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The measurement of light momentum shines the path towards the cell

La medida del momento de la luz ilumina el camino hacia la célula

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ABSTRACT:

After an intense development of optical tweezers as a biophysical tool during the last decades, quantitative experiments in living cells have not found in this technique its best ally, due, in part, to the lack of a standard method to measure forces in complex environments. The existent alternatives either require complicated in situ calibrations, which make their use impossible in the study of dynamic processes, or they lack accuracy. Using an approach completely different from the most extended options, Steven Smith at Carlos Bustamante's Lab at the University of Berkeley developed a method based on the direct measurement of the momentum change of the trapping beam, which has the potential to become the standard for measuring forces in all kind of experiments. Measurements are performed regardless of the physical properties of the sample or the trapping laser, and they only depend on some parameters of the instrument design. As a consequence, the method does not need a continuous calibration and can be used in a wider range of experiments. However, its diffusion has been modest mainly because it requires a counter-propagating optical trapping system, which is difficult to implement and combine with other techniques. Here, we show how this method can be implemented in the more extended trapping configuration, optical tweezers, and how this relates to the well-known position detection technique, back-focal-plane interferometry. We finally discuss the potential of this method to perform experiments inside cells and also for commercial purposes, to make optical trapping available to non-experts.

Key words: Optical Trapping, Optical Tweezers, Optical Manipulation.

RESUMEN:

Después de un intenso desarrollo de las pinzas ópticas como herramienta biofísica durante las últimas décadas, los experimentos cuantitativos en células vivas aún no ha encontrado en esta técnica su mejor aliado, debido, en parte, a la falta de un método estándar para medir fuerzas en entornos complejos. Las alternativas existentes o bien requieren complejas calibraciones in situ, lo cual puede hacer su uso imposible en el estudio de procesos dinámicos, o bien son poco exactos. Utilizando una aproximación completamente diferente a las opciones más extendidas, Steven Smith, en el laboratorio de Carlos Bustamante de la Universidad de Berkeley, desarrolló un método basado en la medida directa del cambio de momento del haz de atrapamiento que tiene el potencial para convertirse en el estándar de medida de fuerzas, no sólo en este tipo de experimentos sino también en general. Las medidas se realizan independientemente de las propiedades físicas de la muestra o del láser de atrapamiento y sólo dependen de algunos parámetros del diseño del instrumento. Así, el método no precisa de una calibración continuada y puede ser usado en un abanico más amplio de experimentos. Sin embargo, su difusión ha sido modesta, principalmente porque requiere de un sistema de atrapamiento óptico contra-propagante, que es difícil de implementar y combinar con otras técnicas. Aquí, mostramos como este método puede ser implementado en la configuración de atrapamiento más extendida, las pinzas ópticas, y cómo se relaciona con la conocida técnica de

detección de posiciones, interferometría en el plano focal trasero. Finalmente, discutimos el potencial de este método para realizar experimentos dentro de células, y también con un propósito comercial, para hacer accesible el atrapamiento óptico a los no expertos.

Palabras clave: Atrapamiento Óptico, Pinzas Ópticas, Manipulación Óptica.

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1. Introduction

Optical traps are a technique developed in the early 1970s by Arthur Ashkin [1]. Taking advantage of the momentum of light, he showed the possibility of moving micron-sized particles without mechanical contact. Using the recently discovered laser, he could confine enough power to a sufficiently tiny spot to accelerate latex particles within a suspension fluid. Despite its success, however, the actual development of the technique did not take place until the discovery, sixteen years later, of a particular trapping configuration, called optical tweezers [2]. Ashkin himself realized that when the same laser was focused by a high magnification lens, such as those used in microscopy, he could not only exert forces on particles but also stably trap them at the focal region (Fig. 1). This is how optical manipulation began.

