The effect of the shutter exposure time in force calibration in Magnetic Tweezers

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Abstract: Magnetic tweezers is a single-molecule technique highly used in biophysics to investigate processes at molecular scale. Obtaining quantitative results relies on a precise force calibration, which depends on the measurement of bead's transversal fluctuations. The later is influenced by the camera shutter exposure time along which the light intensity signal is averaged. Previous works have highlight the importance of reducing the camera's exposure time in short molecules (where the bead's fluctuations are more rapid). Here we carry out experiments with long and short DNA molecules to study the effect of using different shutter exposure times in force calibrations. We find that working at the standard conditions, where the camera exposure time is maximized, the force is highly overestimated. Whereas modulating the exposure time becomes a good strategy to correctly measure forces both in long and short molecules. Finally, we use the results to compare the elastic response of single-stranded and double-stranded DNA molecules.
Keywords: Magnetic tweezers, force calibration, bead's transversal fluctuations, shutter exposure time, single-stranded DNA, double-stranded DNA.
SDGs: This TFG is related with the following Stock Sustainable Development Goals: Quality education (4), Industry/Innovation and Infrastructure (9).

I. INTRODUCTION

Single-molecule techniques, including optical (OT) and magnetic (MT) tweezers and AFM [1] have been widely used in recent years to observe and investigate molecular processes. These techniques allow to manipulate individual molecules by applying mechanical forces to their extremities. In Magnetic tweezers a molecule, such as DNA, RNA or a protein, is tethered between a micron-sized magnetic bead and a glass surface. Two magnets exert a force on the bead enabling to stretch the molecule. Using video-microscopy one can track simultaneously the 3D position of a large number of beads (on the order of 100) and characterize the behavior of a large group of molecules. The parallelization is the big advantage of MT with respect to other single molecule techniques, such as optical tweezers or AFM. However, MT lack a direct measurement of the force. The tension exerted on the bead-molecule system, which can be modeled as an inverted pendulum (Fig.1B), can not be directly determined, but it can be derived using the equipartition theorem: $\frac{1}{2}k_BT = \frac{1}{2}k_x\langle\delta x^2\rangle$, where $\langle\delta x^2\rangle$ is the amplitude of the bead's transversal fluctuations, k_x is the rigidity of the tether in the transversal direction x, k_B is the Boltzmann constant and T is the absolute temperature of the surrounding buffer. Taking into account that the molecule extension is related to k_x as: $L_{ext} = F/k_x$, the force can be estimated as [2, 3]:

$$F = \frac{k_BTL_{ext}}{\langle\delta x^2\rangle}. \quad (1)$$

The measurement of the molecule extension L_{ext} and the amplitude of the transversal fluctuations $\langle\delta x^2\rangle$ are obtained via the analysis of the video camera images at a given frequency, f_{ac}. The camera shutter exposure time τ_{sh}, which is the time the camera is recollecting light for imaging, sets a critical limitation for measuring accurately $\langle\delta x^2\rangle$, and consequently the force. In particular, if the characteristic time of the fluctuations is on the order or shorter than τ_{sh} , $\langle\delta x^2\rangle$ is averaged and its value is underestimated. Therefore, prolonged shutter exposure times result in average imaging, which compromises the tracking of the particle's Brownian motion. To solve this issue, we have reduced the τ_{sh} to decrease averaging, while tuning the LED illumination to have the light intensity trade-off for correct bead's tracking. In this way, $\langle\delta x^2\rangle$ can be accurately measured and the force correctly estimated.

