Soft magnetic tweezers: A proof of principle

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We present here the principle of soft magnetic tweezers which improve the traditional magnetic tweezers allowing the simultaneous application and measurement of an arbitrary torque to a deoxyribonucleic acid (DNA) molecule. They take advantage of a nonlinear coupling regime that appears when a fast rotating magnetic field is applied to a superparamagnetic bead immersed in a viscous fluid. In this work, we present the development of the technique and we compare it with other techniques capable of measuring the torque applied to the DNA molecule. In this proof of principle, we use standard electromagnets to achieve our experiments. Despite technical difficulties related to the present implementation of these electromagnets, the agreement of measurements with previous experiments is remarkable. Finally, we propose a simple way to modify the experimental design of electromagnets that should bring the performances of the device to a competitive level. © 2011 American Institute of Physics. [doi:10.1063/1.3531959]

I. INTRODUCTION

Force spectroscopy was introduced in biology in the early 1990s, through the development of single molecule micromanipulation techniques, such as optical tweezers (OT), magnetic tweezers (MT), and cantilevers-type techniques (AFM, micropipettes). Since then, the response to force of many biological systems has been studied and the mechanical properties of deoxyribonucleic acid (DNA) and proteins have been extensively characterized (see recent reviews on the techniques and on the main results).

Torque is also an important constraint in many biological processes. The adenosine triphosphate (ATP)-producing enzyme F0/F1-ATP-synthase is a reversible rotary motor, and on a larger scale, bacteria such as E. coli are propelled by rotating a flagella which implies the active generation of torque. As far as DNA is concerned, torque has been demonstrated to be a key element to induce structural transitions in the molecule. DNA torsion is so important that nature has evolved a family of enzymes, generally known as topoisomerases, to control the torsion and entanglement of DNA molecules and apply a torque to the DNA molecule. Enzymes that translocate DNA such as FtsK (Ref. 13), EcoR124I (Ref. 14), and RSC (Ref. 15) can also relax or apply a torque on the molecule as it is moved along. Thus, producing a well defined torsional constraint and measuring its associated torque is an important issue with immediate application to a variety of domains in biophysics and biology.

Although the conception and development of torque-sensitive measurements seem natural in biophysics, their poor signal to noise ratio significantly delayed their implementation. This is quite different from the case of force measurement. To measure a force or a torque on a biological molecule, the translational and angular positions of a small probe attached to one extremity of the molecule need to be determined, while its rigidity is independently measured. The force or torque is given by the product of the rigidity by the displacement. From the fluctuation–dissipation theorem we know that in water, noise arises from the viscous dissipation of the probe. Table I reports the dependence of the Brownian noise on the size of the probe in the case of force and torque. Typical forces acting on DNA are in the pN range, while torque lies in the pNm range. Thus, for a one micron diameter bead torque measurements have at least a 100 times more noise than force measurements. The difference is remarkable, and it is mainly due to the power 3/2 dependence of the noise on the size of the probe.

For this reason, previous attempts of torque measurement on biological objects often relied on the use of two separate systems: one to apply the torsional constraint and/or one to measure the torque. For example, without the need to apply a force, Kinosita and co-workers used either a rigid filament (actin) or a small reporter bead to deduce the torque generated by F1-ATPase. The average torque was obtained by multiplying the friction coefficient of the reporter object by its rotational speed. In a second set of experiments, the same group imposed the angle of the motor in order to control ATP synthesis by the motor, but they did not simultaneously measure the torque required for this chemical synthesis induced by the torque. More recently, Yoshida et al. used electromagnets to impose a specific torque and twist F1-ATPase (Ref. 19) without applying a force.

When both torque and force are relevant the task is more difficult, Bryant et al. twisted the DNA molecule by rotating a micropipette linked to a plastic bead at one end of the molecule, while they pulled on the second end using optical tweezers. They measured torque by recording the rotation of a third reporter bead attached in the middle of the molecule. A slightly simpler solution was proposed by the same group

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using magnetic tweezers to stretch and twist the molecule,\textsuperscript{20} with a small reporter bead to measure the torque. Such devices are very efficient since they used a micron size bead to apply the stretching force (forces scale like $r^3$) and a submicron bead as a rotation reporter. This scheme optimizes the experiment signal to noise. However, since these experiments require a DNA construction involving multiple beads and a rather complex setup, they cannot be performed routinely by nonexpert groups.

Light has also been used to apply a torque on micrometeric objects. Berry and co-workers,\textsuperscript{21} measured a torque using dumbbell optical tweezers holding a bead doublet. By rotating the two traps around each other it is possible to twist the doublet. By measuring the angular lag between the traps and the bead doublet one accesses to torque measurements. Angular momentum of photons has also been used to perform rotation with optical tweezers,\textsuperscript{22,23} and the light-induced torque on absorbing microspheres has been characterized.\textsuperscript{24} More recently, Wang’s group in Cornell designed an experiment\textsuperscript{25,26} where the same particle is used both for imposing the torsional constrain and measuring the torque applied. They use optical tweezers with a linearly polarized laser to pull on the DNA molecule and a birefringent particle which orients itself along the polarization direction. By measuring the induced elliptical polarization in the light passing through the particle they directly measure the small torque applied. To change the angular position the authors slowly rotate the linear polarization of the incoming laser. This setup is very convenient, it uses the DNA molecule bound at both ends and it presents a high torque sensitivity. However, the bead needs to be birefringent and its easy axis must be oriented perpendicular to the stretching direction! Although Wang’s group developed microfabricated quartz rod particle designed for this experiment,\textsuperscript{25} their use, together with the polarized laser tweezers setup, is not simple. Very recently Block’s group at Stanford also published results of torque measurement using optical tweezers with polarized light.\textsuperscript{27}