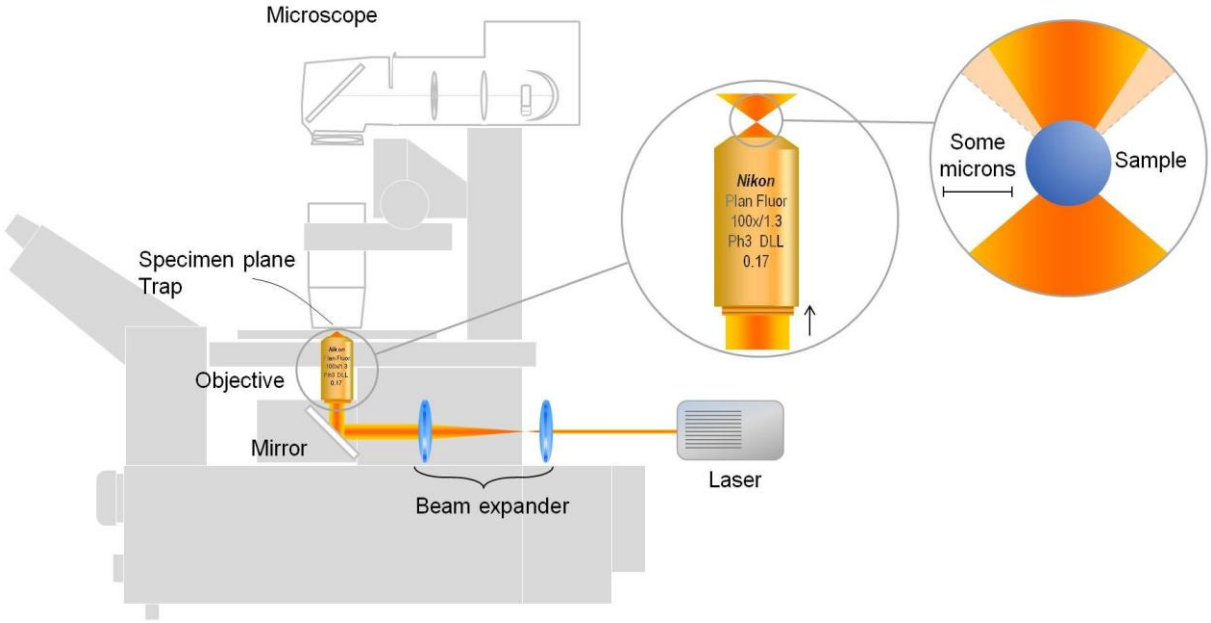

Fig.1. Layout of an optical tweezers system.

Soon after that, optical tweezers started being used by other groups, and a new aspect of the technique arose. The force exerted by the laser in an optical trap depends on the position of the sample inside the trap. For small displacements of the particle, force is proportional to the position of the object, so when the conversion factor between both magnitudes is determined, optical tweezers can be used as a dynamometer to measure the forces exerted on samples.

The range of forces that optical traps are capable of exerting is in the order of 0.1 – 100 pN, which are indeed the typical forces governing interactions between molecules, so the technique rapidly found a good acceptance in the field of molecular biology. One particularly interesting application that quickly showed up was the use of optical tweezers to manipulate intracellular structures inside cells, such as vesicles or organelles. Nevertheless, the difficulty to measure forces in this complex and changing environment prevented the technology from providing further results in this direction.

The technique has been so far increasingly adopted by research groups in different fields and has now gained a lot of popularity, especially in molecular and cellular biophysics. Many advances have been achieved and have equipped optical tweezers with a complete manipulation and measurement toolbox.

One of the recent improvements has been the development of new technology to measure forces inside cells, which was one of the remaining challenging questions. As a result, the interest in experiments within living cells has remarkably increased in the last few years. Despite the enormous progress, at present the problem is still the lack of a standard method to reliably and easily measure these forces in such a complex environment to make this kind of applications available to non-experts. Here, we propose a method based on the direct measurement of the light momentum change to extract the force exerted inside a cell without an in situ and tedious calibration. The method, in addition, allows performing other interesting experiments that were not available before, and has an important commercial application since it eliminates the calibration of the system, which is one of the obstacles for biologists or biophysicists.

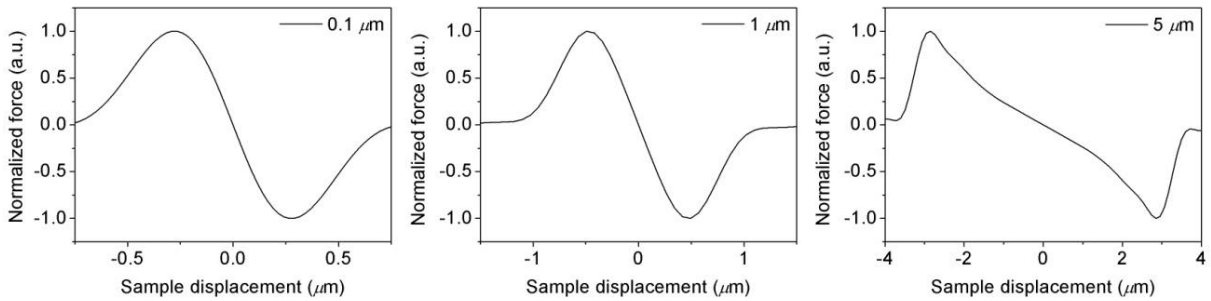

Fig.2. Computer simulation of the optical force produced by a infrared laser focused with a NA=1.2 objective onto microspheres of different diameter. Forces are computed as the particles are moved in the transverse direction across the laser focus.