In this work we have performed force experiments with two different DNA molecules using MT: a double-stranded DNA (dsDNA) and a single-stranded DNA (ssDNA). The dsDNA molecule is long and quite rigid, whereas the ssDNA is much shorter and flexible. By attaching the DNA molecules to a magnetic bead and approaching the magnets, we measure their molecular extension (z position) and their position in the horizontal plane (x, y positions) with the precision of few nanometers. These measurements can be done at different position of the magnets corresponding to different forces. Analyzing the mean z- position and the variance of the transversal motion (Fig.1D and Fig.1F) we are able to determine the applied force, as described in Eq.1. This allows us to obtain a calibration curve of the force as a function of the magnets position, $F(Z_{mag})$. This calibration curve is used to estimate forces in MT assays, where the force can not be directly measured. For the two types of DNA samples, we have measured the force calibration both using a fixed and tunable shutter exposure time. The comparison between the two measurements allow us

to investigate the range of validity of the force calibration measurements. Finally we have used our results to characterize the elastic response of dsDNA and ssDNA using widely used polymer models, verifying that dsDNA is much more rigid and compact than ssDNA.

II. SETUP AND METHODS

A. Magnetic tweezers

The magnetic tweezers setup is schematically shown in Fig.1A. A DNA molecule is tethered between a micron-sized magnetic bead and the glass surface of a microfluidic chamber, with connections done via biotin/streptavidin and digoxigenin/anti-digoxigenin interactions [4]. The chamber is illuminated from above by a LED to facilitate visualization. An inverted microscope, equipped with a CCD camera is used to image the beads. The bead's images are decorated with a set of diffraction rings. The 3-D position of the bead is tracked using the diffraction pattern [2]. The tethered DNA molecule is stretched with a force that depends on the distance between the magnets and the bead, Z_{mag}. The value of the force decreases strongly as the magnets move away, with values ranging from 10^{-3} to 100 pN when the magnets are few mm from the microfluidics chamber [4].

B. DNA substrates and experimental conditions

In this work we have used two DNA substrates. One is the dsDNA molecule from the λ phage that is 48 kilobase-pair (kbp) long, Fig.1C. This is the standard molecule used to do force calibration measurements in MT, because it is very long and presents slow dynamics, which allows to track the bead's transversal fluctuations with good accuracy using standard acquisition frequencies (on the order of 30-100 Hz). The other molecule is a 2700 bases (b) ssDNA which central part is self-complementary and it therefore folds into a hairpin structure (1350 bps long), as shown in Fig.1E. By increasing the force above 15 pN, the base-pairs holding the hairpin break and the hairpin unzips allowing to extend the full ssDNA molecule (Fig.1F step 2, molecular extension h_2). If we next decrease the force again below 15pN the hairpin rezips. However, if we add into the chamber a 50 bases blocking oligonucleotide that is complementary the loop region, the oligonucleotide hybridization acts as a kinetic block hindering the reziping of the hairpin (Fig.1F step 3, molecular extension h_3). This allows to study the ssDNA elastic response. The experiments has been carried out at 1M NaCl solution. We have performed force measurements using an acquisition frequency f_{ac} of 80 Hz at (i) fixed $\tau_{sh} = 1/f_{ac} = 12500\ \mu s$ and (ii) tunable τ_{sh}. The minium exposure time $\tau_{sh} = 775\ \mu s$ used in experiments is given when the LED intensity is maximum.

