Force calibration inside living cells with *Lunam*™ T-40i by IMPETUX

Introduction

The experimentation inside living cells with optical tweezers is finding an increasing interest due to the possibility of remotely exerting and measuring forces on internal components of the cell. However, these measurements entail certain difficulties mainly because of the complex and varying environment inside the cell. The difficulty to extract valuable information about the microscopic motion of an internal probe makes accurate force calibration particularly challenging. This has attracted a special attention during the last years.

The increasing interest in this convoluted problem and the lack of a standard method to obtain reliable outcomes demands new approaches capable of circumventing the traditional limitations of the typical procedures.

Lunam™ T-series by IMPETUX

The technology developed and patented by *Impetux* [1] represents a rupture with the established paradigms in force measurements. The classic indirect determination of trap calibration from sample displacements has proved to provide a precise route to the force, giving good results for many applications. Furthermore, information about position and stiffness is in many cases as important as the force itself. Nonetheless, it has shown very limited when an additional degree of complexity is required.

To capture the advantages of both methods, *Lunam*™ T-40i (Fig. 1) incorporates a dual-measurement system: the traditional calibration approach [2] and, an additional mode based on the detection of the laser beam deflection [1], which offers the possibility of determining forces in more complex conditions since it does not require an *in situ* calibration.

As we will show next, the possibility of measuring forces through these two complementary methods provides us a more powerful approach as they can be combined in a unique solution to obtain very accurate measurements.

Fig. 1. *Lunam*™ T-40i sensor head.

Results

Lipid droplets inside A549 cells were trapped with the laser, and force was calibrated by an active-passive method [3]. Using rheological information from recordings of actively-displaced organelles in the viscoelastic medium, we could obtain a conversion factor from sensor output voltage to force units for different organelles (Fig. 2). From the set of parameters describing the motion of the trapped organelle in response to the external perturbation induced by moving the whole sample with a piezoelectric stage, we could indirectly determine a force calibration that was repeated more than 40 times for different particles. The mean value of these measurements was compared to the absolute force calibration of the instrument, α (Fig. 2):

$$\frac{2k_BT}{P(\omega_S)}\frac{A_P}{\omega_S A_S}\sin(\Delta\phi)=\alpha \qquad (1)$$

where k_B is the Boltzmann constant, T the sample temperature, $P(\omega_s)$ the power spectral density of the passive recording at the oscillation frequency of the stage, A_p and A_s the oscillation amplitude of the particle

and the stage, respectively, and Δϕ the delay between both motions.

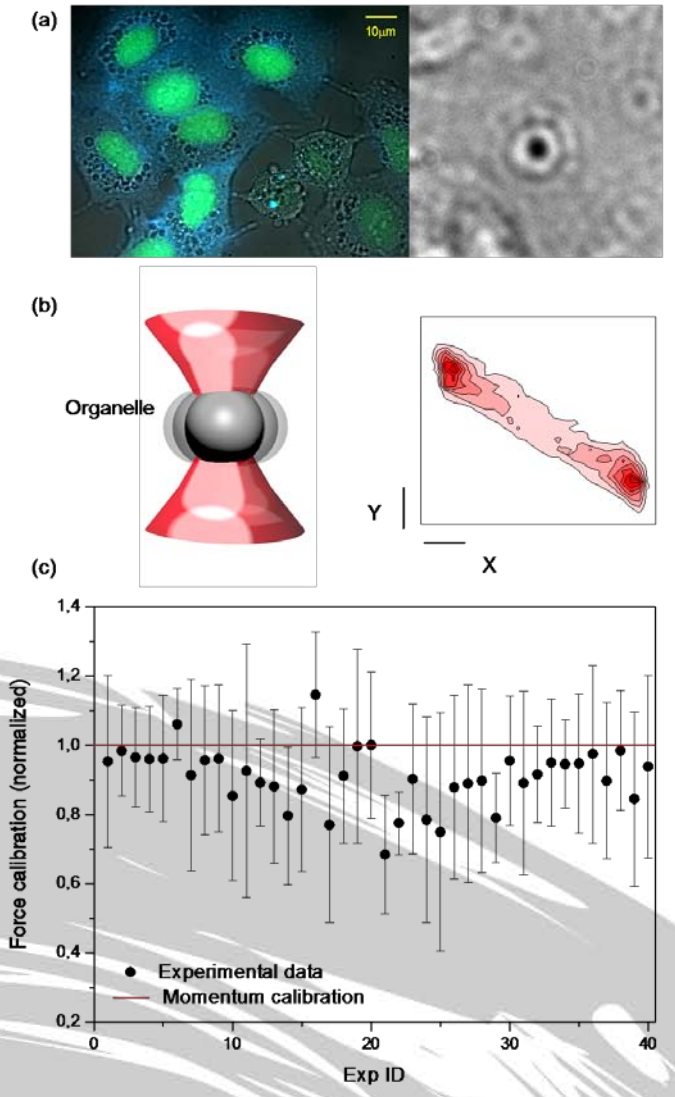

Fig. 2. (a) A549 cells and a zoom into the trapped lipid droplet. (b) From the sample response to an external perturbation, lipid droplets were used as force-sensing probes to extract the conversion from volts to piconewtons (using the left-hand side of Eq. 1). (c) The experimental results were found to be compatible with the absolute calibration of the instrument. Force measurements were normalized to the absolute calibration, α.

Discussion

In light of the results, we can infer two important conclusions for measuring forces inside complex and changing environments such as cells. The data show that complementary approaches relying upon totally different physical models of the system provide compatible calibrations. The determination of the stiffness using the statistical analysis of the microscopic displacements of the probe reveals, rather than a random distribution, the existence of a predictable law that relates the applied force and the sensor output voltage, regardless of the trapped organelle and the trapping region in the cell. Within the standard deviation of the data, the calibrations match a unique and constant factor which, in addition, corresponds to the force calibration based on the beam deflection analysis (a discrepancy of less than 10% is observed).

On the other hand, because trap calibration depends on microscopic information about the interplay of the probe with its surroundings, force readings can be deeply affected by the heterogeneity and the variation of the sample. Although the distribution of data followed a constant function, we found discrepancies of more than a 30% attributable to problems in the *in situ* trap calibration. These large, but undetectable, changes can ultimately lead to large errors during the experiments and therefore in wrong interpretations and conclusions. In this case, the momentum calibration based on macroscopic parameters, completely independent of the exact properties of the specimen, seems to provide a more reliable benchmark for detecting artifacts in the results and eventually for accurately measuring the optical force.

As a conclusion, we can assert that the combination of the two complementary methods integrated in the *Lunam*™ T-40i allows us to accurately determine *in vivo* forces and, by comparing the results from the two methods, the system can make visible artifacts hidden in the traditional calibration approach.

We are indebted to J. Mas from the University of Barcelona for his contribution in the experiments and J. Alcaraz from the same university for kindly providing the cell line.

References

1. M. Montes-Usategui and A. Farré, *US Patent* 8,637,803 (2014).
2. K. Svoboda and S. M. Block, *Annu. Rev. Biophys. Biomol. Struct.* 23, 247-285 (1994).
3. M. Fischer, A. C. Richardson, S. N. S. Reihani, L. B. Oddershede, and K. Berg-Sørensen, *Rev. Sci. Instrum.* 81, 015103 (2010).