2. Optical forces

Forces in optical tweezers are measured from the linearization of the force. The optical force produced by a laser trap can be computed from the integration of the Maxwell stress tensor, **T**:

$$\langle \vec{F} \rangle = \int_V \langle \nabla \cdot \vec{T} dv \rangle = \oint_S \frac{n}{c} I(\hat{n}) \hat{n} ds \,, \qquad (1)$$

where the integration is performed over a spherical surface, *S*, surrounding the object, with normal vector **n**. By applying the Gauss's theorem, we can show that the force is ultimately due to the change in the light momentum, reflected in the changes of the intensity pattern $I(\theta, \varphi)$ when the laser interacts with the sample. This interaction depends on the relative distance between the focus and the particle, so one can extract a force profile for different displacements of the sample.

Synthetic latex or silica microspheres have been traditionally used as handles to manipulate the samples by chemically attaching them to the desired part of the molecule or cell. If we compute the force profile for a microsphere, we can observe that, despite that the shape of the curve changes for different sizes (see Fig. 2) or refractive indexes, for example, there is always a region close to the equilibrium position $(x = 0, F = 0)$ where force and position are proportional [3,4]. This linearization can be then used to easily infer forces from sample displacements. The problem of measuring forces in the range of some piconewtons turns into the search of a clever procedure to measure positions with resolution of some nanometers.

3. Measurement of positions

The first attempts to measure forces taking advantage of the harmonic approximation of the force was in the early 1990s, when the potential of optical traps to study the mechanical properties of motor proteins was explored. After different improvements, the original idea grew into the currently called back-focal-plane interferometry (BFPI), which is the most standard method to measure positions with optical traps. The recent advances in imaging technologies, such as high-speed video cameras, has made this option an attractive alternative, although it still has some limitations for its broad adaptation compared with BFPI. In particular, back-focal-plane interferometry provides a higher position resolution (in the order of 1 angstrom) and a higher temporal bandwidth (up to megahertz) with a small cost.

In this technique, a condenser lens is used to collect the laser light scattered by the sample, responsible (as shown in Eq. 1) of the optical force exerted thereon. The captured light is redirected towards a Si or InGaAs photodetector that records the small changes in the intensity pattern. As shown by Gittes and Schmidt [5], the variations in the light distribution at the back focal plane of the collecting lens change linearly with the displacements of the trapped particle, so when the sample is scanned across the laser beam in the transverse direction, the voltage, Sx, shows a linear region for positions close to the origin (see Fig. 3).

Once the conversion factor between volts and microns is determined, the instrument can be used to measure positions and ultimately forces by virtue of the linear relation between both.

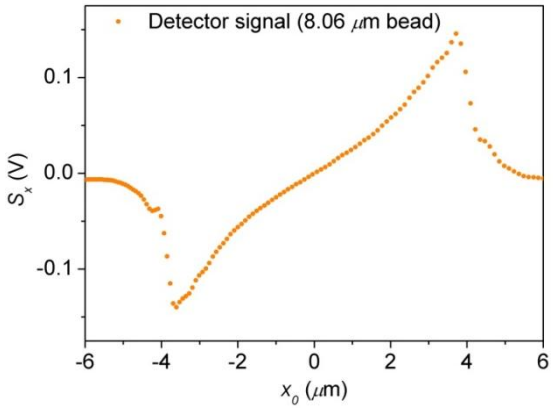

Fig.3. Detector response, S_x, for different positions of an 8 μm microsphere in a direction perpendicular to the axis of propagation of the laser.

4. Force calibration

The calibration of forces in an optical trap, that is, the determination of the trap stiffness, κ, is one of the most cumbersome steps, as it has to be routinely performed and it entails some difficult analysis. The spring constant, which relates positions and forces, is a parameter that depends on the geometric and optical properties of the sample, as well as on the structure of the trapping beam (see Fig. 4).

This strong dependence on the experimental conditions makes the calibration of this parameter essential prior to measuring the force in a new experiment. In addition, not only the stiffness, but also the position calibration factor changes with the sample, so both need to be always measured.

There are different methods to calibrate the spring constant [6], but some of them are barely used due to the associated errors. The most widely spread option is based on the analysis of the motion of the trapped microsphere in the potential well created by the laser, either due to the action of the thermal energy or by applying a known force onto the sample. More specifically, one typically analyzes the power spectrum of the fluctuations in the detector signal due to the Brownian motion of the particle to obtain the spring constant from a fitting of the results to a Lorentzian function (see Fig. 5) [7].