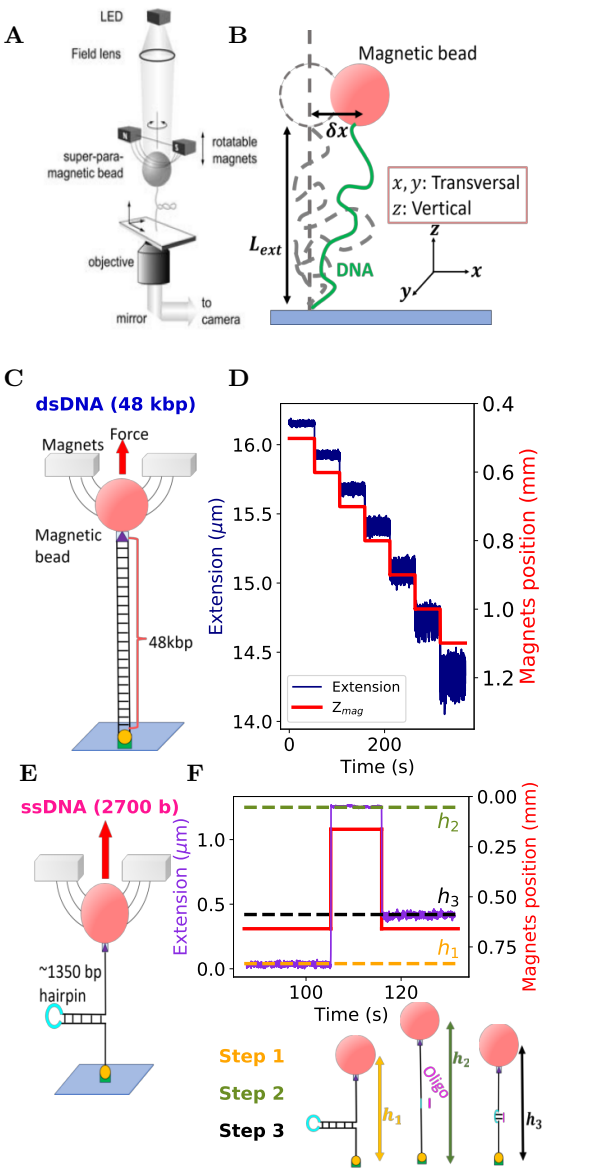

FIG. 1: A) Schematic representation of the MT instrument. B) Schematics of the bead's motion and the measured extension L_{ext} and transversal fluctuations δx. C) Schematics of the MT assays with the dsDNA λ molecule. D) Bead's z position as a function of time at different Z_{mag} values. E) Schematics of the MT assays with the DNA hairpin. F) Bead's z position as a function of time in experiment with the hairpin with the blocking oligo in solution. Initially, the hairpin is formed (step 1), next the force is increased to unzip de hairpin and allow the oligo to hybridize (step 2), and finally the force is reduced at the molecule remains in its ssDNA form.

C. Principle of force measurement

The dynamics of the DNA-bead tethered system can be described as an overdamped pendulum. The bead is subjected to three forces: a stochastic Langevin force, a

restoring force that keeps the bead close to its equilibrium position and a friction force generated by the water molecules in the surroundings. In that situation the bead's motion is described by [4]:

$$k_x x(t) + \gamma\eta r \frac{\partial x}{\partial t} = F_L(t), \tag{2}$$

where k_x is the effective pendulum stiffness ($k_x = F/L_{ext}$, with L_{ext} being the molecule extension), r corresponds to the radius of the bead, γ is a constant which varies depending on the geometry ($\gamma = 6\pi$ for a sphere), η corresponds to the medium viscosity and F_L is the Langevin Force.