MT [see Fig. 1(a)] are a micromanipulation technique that can apply forces in the $[0.05 - 100]$ pN range while imposing a torsional constraint on the trapped molecule. It is, thus, natural to develop an extension of the MT in which a torque of desired intensity can be applied. The traditional implementation of the MT (Ref. 28) uses static magnets to create a horizontally oriented magnetic field with a strong vertical gradient. The interaction between the magnetic dipole of the superparamagnetic bead $\vec{M}_b$ and the magnetic field $\vec{B}$

\begin{equation}
\vec{f} = (\vec{M}_b \cdot \vec{\nabla})\vec{B},
\end{equation}

\begin{equation}
\vec{t} = \alpha \vec{M}_b \times \vec{B}.
\end{equation}

The magnetictorque tends to align the direction of the preferential axis of the bead with the magnetic field, while the force is vertical and tends to attract the bead toward the magnets.

The stiff constraint imposed on the angular direction of the magnetic particle in traditional MT experiments prevents both the application of an arbitrary constant torque and the determination of its value. Given the high stiffness (see below), the particle is always subject to a high transient torque that immediately aligns its direction to the external field. In other words, standard MT generate a torque too high, compared to the biological torques one intends to measure.\textsuperscript{30}

Harada \textit{et al.}\textsuperscript{31} and Pomerantz \textit{et al.}\textsuperscript{32} could observe the rotation of DNA around ribonucleic acid (RNA) polymerases

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Biologically relevant typical value & 1 pN & 1 pN nm \\
\hline
Brownian noise density & $r = 0.5 \, \mu$m Bead, $T = 298 \, K$, $\eta = 10^{-3} \, \text{N} \cdot \text{s} / \text{m}$ & $r = 0.5 \, \mu$m Bead and 3 \, $\mu$m DNA \\
\hline
Cutoff frequency $f_c$ & $k_1/2\pi^2 \eta r$ & $k_0/16\pi^2 \eta^3$ \\
\hline
$\eta = 0.013 \, \text{pN} / \text{s} / \text{m}$, & $f_c \approx 78 \, \text{Hz}$ & $f_c \approx 6.88 \times 10^{-3} \, \text{Hz}$ \\
\hline
\end{tabular}
\caption{Comparison of signal, noise, and cutoff frequency for force and torque measurements at the biological scale. Here, $k_B T$ is the thermal energy, $\eta$ the water viscosity, and $r$ the bead radius. Values for Brownian noise density and cutoff frequency are explicitly calculated in the case of a 1 \, $\mu$m bead attached to a 3 \, $\mu$m DNA molecule. The signal to noise ratio is $\approx 500 \times$ higher for force measurement. The typical bandwidth is also $\approx 10^4 \times$ larger for force measurement than torque. Reducing the bead diameter will improve the torque measurement but drastically reduce the maximum force applicable.}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{(Color online) (a) Scheme of the traditional magnetic tweezers setup. Two permanent magnets, separated by a gap $h$, are suspended at distance $z$ above the sample of an inverted microscope. (b) Superparamagnetic bead immersed in a magnetic field develops a magnetization. The small anisotropy of the bead favors the alignment of its easy axis with the direction of the magnetic field. For small angular shifts, the magnetic torque is directly proportional to the shift between the direction of the bead easy axis and the direction of the magnetic field, giving an approximately quadratic angular potential.}
\end{figure}
using a single magnet version of MT. Thus they were able to apply forces while having very low torques, at least smaller than the one produced by the RNA polymerase. Similarly, two different groups\textsuperscript{33,34} have recently suggested an elegant mean to solve this problem: they use a magnetic tweezers configuration where the magnetic field instead of being perpendicular to the force is nearly vertical. In this situation, the magnetization is nearly aligned with the rotation axis and the torque applied just comes from the small nonvertical component and thus can be decreased very much. Arata \textit{et al.}\textsuperscript{35} successfully used such a configuration to directly observe the twisting steps of the human recombinase Rad51. These configurations, however, have a smaller vertical gradient than the conventional magnetic trap and thus provide a limited pulling force.

In this paper we present a technique that, through a reduction of the angular stiffness of the trap, achieves both the application of an external arbitrarily small torque and the direct measurement of such imposed torque. The advantages of this proposal are in its simplicity and its applicability to low force regimes.

II. THEORY

A. Angular potential and torque measurement

Consider a magnetic dipole immersed in a magnetic field [see Fig. 1(b)]. Let $\theta$ be the orientation of the dipole, $\theta_0$ the orientation of the magnetic field and $\phi = \theta - \theta_0$ the displacement angle between the two. For small angles, the orientation of the dipole is subject to an approximately quadratic angular potential, and it will tend to sit at the bottom of a quadratic potential well in the equilibrium position defined by the orientation of the magnetic field. For a small displacement from the equilibrium position the dipole is subject to an elastic torque proportional to the size of the shift:

$$\tau = k_\phi (\theta - \theta_0),$$

(2)

where $k_\phi$ determines the stiffness of the elastic response.

Conversely, if a torque $\tau$ is applied to a dipole sitting in the equilibrium position $\theta_0$, this will produce a rotation to a new equilibrium position $\theta$ such that Eq. (2) holds. Thus, if the equilibrium position and the stiffness are known, a measure of torque is obtained from an angular position measurement. Considering a Langevin description of the system, where a random angular noise acts on the dipole, the equipartition relation can be used to determine the stiffness of the angular potential form the average size of the angular Brownian fluctuations (see Ref. 36):

$$k_\phi = \frac{k_B T}{\langle \delta \theta^2 \rangle}.$$  

(3)

Optimal torque measurements should only be limited by the intrinsic rotational Brownian motion of the bead which is inversely proportional to $k_\phi$. When $k_\phi$ is too large, the rotational Brownian motion may become too small to be measured. Let us then estimate the angular stiffness of a traditional MT.