The fit has two free parameters that allow to determine both the stiffness, κ, and the position conversion factor from volts to micrometers, β. Thus, from the same experiment one can extract the two constants needed to measure forces:

$$F_X = -\kappa x = -\kappa\beta V_X \,. \qquad (2)$$

Besides the tedious procedure of calibrating the position and the force, the main drawback of this approach is the limited variety of experiments that can be tackled. In particular, there are some requirements that have to be met in order to apply this method. The calibration can only be performed with homogeneous spherical particles of known size in a viscous medium. Measurements, for example, in a complex environment, such as the cytoplasm of a cell, are not feasible since the medium does not have a viscous behaviour.

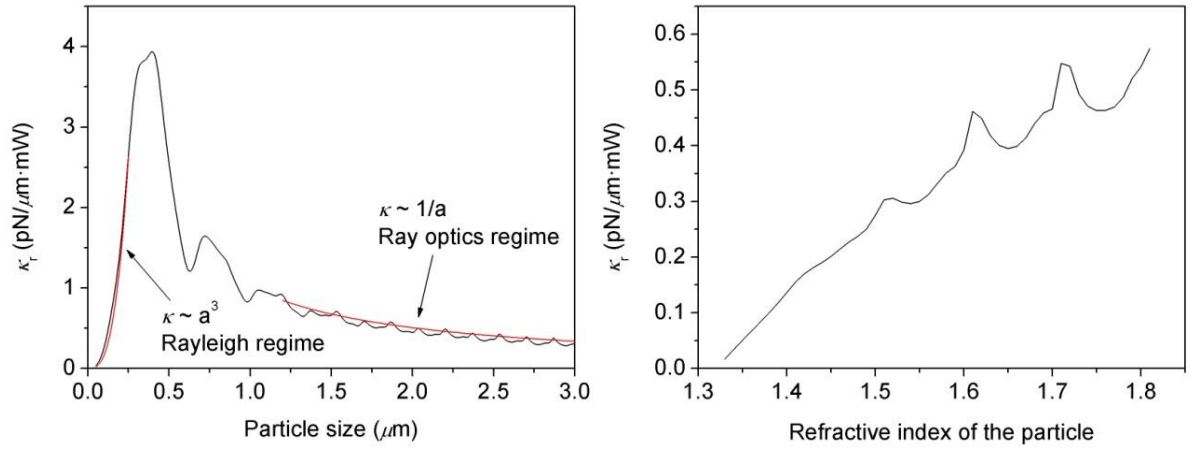

Fig.4. Dependence of the trap stiffness with particle size and refractive index of the sample.

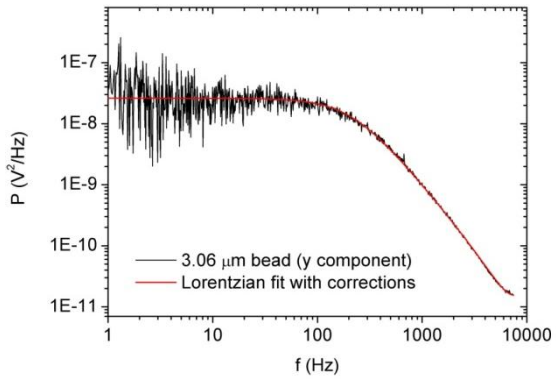

Fig.5. Example of a typical power spectrum of the detector signal for a trapped particle moving due to the thermal energy.

Different strategies have appeared in the recent years to overcome these limitations. One of these proposals exploits the measurement of the momentum change of the light of the trapping beam to obtain a robust calibration measurement valid in a wider range of conditions.

5. Momentum measurements

This method was originally devised for counter-propagating beam traps, a much more complex configuration than optical tweezers (or single-beam traps). In this scheme, two lasers propagating in opposite directions are used to trap objects at their coincident focuses. This alternative approach has been barely used due to the more difficult implementation and limited possibilities when combined with other techniques, such as fluorescence.

The method, developed by Smith *et al.* [8], is based on the detection of the light momentum change at the back focal plane of a collecting lens, similarly to the configuration used in back-focal-plane interferometry with optical tweezers. The difference between the two methods is that in order to implement the momentum measurements, the light scattered by the sample has to be captured in whole to record the correct change in momentum, and this was believed to be impossible for the high-numerical aperture beams used in optical tweezers. The solution of Smith *et al.* was to use low-NA beams (for which the stable trapping is not possible) and combine them in a counter-propagating geometry. The high-NA version of the method for optical tweezers was restricted to the measurement of positions, where it was not necessary to collect all the light leaving the trap, although this was at the expense of a previous calibration of the trap before every experiment, as explained earlier.