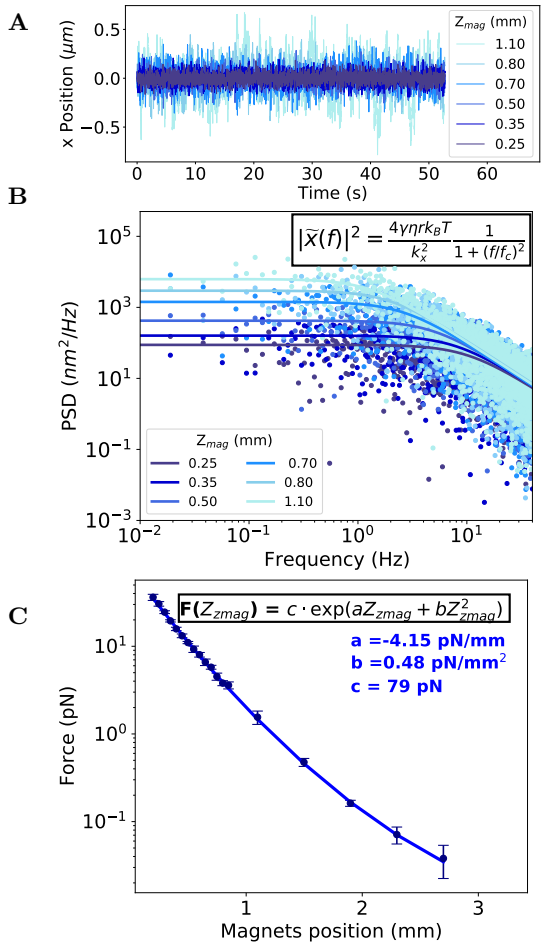

FIG. 2: (A) Bead's x position as a function of time at different Z_{mag} values for the λ DNA molecule. (B) One-sided power spectrum (PSD) for the signals shown in panel A. The continuous lines are Lorentzian fits to Eq.3. (C) Force obtained from as a function of Z_{mag} in a logarithmic representation. The results are fitted to an exponential function, with $a = -4.15 \pm 0.08$ pN/mm, $b = 0.48 \pm 0.04$ pN/mm^2 and $c = 79 \pm 2$ pN.

Modeling the Langevin force as a white noise, with $\langle F_L(t)\rangle = 0$ and $\langle F_L(t)F_L(t')\rangle = 4k_BT\gamma\eta r\delta(t-t')$, one can compute the one-sided power spectrum in the Fourier space as [4]:

$$|\widetilde{x}(f)|^2 = \frac{4\gamma\eta r k_B T}{k_x^2} \frac{1}{1+(f/f_c)^2}, \tag{3}$$

where f_c is the corner frequency given by the inverse of the characteristic response time of the system: $\tau_b = (2\pi f_c)^{-1}$. From the experimental bead's position as a function of time traces, we measure the power spectrum of the x transversal fluctuations and fit it to the Lorentzian function given in Eq.3, getting a value for the k_x to calculate $\langle \delta x^2 \rangle$ from the equipartition theorem: $\frac{1}{2}k_BT = \frac{1}{2}k_x \langle \delta x^2 \rangle$. Next, we measure the molecule extension L_{ext} as the mean bead's z position. Finally, we can directly obtain the force using Eq.1.

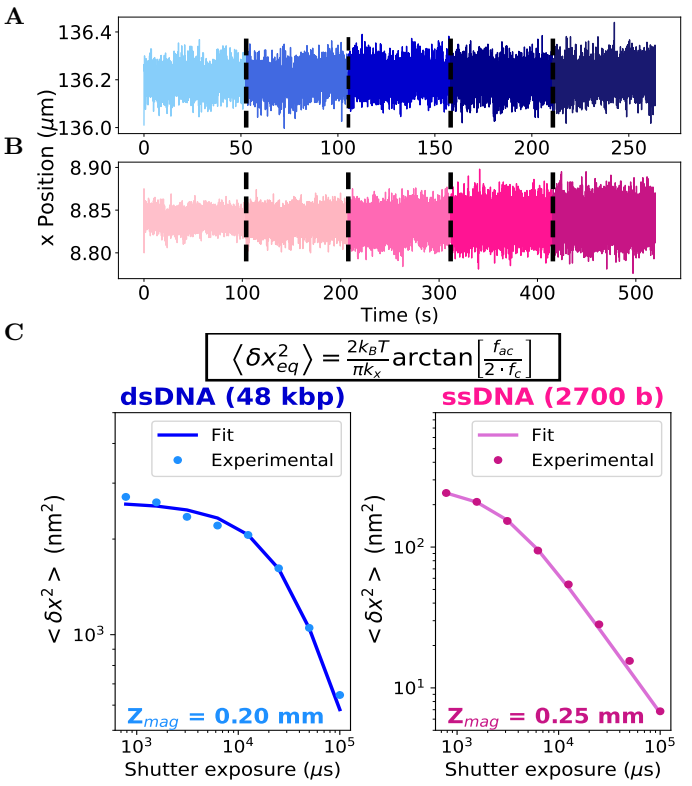

FIG. 3: (A, B) Bead's x position as a function of time for different τ_{sh}, for a λ DNA tether (panel A) and for a ssDNA tether (panel B). The discontinuous vertical lines limit the different τ_{sh} regions. (C) Measured $\langle \delta x^2 \rangle$ at different τ_{sh} for a λ DNA tether at $Z_{mag} = 0.2$ mm (blue) and for a ssDNA tether at $Z_{mag} = 0.25$ mm (pink). Fits to Eq.4 are shown as continuous lines.