B. The torsional stiffness of a magnetic tweezers

In this section, we estimate torques and forces exerted by magnetic tweezers to demonstrate that the torsional stiffness is very strong. For the sake of simplicity we shall discuss only the asymptotic cases: low field, where the magnetization of the bead varies linearly with $B$, and high field, where the magnetization is saturated.\textsuperscript{37}

$$M_s(B) = \begin{cases} 
V_b \chi / \mu_0 & (B \ll B_{sat}) \\
M_{sat} & (B \gg B_{sat}),
\end{cases}$$

(4)

where $V_b$ is the volume of the magnetic material of the bead and $\chi$ its magnetic susceptibility. We shall show that in both cases torsional stiffness is directly proportional to force. In fact the bead magnetization can be written $M_s(B) = M_{sat} \mathcal{L}(B/B_L)$ with $\mathcal{L}(B)$ the Langevin function\textsuperscript{38} and $B_L$ the magnetic field value separating the two magnetic field regimes. This leads to the relation $V_b \chi / \mu_0 = M_{sat} / (3 B_L)$ for the low field case. At high field, the high torsional stiffness saturates as demonstrated by Klaue and Seidel\textsuperscript{30} and a different analysis has to be developed. The force exerted by magnetic tweezers corresponds to the derivative of the magnetic energy along the vertical coordinate $z$ while the torque to derivative with respect to the angle $\theta$. The former has a simple expression if we assume, for the sake of simplicity, that the magnetic field decreases exponentially with the distance from the magnets (nearly true in experiments):\textsuperscript{38}

$$B(z) = B_{Max} e^{-z/r},$$

(5)

where $B_{Max}$ is the maximum strength of the field, $z$ is the distance between the magnet and the bead, and $b$ is the decay length of the field $[b$ is typically the gap size of the polar pieces, see Fig. 1(a)]. The magnetic force acting on the bead may be deduced in both regimes from Eq. (1), Eq. (4), and differentiation with respect to the distance variable $z$:

$$F = \begin{cases}
\frac{M_{sat}}{3 B_L} \frac{\partial B(z)}{\partial z} = \frac{M_{sat} B(z)}{3 B_L} & (B \ll B_{sat}) \\
M_{sat} \frac{\partial B(z)}{\partial z} = M_{sat} B(z) / b & (B \gg B_{sat}).
\end{cases}$$

(6)

To obtain force one needs to know the bead properties $M_{sat}$ and $B_L$ and the tweezers magnetic field properties $B_0$ and $b$. Values for Myone (and M450) beads used in this work are, respectively (data obtained from Invitrogen Inc.): $M_{sat} = 25.92 (1450) \times 10^{-15} \text{ A} \cdot \text{m}^2$ and $B_L = 12(8) \text{ mT}$. The electromagnets used here have $B_{max}(f = 0) = 10 \text{ mT}$ for static field and $B_{max}(f = 1 \text{ kHz}) \approx 3 \text{ mT}$ while $b = 1 \text{ mm}$.

Notice that $b$ is large compared to the microscopic scales involved and thus the force is nearly constant as long as the bead does not move by a significant distance ($b \gg L$ with $L$ the molecule length). If the superparamagnetic bead were perfectly center symmetric, its magnetization would tightly follow the magnetic field being completely decoupled from the bead angular position. No torque would then be exerted on the bead. Superparamagnetic beads are made by aggregating very small (5–10 nm) crystals of iron oxide with a random orientation.\textsuperscript{30} Since superparamagnetic materials present
a crystalline anisotropy and also some shape anisotropy, the crystal cluster averages these anisotropies like the square root of the number of small crystals. As this number is in the $10^4$ range, micron size beads always present a small inhomogeneity in their magnetic susceptibility versus the field direction. In a simplified view, the bead susceptibility presents a maximum along one specific direction: the easy direction. In this direction the susceptibility may be written $\chi_\parallel = \chi_0(1 + \alpha)$, while in the perpendicular direction it is $\chi_\perp = \chi_0$. Typically $\alpha$ is of the order of a few percent, thus the bead magnetization is just slightly increased when the magnetic field is aligned with the bead easy direction. A more accurate understanding of $\alpha$ value requires a deeper investigation of the bead magnetic anisotropy which is not necessary for our purposes.

A magnetic field forming an angle $\phi$ with the easy direction leads to a magnetization which is the sum of two vectors: one parallel to the field direction of magnitude $M_b = \chi_0 B$ where $\chi_0 = \chi_0 V_b / \mu_0$ at low forces to recall with the previous notation and a second one along the easy direction given by the vector $\vec{e}_1$ with an amplitude $\alpha \chi_0 B \cos \phi$ [see Fig. 1(b)].

The torque on the bead arises from the vector product of this second component with the magnetic field. Despite the small value of $\alpha$ it produces an extremely strong rotational stiffness compared to molecular torques.

The torque applied to the bead by the magnetic field reads

$$\vec{\tau} = \begin{cases} \alpha M_{sat} B \cos(\phi) \vec{e}_1 \times \vec{B} = \alpha M_{sat} B^2 \sin(2\phi) \vec{e}_z & \text{for } (B \ll B_{sat}), \\ \alpha M_{sat} \cos(\phi) \vec{e}_1 \times \vec{B} = \frac{\alpha M_{sat} B^2}{2} \sin(2\phi) \vec{e}_z & \text{for } (B \gg B_{sat}). \end{cases} \tag{7}$$

The torsional stiffness $k_\phi = \partial \tau / \partial \phi$ is easily related to the applied force using Eq. (6):

$$k_\phi = \alpha F_b \cos(2\phi) \approx \alpha F_b, \tag{8}$$

where the last approximation holds for small angles $\phi \ll 1$. Since $\alpha$ is small, the force is mainly produced by the first anisotropization component aligned with the field. The second anisotropic component adds a minor modulation to the force. Note that the force depends only slightly on $\phi$: $F(\phi) = F_0[1 + \alpha \cos(2\phi)]$ while torque is directly proportional to $\alpha$.