The existence of the two methods as separate options has remained intact until recently, when

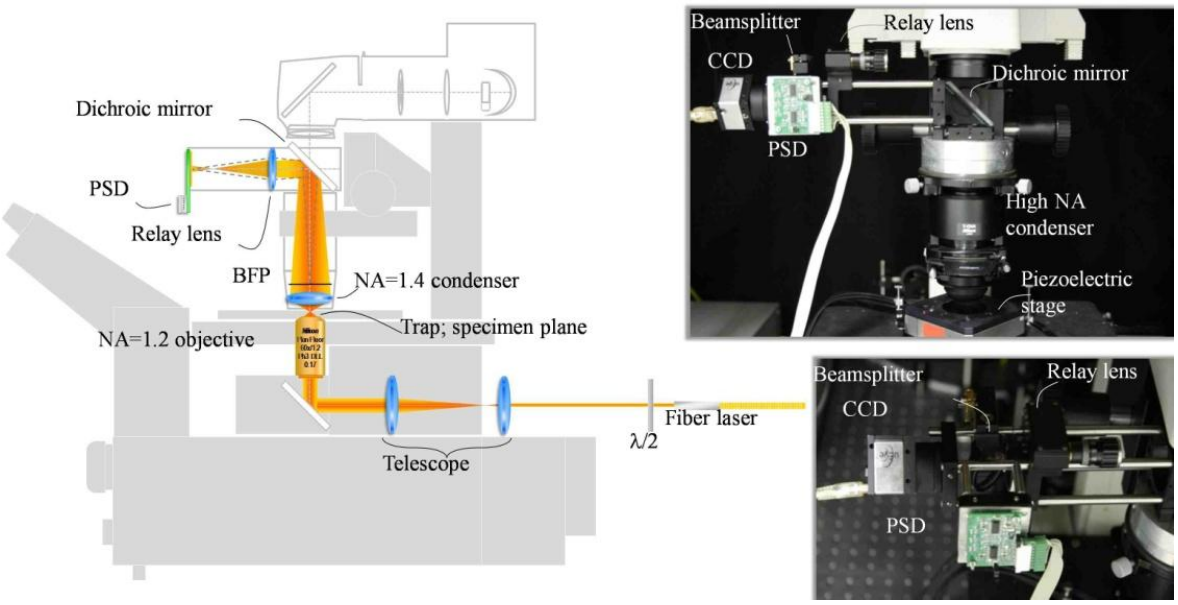

Fig.6. Layout of the detection scheme.

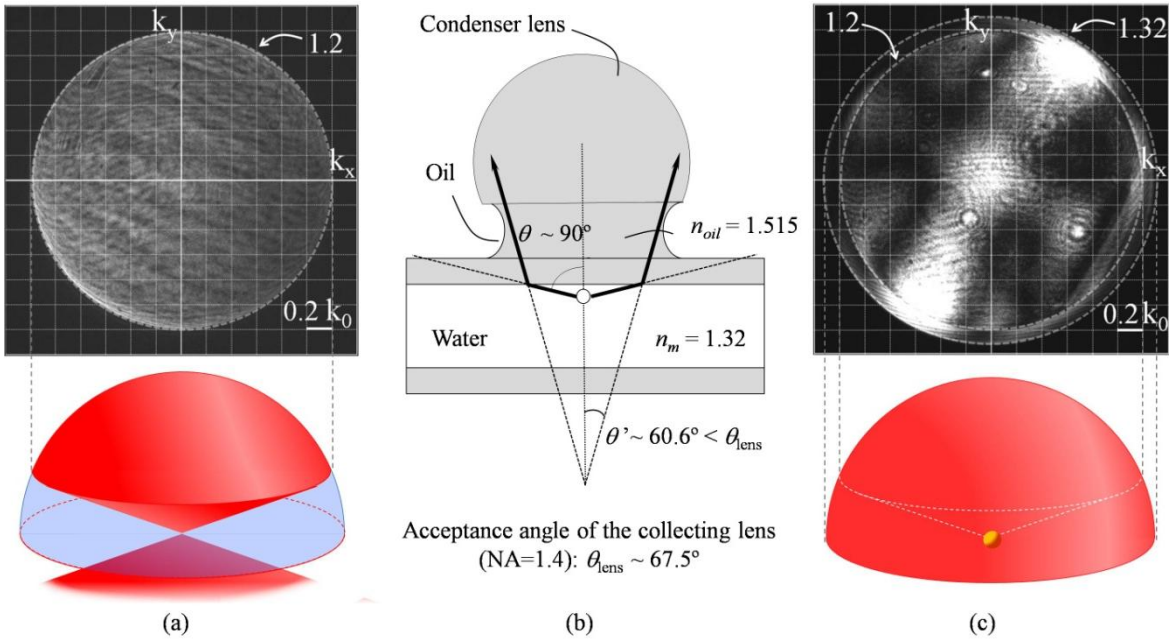

Fig.7. (a) Light pattern at the back focal plane of the collecting lens when there is no trapped sample. The disk has a diameter given by the numerical aperture of the objective (NA = 1.2). In this case, there is no light propagating at larger angles. (b) Detection configuration for the implementation in optical tweezers. When the sample is trapped close to the upper glass surface of the microchamber (within 50 μm, approximately) and the collecting lens has a numerical aperture larger than the refractive index of the suspension medium, all the light propagating in the forward direction (even at ~90º) can be captured. (c) Light pattern when a particle is held in the optical trap. Scattering at angles of ~90º show up as a second disk with an associated numerical aperture of 1.32, which corresponds the refractive index of water.