A shutter exposure time τ_{sh} much shorter than the response time of the system τ_b is needed to correctly measure the amplitude of the fluctuations. Indeed, the measured transversal fluctuations as a function of τ_{sh} is given by [3]:

$$\langle \delta x^2 \rangle = \frac{2k_B T}{\pi k_x} \arctan\left[\frac{\pi \cdot \tau_b}{\tau_{sh}}\right]. \quad (4)$$

For $\tau_{sh} \to 0$, $\langle \delta x^2 \rangle \to \frac{k_B T}{k_x}$ and $\langle \delta x^2 \rangle$ is correctly measured. In the standard configuration τ_{sh} is set to its maximum value, given by the inverse of the camera acquisition frequency ($\tau_{sh} = 1/f_{ac}$). However, for a fixed τ_{sh} the measured $\langle \delta x^2 \rangle$ might differ from the real value (Eq.4) and so the estimated force. In order to get the best estimation with a finite τ_{sh}, we perform measurements at different shutter times τ_{sh}, going from $\tau_{sh} = \frac{1}{f_{ac}}$ to $\tau_{sh} = \frac{1}{20 f_{ac}}$, while increasing the light illumination to maintain a good imaging. We fit the power spectrum at the different τ_{sh} to get k_x and the amplitude of the fluctuations. We next fit $\langle \delta x^2 \rangle$ as a function of τ_{sh} to Eq.4 to get the asymptotic behavior at $\tau_{sh} \to 0$.

III. RESULTS

A. Force characterization

We have performed a force calibration by measuring the force applied on the λ DNA molecule at different positions of the magnets, Z_{mag}, at the maximum shutter exposure time $\tau_{sh} = 1/f_{ac}$ (Fig.2A). From fitting the power spectrums for the x bead's position we estimate $\langle \delta x^2 \rangle$ (Fig.2B) and from the average z bead's position we obtain L_{ext}. The force is then given by Eq.1 . As shown in Fig.2C the force strongly decreases with Z_{mag} almost exponentially. The measurement of $F(Z_{mag})$ is used as a calibration curve to estimate forces in MT assays.

In order to investigate whether the forces are correctly estimated we have performed the same measurements but tuning the value of τ_{sh}. As discussed in Section "Principle of force measurement", the value of $\langle \delta x^2 \rangle$ depends on the shutter exposure time τ_{sh}, as given by Eq. 4. We then track the bead's position at different τ_{sh} (Fig.3A) and fit the $\langle \delta x^2 \rangle$ results to Eq.4, Fig.3C (in blue). The same measurements have been also carried out with a shorter ssDNA molecule, as shown in Fig.3B and C (in pink). We next compare the forces estimated using the shutter analysis (Fig.3) with those measured in the standard way in which the shutter exposure time is fixed to $1/f_{ac}$ (Fig.2). As shown in Fig.4A the forces measured with the the two methods differ, especially at small Z_{mag} values. This difference is much more evident for the short ssDNA molecule (pink). The reason is that when the molecule is shorter the transversal motion becomes more rapid and the averaging effect due to a finite exposure time is more relevant (Eq.4). In the later case, using a fixed shutter exposure time underestimates $\langle \delta x^2 \rangle$ because the camera integrates the bead's displacement. However, even for the long λ DNA the effect is important for $Z_{mag} < 0.35$, which was not considered significant in previous studies.