Since one needs to apply a force of the order of $1 \text{ pN}$, in the case of small angle $\phi$ the torsional stiffness will be this force multiplied by ($\alpha b$). The parameter $b$ is in the millimeter range, leading to an estimated torsional stiffness value of $k_\phi = 10^4 \text{ pN} \cdot \text{nm} / \text{rad}$. (This value is in agreement with Klaue and Seidel who measured a torsional stiffness ten times higher at a force of $10$–$20 \text{ pN}$.)

The minimal angular resolution for micron size objects depends on the measuring technique but it is typically limited to $\theta = 0.01 \text{ radians}$. Combined with the high rotational stiffness, this limits torque measurement to values considerably too strong as compared with biological values.

The focus of the present study is now clear: torque measurement via Eq. (2) needs a reduction of the stiffness of the MT, so that the rotational Brownian motion becomes accessible. Once this is done, the stiffness can be calibrated using Eq. (3) and the equilibrium position can be independently determined, so that a direct correspondence between the angular position of the bead and the applied torque is obtained.

Our device achieves this purpose and it can be used to study DNA under constant torque or constant torsion, depending on the protocol used. With this picture in mind, we can proceed to a step by step description of the protocol used to produce soft magnetic tweezers.

III. SOFT MAGNETIC TWEEZERS

Reduction of the torsional stiffness while keeping a finite stretching force (in the pN range) on the system is achieved by using a nonlinear coupling regime of a damped rotator system.

First, we shall show that in this regime the system behaves as a constant torque generator. However, one usually prefers to impose rotational position while measuring torque. We shall discuss later how to achieve this goal.

A. Finite constant torque generator

Using Eq. (7), the equation describing the motion of a magnetic bead in a viscous medium (where inertia terms can
where the angular velocity of the magnetic field is increased and a synchronous rotation would induce too large a friction [see Appendix C].

Above a threshold velocity, the bead can follow the rotation of the magnetic field in a perfectly synchronous manner (this behavior corresponds to the normal use of a compass). The second behavior appears when the angular velocity of the magnetic field is increased and a synchronous rotation would induce too large a friction [see Appendix A for the detailed calculation]. The first one is the synchronous mode where the bead follows the rotation of the magnetic field in a perfectly synchronous manner (this behavior corresponds to the normal use of a compass). The second behavior appears when the angular velocity of the magnetic field is increased and a synchronous rotation would induce too large a friction [see Figs. 3(a) and 2]. Above a threshold velocity, the bead cannot follow the magnetic field any longer and it exhibits a new behavior with a motion made of a fast oscillations combined with a slow rotation: this phenomenon has already been described\(^{39-45}\) and we call it “slippage” to indicate that the orientation of magnetization vector of the paramagnetic bead is no longer coupled to the orientation of the bead itself. This happens because the bead is immersed in a viscous fluid, exerting a drag that depends on the angular velocity of the bead.

This effect was characterized for superparamagnetic beads by Janssen et al.\(^{46}\)

For practical reasons in many experiments we did not use an isolated bead but rather two beads attached one to the other: a bead doublet. Figure 2 shows the dependence of the average angular velocity of a bead doublet as a function of the magnetic field angular velocity.

In the synchronous mode (slowly rotating field) the bead easy direction and the magnetic field form an angle \(\phi\) such that the magnitude of the torque applied increases and it compensates the viscous drag. The motion is described by

\[\theta(t) = \Omega t - \phi,\]

where \(\chi(t) = 2\gamma \Omega \theta(t)\) and \(\gamma\) is the rotational viscous drag coefficient (we do not consider here the influence of the surface) and \(\Omega\) is the angular velocity of the magnetic field. The factor 8 is valid for a sphere, but it becomes higher for other configurations (see Appendix C).

Equation (9) has two characteristic regimes\(^{39-42}\) (the interested reader is referred to Appendix A for the detailed calculation). The first one is the synchronous mode where the bead follows the rotation of the magnetic field in a perfectly synchronous manner (this behavior corresponds to the normal use of a compass). The second behavior appears when the angular velocity of the magnetic field is increased and a synchronous rotation would induce too large a friction [see Figs. 3(a) and 2]. Above a threshold velocity, the bead cannot follow the magnetic field any longer and it exhibits a new behavior with a motion made of a fast oscillations combined with a slow rotation: this phenomenon has already been described\(^{39-45}\) and we call it “slippage” to indicate that the orientation of magnetization vector of the paramagnetic bead is no longer coupled to the orientation of the bead itself. This happens because the bead is immersed in a viscous fluid, exerting a drag that depends on the angular velocity of the bead.

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optimal, because it requires an infinitesimal response time that cannot be achieved by the electromagnets. A smoother inversion is obtained introducing a Hanning window modulation of the amplitude of the rotating field, such that it goes to zero when rotations reverse and it presents a maximum at the middle of the rotation phases.

This procedure adds an extra high frequency torque modulation (the rotation switching frequency); this modulation is considerably damped by the viscous drag of the bead especially when the rotation frequency and its modulation component are set to high values. This procedure also adds a small modulation on the stretching force at high frequency but again this effect has a magnitude proportional to \( \alpha \), and it is not a problem if the modulation frequencies are high enough. Ideally, we obtained a condition where there is no angular potential and the trap does not impose a preferential direction to the bead easy axis. However, the practical implementation of such scheme reveals the imperfections of our experimental device. For instance, a small off-centering of the magnets breaks the rotational symmetry inducing a preferential orientation for the bead. Once most experimental defects are compensated we are left with a very small stiffness along a preferential direction (see Fig. 6).