we showed that momentum measurements can actually be implemented in optical tweezers [9-12]. There are some requirements in the setup used to capture and analyze the light, but it is essentially the same as a back-focal-plane interferometry instrument (see Fig. 6). In short, a lens collects the laser light from the trap and a relay lens system projects an image of the light pattern at the back focal plane of the first lens onto a position sensitive detector, PSD.

Light is collected by the condenser lens used for illumination of the sample. The back-scattered contribution, which is discarded in our measurements, has typically a small impact on the total force, since it represents a tiny fraction (1-3%) of the whole power. Only the forward-scattered light is captured and analyzed to obtain the momentum measurement. In order to get a correct measurement from this forward-scattered light, the system must fulfill some conditions. The lens, as well as the rest of the optical design, must follow the Abbe sine condition to assure that the momentum structure of the beam is correctly observed at the detector plane. In this case, the Fourier transform relation between the back focal plane and the specimen plane can be extended to the largest angles at the sample, θ. Then, besides a constant factor for the whole plane, positions on the detector correspond in fact to transverse components of the momentum, $p = x/\lambda f'$.

On the other hand, the numerical aperture of the collecting lens must be larger than the refractive index of the suspension medium, so all the light propagating in the forward direction is captured by the lens, as it refracts to a smaller cone at the water-glass interface (see Fig. 7). For the same reason, the particle has to be trapped close to the upper surface of the microchamber to avoid the loss of light before being refracted at the glass surface.

A further requirement of the instrument is that the photodiode must be a position sensitive detector and not a quadrant photodiode, typically used for position measurements, as this kind of sensors does not measure the exact position of the center of mass of the light distribution; it measures a derived magnitude which can change from sample to sample.

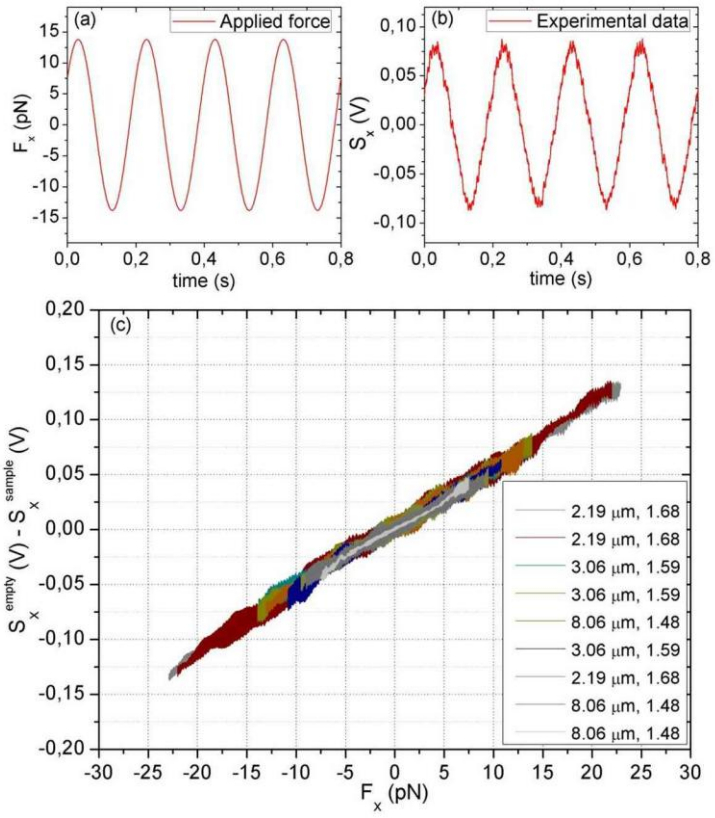

Fig.8. (a) Viscous force applied to the trapped samples. (b) Recorded signal. (c) Relation between both magnitudes for different experimental conditions.

When all these conditions are fulfilled, the instrument has a conversion factor between voltage and force that is constant and permanent regardless of the physical properties of the sample. Figure 8 illustrates this property in an experiment where viscous forces of known magnitude were exerted onto different trapped particles, and the signal from the photodetector was plot against the applied forces. We can observe that the relation between both scales is the same for different samples, despite that we were using microspheres of varying sizes and refractive indexes and the laser power changed between experiments.