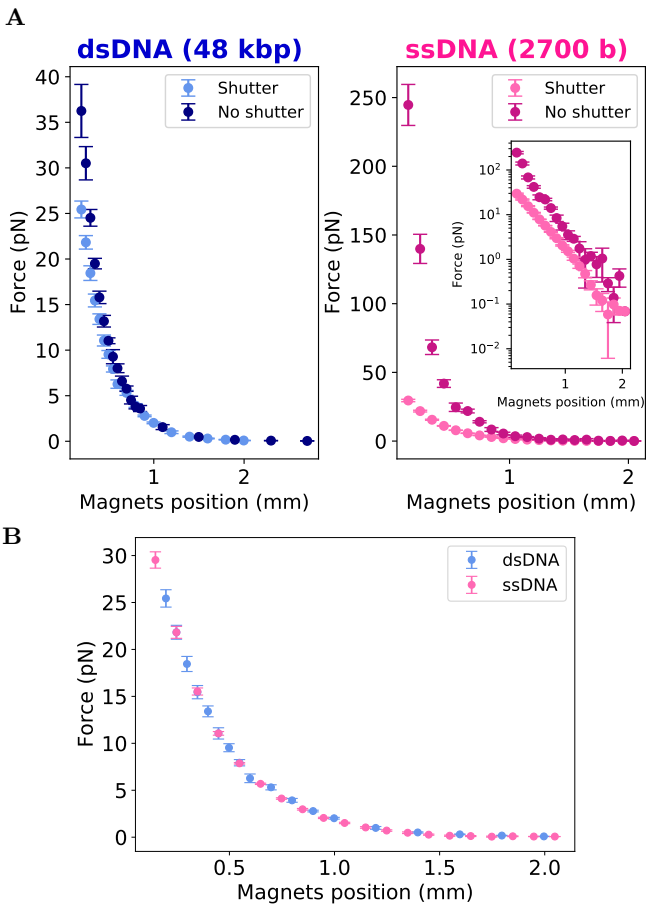

FIG. 4: (A) Comparison between the two force measuring methods: with a fixed and a tunable τ_{sh}. The inset in the right panel shows the ssDNA force measurements in logarithmic scale. (B) Comparison between the force calibrations for dsDNA and ssDNA molecules obtained using the using the shutter analysis.

B. dsDNA versus ssDNA elasticity

Measuring the force versus the extension allows to characterize the elastic response of molecules. In order to compare the dsDNA and ssDNA molecules, that have very different lengths, we represent the force as a function of the extension per base or base-pair. As shown in Fig.5, the elastic response of the two molecules is very different. The dsDNA molecule has less extension at high forces than the ssDNA molecule, due to the compaction generated by base-pairing. Whereas the dsDNA has more extension than ssDNA at low forces, indicating that is more rigid. In order to quantify these results we use the Worm-Like-Chain model (WLC). This is a model widely used to describe the elastic response of polymers, that uses two characteristic lengths: the contour (l) and persistence (p) lengths. The former is the maximum length between monomers (bases for ssDNA and base-pairs for dsDNA) when the polymer is extended. The later is the typical length at which the polymer can bend. For this

model, the relation between the force F and the molecule extension per monomer z_m, $z_m = z/N$ (z being the measured extension and N the number of monomers), can be approximated as [5]:

$$F = \frac{k_B T}{p}\left[\frac{1}{4}\left(1-\frac{z_m}{l}\right)^{-2} - \frac{1}{4} + \frac{z_m}{l}\right]. \qquad (5)$$

As shown in Fig.5, the experimental data can be fitted to this equation, obtaining a contour length for dsDNA that is half of that for ssDNA and a persistence length that is almost 100 times larger for dsDNA than for ssDNA.

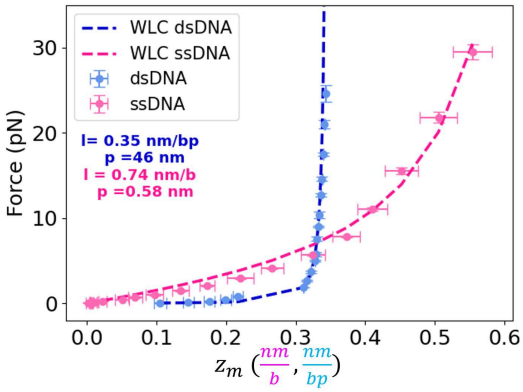

FIG. 5: Force versus normalized molecular extension (extension per base for ssDNA and extension per base-pair for dsDNA) obtained with the tunable τ_{sh}. Fits to the WLC model are shown as discontinuous lines with $l = 0.35 \pm 0.01$ nm/bp and $p = 46 \pm 8$ nm for dsDNA and $l = 0.74 \pm 0.01$ nm/bp and $p = 0.58 \pm 0.04$ nm for ssDNA. The crossover point is around 6 pN.