### C. Fixed angular position with torque measurement

In Sec. III A we showed how to obtain a constant low torque configuration and in Sec. III B how such torque can be canceled on average, yielding magnetic tweezers which impose no angular constraint (zero average stiffness). Here we pledge to reintroduce a small angular potential along a defined direction, in order to be able to impose a rotational constraint and to obtain a torque measurement by means of Eq. (2).

Such a goal is achieved by superposing an elliptical deformation of the magnetic field amplitude to the previously described alternating modulation [see Fig. 3(c)]. This breaks the rotational symmetry of the configuration and it generates an approximately quadratic angular potential centered along the ellipse major axis. Rotating the ellipse we rotate the bead and adjusting the ellipticity we tune the rotational stiffness, i.e., the curvature of the potential. A small ellipticity leads to a very low rotational stiffness \( k_\theta \) ideal for torque measurement via Eq. (2).

With low torsional stiffness, one must not rotate the major axis of the elliptical modulation too fast otherwise the bead will not follow. Using this nonlinear effect we completed the first step in the procedure outlined in Introduction, i.e., we reduced the trap stiffness to a calibrated small value.

### D. Angular position measurement

To complete the procedure outlined above, we need to be able to track the angular position of the probe bead in real time. Various methods may be used for this purpose. A very simple scheme consists in using the fact that the attachment point is usually off-centered. The bead center follows a circle as the bead rotates. This elegant method suffers from two limitations: (i) the bead center is also affected by the translation Brownian fluctuations which must be smaller than the circle radius for unambiguous measurements; (ii) if the bead rotates fast enough, it spontaneously recenters as the rotation frequency approaches the translational cutoff frequency of the bead molecule system.

Alternatively, smaller beads can be attached to large ones, providing an anisotropic image that facilitates angular position tracking. For the purpose of this proof of principle, we used two beads stuck together, the previously named doublet, rotating about one molecule [see Fig. 4(b)], the angular position is then easily deduced tracking the centers of the two beads. The limitation of this method is the larger viscous drag of the bead doublet.

The angular position measurement obtained with two beads tracking is affected by the center tracking error, which is of the order of the size of 0.05 pixel. If the distance between the two centers is small, two unwanted effects appear: (i) a huge uncertainty on the value of the angle and (ii) the angular fluctuations are polluted by noise from the bead displacement leading to inaccurate values of the stiffness obtained via Eq. (3). The magnification of the microscope has to be chosen so that the distance between the two centers is much larger than the pixel size for this effect to be negligible.

### IV. IMPLEMENTATION

The soft magnetic tweezers apparatus is implemented substituting the permanent magnets of magnetic tweezers with electromagnets. Other characteristics are the same as in standard MT. Briefly, a glass chamber is prepared, fixed on the stage of an inverted microscope, and illuminated with a parallel light beam using a red light emitting diode (LED) as a source. Electro-magnets are placed just above the chamber in order to provide a vertical pulling force and a horizontally aligned magnetic field. Light is collected through a 100 × 1.25 oil immersion microscope objective (Olympus, France) and the image is formed on 25 Hz charge coupled device camera (Sony, France). DNA is attached to the bottom of the glass chamber on one end and on a paramagnetic bead on the other end. The image of the bead is used to track its position in the three dimensions.

If a static current is applied to the electromagnets, this apparatus behaves as the traditional MT. It is possible to change the orientation of the magnetic field by using an alternative current with the desired phase between the different coils. This allows rotating the magnetic field at higher frequencies than the MT, and the absence of moving parts also eliminates mechanical vibrations. The current necessary to generate a magnetic field of desired intensity is provided to the six coils by six power amplifiers that take an input signal generated by a computer controlled DAC card [see Fig. 4(c)].

The six coils (Lima 600880, Vicenza, Italy) [see Fig. 4(a)] are disposed on the six corners of a hexagon. They are made of copper wire and have a resistance of 10 ± 2 Ω. Each of them encloses a cylinder of mu-metal (Goodfellow, Cambridge, U.K.) slightly longer than the coil. These six polar pieces are inserted in a soft steel cap on the top and bent toward the center at the bottom. The XC15 soft steel cap (Tonnetot Metaux, Fontenay-sous-Bois, France) is designed to close the field lines in the system, thus increasing the...
magnitude of the magnetic field. Finally, the bent tips are also
inserted in a PMMA ring which confers overall stability to
the ensemble.

The coils are driven by six voltage amplifiers [see
Fig. 4(c) for a schematic diagram of one channel]. We
preferred a voltage feedback to a current feedback because
the signal appeared to be more stable in this configuration. We
modulate the current driving the coils as explained in Sec. III.
The details of the algorithm are reported in Appendix B.
The resulting signal has a frequency of 1–2.5 kHz and it is
modulated in amplitude and phase at 100–250 Hz. At these
frequencies, the coil inductance dominates and the voltage in-
put to the coils is adapted to maintain the current at the desired
level.

The voltage signal is generated by a digital to analog
output computer board (NI-PCI6733, National Instruments,
USA), which generate six analog signals between −10 and
10 V for the coils, with a maximal sampling frequency
of 1 MHz. We use memory buffer to prepare the various
modulations. Active regeneration of this buffer is used in
order to produce a signal with a frequency in the range of
1–10 kHz, modulated in amplitude and shape in real time
without interruption. Since the new samples are calculated
in real time, the signal can be modified with an update
frequency given by

\[ f_u = \frac{2 f_s}{N} \]

where \( f_s \) is the sampling frequency and \( N \) is the number of samples transferred to the
 card at each update.