The existence of such a fundamental calibration between the instrument signal and the optical force is thanks to the actual nature of this detection method. As shown by Smith *et al.*, deflections of the beam, $\langle\theta\rangle$, due to the interaction with a trapped object recorded by a position detector at the back focal plane of a collecting lens (where the Abbe sine condition is fulfilled, $x' = f'n\sin\theta$, f' being the focal length of the lens and n the refractive index of the suspension medium), correspond in fact to measurements of the optical force.

$$S_X = \frac{S_{SUM}}{R_D}\langle x'\rangle = \frac{S_{SUM}}{R_D} f' n \sin\langle\theta\rangle = \\ = \frac{\psi P}{R_D} f' \frac{cF_x}{P} = \frac{\psi f' c}{R_D} F_x \propto F_x , \quad (3)$$

where S_{SUM} is the total power recorded by the sensor, R_D its radius, P the laser power at the sample and ψ the relation between laser power and detector response in volts. We can observe that the voltage is proportional to the force through different parameters that are related only to the instrument design, so the conversion from volts to piconewtons is in this case independent of the sample properties.

Then, in contrast to how the principle of back-focal-plane interferometry was initially demonstrated, sample displacements, *x*, should be understood as a derived magnitude of the momentum measurements [11]. Positions are

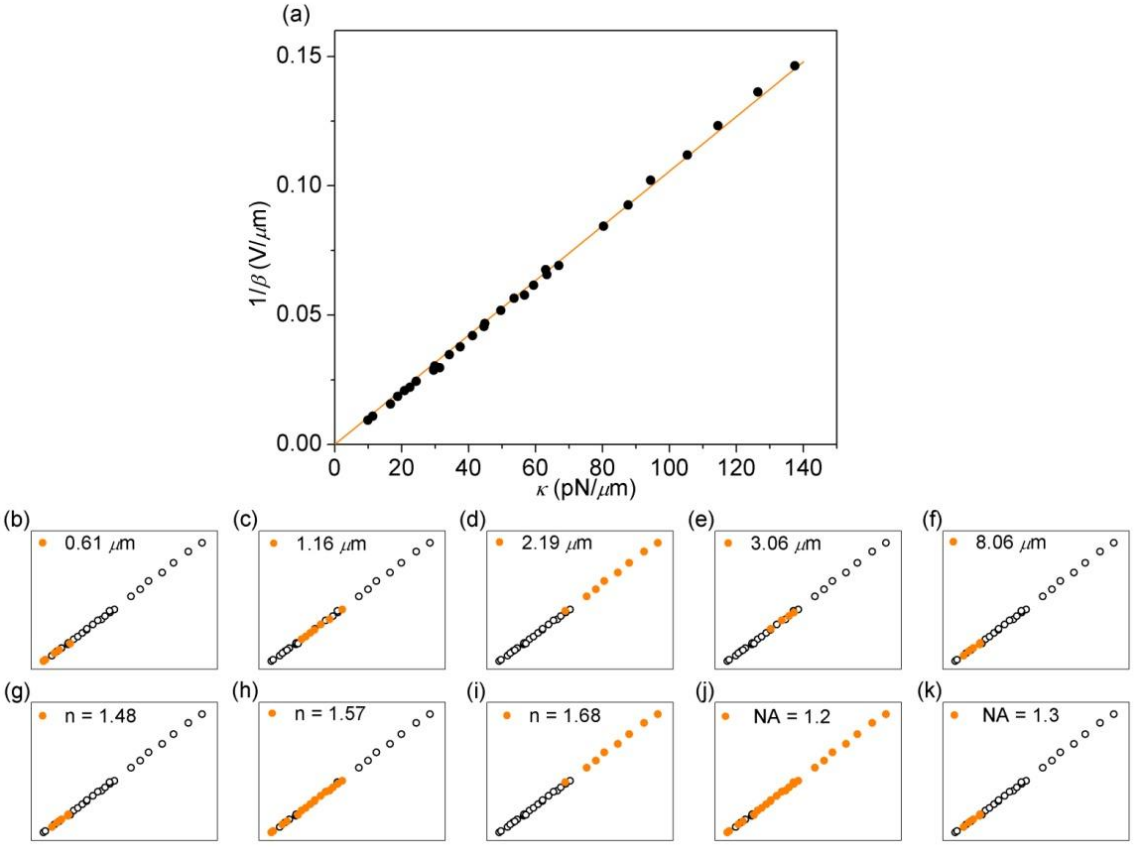

Fig.9. Linear relation between position sensitivity and trap stiffness for a wide range of experimental conditions.

available due to its linear relation (in the harmonic region) with the force:

$$x = \frac{F_x}{\kappa} = \frac{R_D}{\kappa \psi f' c} S_x = \beta S_x \propto S_x \,. \qquad (4)$$

In order to show that the position calibration factor β is in fact derived from the trap stiffness κ, we calibrated the instrument using the power spectrum analysis of the thermal motion of trapped microspheres, and we plotted one parameter against the other. The result is shown in Fig. 9. We can observe how there is a perfect proportionality between both magnitudes for different particle sizes (0.61 μm, 1.16 μm, 2.19 μm, 3.06 μm, 8.06 μm), different refractive indexes (1.48, 1.57, 1.68) and different numerical apertures of the trapping objective (1.2, 1.3).