IV. CONCLUSIONS

We have performed magnetic tweezers experiments to investigate how the shutter exposure time τ_{sh} affects force measurements using two different DNA molecules. We have tracked the 3D position of the DNA-tethered beads. From the measurement of the bead's x position we have extracted the amplitude of the bead's transversal fluctuations and estimate the applied force using Eq.1. The measurements have been performed using a fixed and a tunable τ_{sh}. The comparison between the two methods allows us to determine the quality of the force estimation. We find that the force measurements performed in the standard way (fixed τ_{sh}) differ from those in which the shutter exposure is tuned, specially when the magnets are close to the bead (large forces). This difference is more important for short ssDNA molecule (due to its faster Brownian dynamics). Nevertheless, even for long DNA molecules (such as λ DNA), the correction is significant. As the λ molecule is the typical molecule used to calibrate the force in MT, using the shutter analysis proposed should be consider to obtain a correct calibration. We aim to extend this study by using shorter molecules to investigate the limitations of the method. The correct measurement of forces in shorter molecules may require further reduction in τ_{sh}. However, with the actual LED is not possible reducing further τ_{sh} without compromising the quality of imaging required for a good bead tracking. Consequently, working with shorter molecules might require to implement a more powerful illumination system enabling working with smaller shutter exposure times.

Besides, we have use the force extension measurements to characterize the elastic response of dsDNA and ssDNA molecules, finding the double-stranded DNA form is more compact and rigid than the single-stranded one.

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L'efecte del temps d'exposició de l'obturador en el calibratge de la força en Pinces Magnètiques

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Resum: Les pinces magnètiques són una tècnica de molècula única àmpliament utilitzada en biofísica per investigar processos a escala molecular. L'obtenció de resultats quantitatius es basa en una corba de calibratge, que depèn de la mesura de les fluctuacions transversals de la bead, en la qual la força quedi descrita de forma precisa. La mesura d'aquestes fluctuacions es veu influïda pel temps d'exposició de l'obturador al llarg del qual es fa la mitjana del senyal d'intensitat de la llum. En estudis previs s'ha destacat la importància de reduir el temps d'exposició de l'obturador quan es treballa amb molècules curtes (on les fluctuacions de la bead són més ràpides). Els experiments portats a terme s'han realitzat amb una molècula llarga i una molècula curta de DNA per estudiar l'efecte d'utilitzar diferents temps d'exposició de l'obturador en el calibratge de forces. Hem vist que treballant en condicions estàndards, és a dir, en les quals es maximitza el temps d'exposició de la càmera, la força calculada és altament sobreestimada. Mentre que la modulació del temps d'exposició esdevé una bona estratègia per mesurar correctament les forces sobre molècules llargues i curtes. Finalment, a partir dels nostres resultats hem comparat la resposta elàstica de les molècules de DNA de cadena doble i cadena simple.

Paraules clau: Pinces magnètiques, corba de calibratge de forces, fluctuacions transversals de la bead, temps d'exposició de l'obturador, DNA de cadena simple, DNA de cadena doble.
ODSs: Aquest TFG està relacionat amb els següents Objectius de Desenvolupament Sostenible: Educació de qualitat (4), Indústria/Innovació i Infraestructures (9).

Objectius de Desenvolupament Sostenible (ODSs o SDGs)

1. Fi de les desigualtats		10. Reducció de les desigualtats	
2. Fam zero		11. Ciutats i comunitats sostenibles	
3. Salut i benestar		12. Consum i producció responsables	
4. Educació de qualitat	X	13. Acció climàtica	
5. Igualtat de gènere		14. Vida submarina	
6. Aigua neta i sanejament		15. Vida terrestre	
7. Energia neta i sostenible		16. Pau, justícia i institucions sòlides	
8. Treball digne i creixement econòmic		17. Aliança pels objectius	
9. Indústria, innovació, infraestructures	X		

Resum Visual