All routines are written in C using the NIDAQmx API
(National Instruments, USA). The interested reader is referred
to Appendix A for the details of the generation of the rotating
magnetic field signal.

![FIG. 4. (a) Scheme of the electromagnets. The coils are disposed on the cor-
ner of a hexagon. (Figure adapted with permission from Ref. 47.) The soft
magnetic material is made of permalloy rods. (b) Image of the bead doublet
inserted in a PMMA ring which confers overall stability to
the ensemble.

The coils are driven by six voltage amplifiers [see
Fig. 4(c) for a schematic diagram of one channel]. We
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All routines are written in C using the NIDAQmx API
(National Instruments, USA). The interested reader is referred
to Appendix A for the details of the generation of the rotating
magnetic field signal.

![FIG. 5. (Color online) (a) Comparison of the angular fluctuations of a 4.5 µm
bead doublet in the traditional MT and in the soft MT. In the latter case, the
amplitude of Brownian fluctuations is increased by a two orders of magnitude
demonstrating the stiffness reduction. (b) Averaged power spectral density of the
two trajectories.

V. CHARACTERIZATION

A. Stiffness calibration

Figure 5 shows a comparison between the angular fluctu-
ations a 4.5 µm bead doublet in a static MT and in the soft
MT. The dramatic increase in the size of fluctuations con-
firms that the stiffness of the trap is reduced by about two
orders of magnitude. Due to the implementation with the el-
liptical field, the stiffness of the asynchronous trap can be
continuously tuned from high to low values by varying the
ellipticity parameter \( e \). Figure 7 shows the linear relation be-
tween the stiffness and the ellipticity parameter. The nonzero
minimum value obtained indicates the presence of a residual
magnetic field due to mechanical imperfections in our setup
and it is not a problem for the following measurements, its
value being very low.

B. Torque calibration

The elliptic field implementation, as outlined before, al-
lows us to arbitrarily choose the orientation of the minimum
of the angular potential well.

Different calibration schemes are possible, a simple one
consists in calibrating the torque against viscous drag and
checking that this calibration is independent of the tweezers
stiffness. We can, thus, perform an absolute torque calibration
with a synchronous detection measurement as follows. First
we reduce the stiffness of the trap. Then we let the orientation
of the ellipse major axis vary between two angular position
in a sinusoidal fashion. The average orientation of the bead
doublet will follow the modulated signal with a phase shift \( \phi \)
subject to the condition:

\[ \tan \phi = \frac{\gamma \Omega}{k_0}, \]  (11)
FIG. 6. (Color online) The angular stiffness of the trap can be obtained from the angular fluctuations trajectory through the equipartition relation. The stiffness is plotted here as a function of the ellipticity imposed on the rotating field. As expected, reducing the ellipticity reduces the stiffness of the trap to very low values. Inset: a very small stiffness remains even for null ellipticity. Probably due to geometrical imperfections of the trap, the residual stiffness size is negligible for the purposes of the device.

where $\Omega$ is the oscillation frequency of the ellipse main axis, $k_\theta$ is the imposed stiffness of the trap [determined through Eq. (3)], and $\gamma$ is the viscous damping coefficient due to the presence of the fluid. The latter is independent from the stiffness used to measure it and can be extracted by plotting the quantity $(k_\theta \tan \phi)$ as a function of the oscillation frequency.

Figure 7 shows that the value of such viscous component is insensitive to the value of the stiffness imposed and thus proves that an absolute measurement of the viscous coefficient is possible with our device.

C. Validation on DNA

We proceed to the final step in the validation of our device, performing an absolute measurement of a biological torque. DNA has a chiral geometry and its twist rigidity has been characterized in a number of experiments. In particular, a torsionally constrained DNA molecule, subject to an external pulling force, is expected to respond with a torque that increases linearly as a function of the number of turns added to the molecule. When the number of added turns overcomes the critical value for buckling, the torque stabilizes to a fixed value and it remains constant.

DNA molecules (5.4 $\mu$m long, see Ref. 51 for details) were attached to superparamagnetic beads and incubated in the flow cell using the procedure described in Ref. 52. Angular deviations from the equilibrium position were measured as a function of the number of turns applied. As previously described, in order to facilitate angular tracking we searched for DNA molecules attached to bead doublets.

In Fig. 8 we compare the experimental torque signal of a 4.5 microns bead doublet (spiky line) with the size of the biological torque we intend to measure (straight line). This clearly demonstrates that the torque Brownian fluctuations are enormous, due to the large beads used in this proof of principle. This really unfavorable signal to noise ratio requires long time averaging. The experimental procedure was, thus, designed as follows: a molecule attached

FIG. 7. (Color online) Viscous torque extracted from synchronous detection measurement. The extracted torque does not depend on the strength of the angular potential used in the measurement. The linear fit yields the viscosity constant. This curve was measured with a doublet composed of a 4.5 $\mu$m bead and a 2.8 $\mu$m bead rotating about the central axis of the larger bead. The expected geometric coefficient for this configuration is $g \approx 19.75$ very close to the measured value $g \approx 21$.

