIG. 4: (a) Boxcar averages and the fits to Eq.(8) for three beads. (b) Boxcar averages and the fits to Eq.(8) for three RBCs.

V. RESULTS AND DISCUSSION

For each fit to a power spectrum, we obtain a couple of k and γ values. In Table 1, we present the results obtained with their corresponding uncertainties.

	$k \times 10^{-2}(pN/nm)$	$\gamma \times 10^{-5}(pN \cdot s/nm)$
Bead 1	2.19 ± 0.11	2.38 ± 0.18
Bead 2	2.21 ± 0.11	2.44 ± 0.19
Bead 2	2.23 ± 0.11	2.50 ± 0.19
RBC 1	0.48 ± 0.05	5.0 ± 0.8
RBC 2	0.71 ± 0.07	4.6 ± 0.7
RBC 2	0.66 ± 0.09	6.2 ± 1.2

TABLE I: Stiffness and friction coefficient results obtained for beads and RBCs.

In Figures 5a and 5b, we plot the k and γ values, respectively, to give a more visual sense of the results. In these figures there is also plotted the average k and γ values in order to provide a quick evaluation of the dispersion of the results.

As already discussed in the previous section, the results for the beads are compatible with each other (and here it is even clearer than in Figure 4a). In fact, this compatibility is expected as the dispersion in the bead's radius is very small (r= $1.50 \pm 0.01 \mu$m).

On the other hand, RBCs present a larger heterogeneity due to the exogenous processes that suffer in vivo. For that reason, the dispersion in both stiffness and friction coefficient are also expected. The fact that we are able to observe this heterogeneity is a proof of the resolution of the noise force spectroscopy measurements to characterized complex systems.

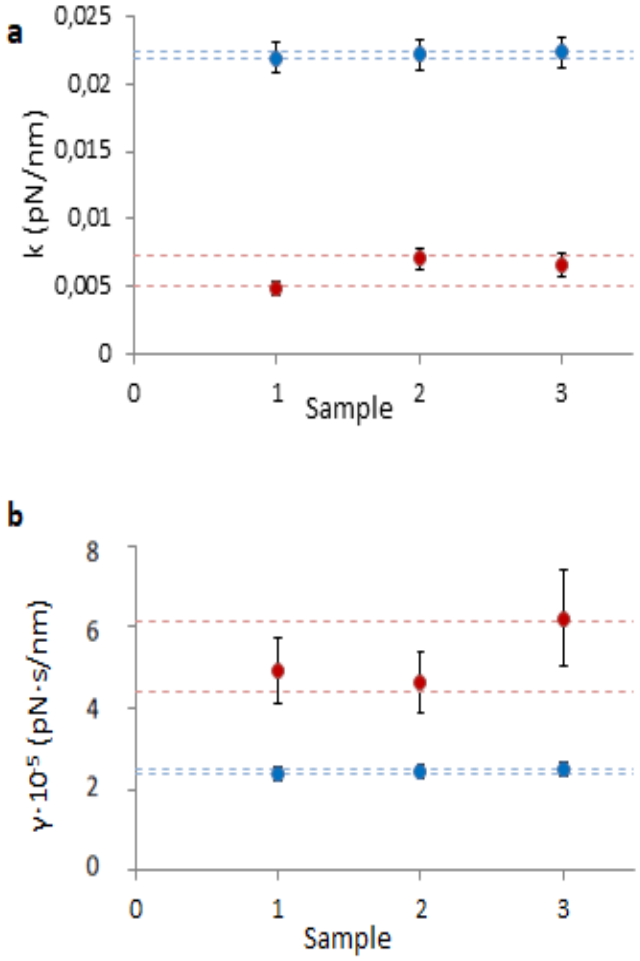

FIG. 5: (a) Stiffness, k, of three beads (in blue) and three RBCs (in red) together with their uncertainties. With dashed lines a representation of the average values for both systems. (b) Friction coefficient, γ, of three beads (in blue) and three RBCs (in red) together with their uncertainties. With dashed lines a representation of the average values for both systems.

The average results for the beads are:

$$k_{bead} = (2.21 \pm 0.02) \times 10^{-2} \frac{pN}{nm} \tag{11}$$

This value is compatible with the trap stiffness mentioned in section IV, which is $k = (2.20 \pm 0.08) \times 10^{-2} pN/nm$.

And for the friction coefficient,

$$\gamma_{bead} = (2.44 \pm 0.06) \times 10^{-5} \frac{pN \cdot s}{nm}. \tag{12}$$

In hydrodynamics, the friction coefficient of a spherical object inside an incompressible fluid has the following expression,

$$\gamma = 6\pi\eta r \tag{13}$$

where η is the fluid viscosity and r is the radius of the object. In our case, the fluid is mQ water, which has a viscosity of $0.8904cp$ at $25°C$, and the radius of the beads is $1.5\mu m$. Therefore the theoretical value for γ is $2.52 \times 10^{-5} pN \cdot s/nm$, which is compatible with our result.

The average results for the RBCs are:

$$k_{RBC} = (6.2 \pm 1.2) \times 10^{-3} \frac{pN}{nm} \tag{14}$$

This value is compatible with the stiffness obtained in RBC pulling experiments using optical tweezers [7].

While for the friction coefficient,

$$\gamma_{RBC} = (5.3 \pm 0.9) \times 10^{-5} \frac{pN \cdot s}{nm}. \tag{15}$$

To evaluate this result we can do a rough approximation considering the RBC as an sphere with a diameter of $6\mu m$. Using Eq.(13), we obtain a theoretical value of $\gamma \approx 5 \times 10^{-5} pN \cdot s/nm$. Although it is an inaccurate approximation, it gives us a first estimate of the value for γ that we should expect and, actually, the result turns out to be compatible.

In order to emphasize the variability in the friction coefficient between beads and RBCs, in Figure 6 we show an histogram of the friction coefficient. In particular, we present 7 different beads (blue, measured at high laser power, notice that γ is independent from the laser power), and 7 RBCs (red).

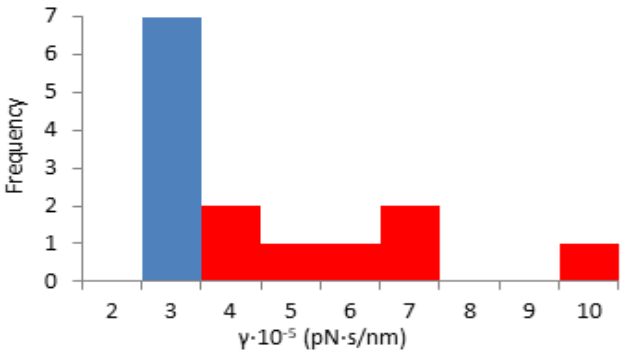

FIG. 6: Histogram of the friction coefficients, γ, obtained for seven beads (in blue) and seven RBCs (in red).

VI. CONCLUSIONS

- We have obtained the stiffness and the friction coefficient for beads and red blood cells (RBCs) by measuring their force signal with the optical tweezers and performing a power spectra analysis.
- We have shown that power spectra analysis is a very precise method for the calibration of the trap stiffness at zero force and the characterization of the bead geometry through their friction coefficient.
- In terms of the study of biological systems such as RBCs, we have shown that the power spectrum analysis is capable of characterizing their heterogeneity together with the precise measure of their stiffness and their friction coefficient.
- The results are compatible with the literature and measurements obtained with optical tweezers, such as force-distance curve calibration in pulling experiments.
- Optical tweezers are a well-established instrument in the fields of molecular and cell biophysics, and this work is another proof of their important role regarding single-molecule experiments.

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