Equation 4 indicates that, in addition, the constant relating stiffness and position calibration can be determined from the measurement of parameters related with the detection instrument, namely the detector radius, the instrument response and its focal length:

$$\alpha_{trap} \equiv \kappa\beta = \frac{R_D}{\psi f' c} \equiv \alpha_{detector} \,. \qquad (5)$$

For our apparatus, we measured these parameters to compute $\alpha_{detector}$ and compared it with the product of the two constants $\kappa\beta$ for different experimental conditions, and found a perfect agreement between them (see Fig. 10), both in the *x* and *y* directions.

The results confirm that force measurements based on the detection of the light momentum can be implemented in optical tweezers through an optimized version of a back-focal-plane interferometry instrument. Positions can also be measured by virtue of the linear relation with force for small displacements of the sample. The properties of the momentum measurement are retained as long as the requirements for this are fulfilled. The deviation from the ideal conditions lead to a worsening of the quality of measurements.

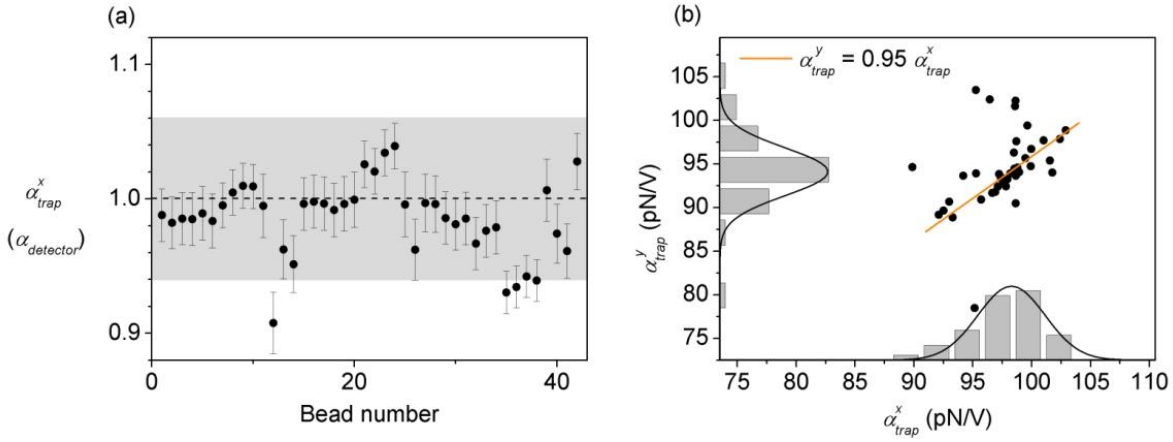

Fig.10. (a) The value of κ·β equals the force calibration for different samples, (b) both in *x* and *y*.

6. Conclusions

These results allow us to establish a method to measure forces that does not require an *in situ* calibration prior to performing experiments. Although it is an optimized version of the well-known back-focal-plane interferometry position detection technique and it hence shares many points with it, the possibilities offered by that the new method are very promising. In particular, the absence of previous calibration opens the door to the experimentation *in vivo*. The measurement of forces inside cells is very complicated and it was impossible until recently. The requirement of a complex calibration inside the cell makes this kind of experiments difficult and has partially prevented the field of optical trapping from developing in this direction. However, there is an increasing need for performing molecular biology experiments inside the natural environment of molecules, the cell, since discrepancies between results obtained *in vitro* in the past years have been attributed to the different experimental conditions used by the groups. In this direction, we have recently shown that momentum measurements can be easily extended to *in vivo* conditions [13,14]. We calibrated the instrument following a different approach and found that the absolute force calibration remained constant, even when biological particles such as vesicles and organelles were used to measure forces inside a non-viscous medium like the cytoplasm of the cell.

A second important application of the force method explained here is its capability to bring optical trapping closer to biologists and biophysicists, since the need of an expertise in the calibration of forces, which is one of the main obstacles for a non-expert, is reduced. The commercialization of this technology could in the near future make optical trapping become a mainstream tool in fields like molecular biology. The possibilities that force measurements based on this method can offer still need to be explored, but the problems that can already be solved stimulate us to continue investigating [15,16].

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