FIG. 8. (Color online) Size of torque fluctuations for a 4.5 microns bead doublet obtained by means of Eq. (2), after $k_\theta$ has been calibrated. The straight line represents the size of biological torque we intend to measure. The poor signal to noise ratio implies long averaging times.
to a bead doublet is identified, a fixed force is imposed, and
the extension of the molecule versus rotation at fixed force is
measured, this produces a bell shaped curve that presents a
maximum when the molecule is torsionally relaxed, we then
use the position of this maximum to fix the rotation reference.
Then the angular fluctuations of the beads are recorded when
−75, −45, −15, 15, 45, 75 turns are added to the molecule.
Each position was maintained for about 7 min and then 30
turns were added to switch to the next position. The whole se-
quence was repeated 100 times in order to accumulate enough
statistics to refine the sampling of the average position. The
use of bead doublets significantly increased the relaxation
times of the system (viscous drag for a two bead system is
not $8\pi\eta r^3$ but $28\pi\eta r^3$, see Appendix C). The choice of inte-
gration times of 7 min is a compromise between the need to
average the angular fluctuations long enough and the risk of
introducing low frequency $1/f$ noise in the measurement due
to thermal drifts.

In Fig. 9 we compare the measured values of absolute
torque as a function of rotation with other measurements ob-
tained either with OT (Ref. 50) or from an estimation from
MT data51 for comparable values of applied force and similar
buffer conditions. The agreement between the three different
measurements supports the validation of our technique.

VI. DISCUSSION

The current limitation of our apparatus consists of the
long time required for measurements. Figure 9 required
3 days of continuous acquisition on a single DNA molecule.
This limitation is severe if the action of an enzyme is to be
studied in real time, but fortunately it is mainly due to
technical factors. Table I reports the dependence of the cut-
off frequency for Brownian fluctuations, which also depends
on the inverse of the cubic radius of the bead. In our case,
the use of a bead doublet for ease of tracking and the high
viscous drag of the large $4.5 \mu m$ beads conspired to enor-
mously increase the averaging time. The use of smaller size
single beads will dramatically improve this aspect. Neverthe-
less, smaller beads will also experience smaller forces. So in
order to keep the forces as high as possible a better design of the
electromagnets is required.

Indeed most of these limitations come from the inap-
propriate electromagnets that have been originally designed
for slow varying field. A reduction of the pulling force is
observed when the rotation frequency of the magnetic field
is increased to values higher than 1 kHz. This could be due
to the inductance of the coils that depends on the frequency
of the alternating current and also to Foucault dissipative
currents developed in the metallic cores. With the current
implementation of the device, it was not possible to apply a
pulling force to beads smaller than $4.5 \mu m$ diameter. A new
magnets design should overcome this.

Instead of using a permalloy plain rod we intent to use
magnetic cores suitable for medium frequencies in the
form of thin metallic sheets or an aggregate of iron iso-
lated particle. Coils should be made of thicker wires with
less turns in order to reduce the inductance. As described
above, the present electromagnets have very poor tweezers
properties: $B_{\text{max}}(f = 0) = 10 \text{mT}$ for static field and $B_{\text{max}}(f
= 1 \text{kHz}) \approx 3\text{mT}$ while $b = 1 \text{ mm}$. This is a design based
on an old MT configuration with poor performance com-
pared to the current implementation of standard magnetic
tweezers. In fact, we have recently improved permanent
magnets design reaching $B_{\text{max}}(f = 0) = 250 \text{mT}$ and $b =
0.3 \text{ mm}$ boosting the maximum force by more than a fac-
tor $25$.53 We need to perform the same kind of work for
the electromagnets. It is difficult to evaluate the performance
of such a new design, but there is no reason to suppose
that electromagnets could not produce a force of at least
1/10 of that obtained with permanent magnets as it was the
case for the design used here. Thus, a force of 1 pN for a
one micron bead should be attainable. In such a case, the
noise level of our system would decrease by a factor $4.5^{3/2}\approx
10$.

Another limitation of the current setup is the heat devel-
oped by the coils when high frequency fields are used. This
heat produces thermal drifts of the sample and complicates the
longitudinal tracking required for force measurement. Typi-
cally a bead will drift of $0.5 \mu m$ in 20 min. Differential track-
ing between the beads of interest and a bead fixed on the glass
surface will reduce this effect by a factor 10 leading to small
error in molecule extension. However, the angular tracking
will only work in a reduced range of focus position, ($\approx 2 \mu m$)
and thus the vertical drift will not allow very long tracking
without refocusing. This problem will also partly be improved
with new magnets that will not produce as much heat as the
current cores.
In conclusion, we built a new apparatus that allows the application and measurement of torques of biological size. Our device is an extension of the magnetic tweezers apparatus and it was validated with the measurement of torque on a single DNA, showing a remarkable correspondence with data obtained with other currently available methods. Technical improvements are on the way in order to overcome the limitations of dynamic range.

APPENDIX A: APPROXIMATE SOLUTION OF EQ. (A5)

A simple view of the slippage phenomenon is obtained in the limit where the magnetic field rotates very fast. In this case we can treat the rotating field as a perturbation and look for a solution of Eq. (9) in the form

$$\theta(t) = \theta_0 + \epsilon \theta_1(t) + \epsilon^2 \theta_2(t) + \ldots.$$  \hspace{1cm} (A1)

The perturbation solution is easily obtained rescaling the time variable from \( t \) to \( t' = \Omega t \). Equation (9) becomes

$$\dot{\chi}(t) = \frac{A}{\Omega} \sin[2(t - \chi(t))],$$  \hspace{1cm} (A2)

with \( \chi(t) = \theta(t)/\Omega \). Increasing the value of \( \Omega \) can be viewed as effectively decreasing the value of the coupling constant \( \epsilon \) that appears before the sine. For \( \Omega \to \infty \) the coupling constant tends to zero.

We then look for a perturbation solution with the form, correct to second order in \( \epsilon = A/\Omega \):

$$\chi(t) = \chi_0 + \epsilon \chi_1(t) + \epsilon^2 \chi_2(t) + \epsilon^3(\text{epsilon}^3).$$  \hspace{1cm} (A3)

The L.H.S. of Eq. (A2) becomes

$$\dot{\chi}(t) = \epsilon \dot{\chi}_1(t) + \epsilon^2 \dot{\chi}_2(t) + \epsilon^3(\text{epsilon}^3),$$  \hspace{1cm} (A4)

while the R.H.S. becomes

$$\epsilon \sin[2(t - \chi(t))] = \epsilon \sin[2(t - \chi_0)] - 2[\epsilon \chi_1(t) - \epsilon^2 \chi_2(t) - \ldots].$$

We then expanded \( \sin[2(t - \chi(t))] \) using the identity \( \sin(a - b) = \sin(a) \cos(b) - \cos(a) \sin(b) \) and then approximated the sine and cosine for small values of \( \epsilon \). The approximate solution is calculated at the desired order in \( \epsilon \) by grouping the terms of the same order. Here we consider only terms up to order \( \epsilon^2 \), obtaining

$$\chi(t) = \chi_0 - \frac{\epsilon}{2} \cos[2(t - \chi_0)] + \frac{\epsilon^2}{2}(t - \chi_0) + \frac{\epsilon^2}{8} \sin[4(t - \chi_0)].$$  \hspace{1cm} (A6)

The average angular velocity of the bead is obtained considering the derivative with respect to time of the linear term: \( \bar{\omega} = A^2/(2\Omega) \).

APPENDIX B: ROTATING FIELD CONSTRUCTION

In order to apply a determined magnetic field, a relationship between the voltage applied to each channel and the total magnetic field must be established. Consider a couple of opposed electromagnets. A static magnetic field can be generated providing opposite continuous currents to the two coils. The generated magnetic field intensity will be proportional to the electric current passing through the copper coils, which is determined by the voltage applied and the impedance of the solenoid. The total magnetic field generated by the three couples of magnets can be viewed as a linear combination of three magnetic fields generated by coupled opposite pairs of solenoids. The six electromagnets are grouped in three couples of inverse polarities. This allows us to drive the six electromagnets with three signals, reducing the number of output channels required.

1. Rotating phase

In order for the slippage phenomenon to appear, the magnetic vector field \( B \) has to be rotated sufficiently fast for the viscous drag to prevent the magnetic bead from following. The rotating phase \( \theta(n, t) \) is defined as a function of time and of the number of turns \( n \). The rotating phase is the junction of two pieces: a uniform antiviscous motion for \( n \) turns, followed by a uniform antiviscous motion:

$$\theta(n, t) = \begin{cases} 4n\pi(t - |t|), & t - |t| < 0.5, \\ 4n\pi(2 - t - |2 - t|), & t - |t| > 0.5, \end{cases}$$  \hspace{1cm} (B1)
where \( \lfloor t \rfloor \) means Floor\([t]\), i.e., the integer part of \( t \) (for positive \( t \)).

This form of rotating field is not sufficient to reduce the angular stiffness of the trap to the desired value.

2. Amplitude modulation

The cosine amplitude modulation is defined as

\[
B(t, b) = 1 - b \cos\left(\frac{2\pi(t - \lfloor t \rfloor)}{0.5}\right).
\]

This depends on a parameter \( b \in [0, 1] \), which allows to switch continuously from no modulation to full modulation.

Here, \( b = 0 \) means no modulation.

3. Ellipse deformation

Figure 10 shows the curve described by the magnetic field vector in XY plane.

An elliptical distortion of this figure is defined as

\[
E(e) = \begin{pmatrix}
1 + e & 0 \\
0 & 1 - e
\end{pmatrix}.
\]

And to allow for arbitrary rotation of the axes of the ellipse, the matrix

\[
T(e, \eta) = R(\eta)E(\eta)R(-\eta)
\]

\[
= \begin{pmatrix}
1 + e \cos[2\eta] & e \sin[2\eta] \\
e \sin[2\eta] & 1 - e \cos[2\eta]
\end{pmatrix}
\]

can be applied to the rotating field vector. The relevant parameters are \( \eta \) controlling the angle formed by the ellipse major axis with the X axis and \( e \) controlling the ellipticity.

**APPENDIX C: APPROXIMATE CALCULATION OF THE GEOMETRIC VISCOS DRAG FACTOR**

A sphere that rotates about its axis with angular velocity \( \omega \) in a viscous fluid is subject to a viscous torque:

\[
\tau_c = 8\pi \eta R^3 \omega.
\]

(C1)

If the axis of rotation does not pass through the center, but it is at a distance \( d \), the torque generated by the fluid is still proportional to the cube of the radius of the sphere, to the angular velocity, and to the viscosity but the geometric factor will be larger than 8. A first order estimate of the geometric factor for this configuration can be obtained considering the viscous force acting on a sphere that moves with a linear velocity \( \nu \) in the fluid:

\[
F_t = 6\pi \eta R \nu.
\]

(C2)

The linear velocity of the center of mass of the off-centered sphere is \( \nu = od \), and the torque corresponding to the viscous force is \( \tau_t = d F_t \). Thus, the total viscous torque acting on the sphere is

\[
\tau = \tau_c + \tau_t = 8 + 6\left(\frac{d}{R}\right)^2 \pi \eta R^3 \omega = \gamma \pi \eta R^3 \omega.
\]

(C3)

This formula allows us to determine the geometric factor \( \gamma \) for various special configurations. A maximally off-centered sphere, for which \( d = R \) will give \( \gamma = 14 \). A bead doublet rotating about its center of mass has \( \gamma = 28 \). A bead doublet rotating about the center of one of the two beads has \( \gamma = 40 \). Finally, the configuration of a large 4.5 \( \mu \)m bead with a smaller 2.8 \( \mu \)m bead attached, rotating about the axis of the larger one, is subject to a viscous torque \( \tau \approx 19.75 \pi \eta R^3 \), where \( R \) indicates the radius of the larger bead.

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