

# Real-time actin-cytoskeleton depolymerization detection in a single cell using optical tweezers

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**Abstract:** The cytoskeleton provides the backbone structure for the cellular organization, determining, in particular, the cellular mechanical properties. These are important factors in many biological processes, as, for instance, the metastatic process of malignant cells. In this paper, we demonstrate the possibility of monitoring the cytoskeleton structural transformations in optically trapped yeast cells (*Saccharomyces cerevisiae*) by tracking the forward scattered light via a quadrant photodiode. We distinguished normal cells from cells treated with latrunculin A, a drug which is known to induce the actin-cytoskeleton depolymerization. Since the proposed technique relies only on the inherent properties of the optical trap, without requiring external markers or biochemical sensitive spectroscopic techniques, it can be readily combined with existing optical tweezers setups.

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## 1. Introduction

The cytoskeleton is a dynamical structure made up by a network of protein filaments [1]. One of these is the actin which is present in all the cytoplasm and in particular in the cell cortex. The cytoskeleton provides the cell with mechanical rigidity and takes part in many important cellular functions, such as cell mobility, cell division and vesicle transport. Cytoskeleton alterations are related to several disease, including infectious processes, circulatory problems, blood diseases (anemia), and even aging related (Alzheimer) and motor neuron (sclerosis) diseases. Often the cell malignant transformation is associated with a change in the cytoskeleton viscoelastic properties [2, 3]. The changes include alterations in cellular elasticity and viscosity, that may be used as cellular markers and diagnostic parameters [4].

There are only few experimental techniques which are able to give information about cellular mechanical properties. Historically, the prevalent technique has been micropipette aspiration [5]. More recently, micro-rheological methods have been used to make local measurements of viscoelastic properties inside cells [6, 7].

In such experiments micro-spheres are employed to probe the medium response using either video or laser tracking [8, 9]. The tracked particle motion is often simply related to its Brownian motion (passive technique), while, in other cases, it is driven externally (active technique). Mechanical response of living cells can also be obtained by stretching the whole cell using atomic force microscopes, magnetic tweezers [10], or optical stretchers [11]. However, all these techniques are quite complicate to realize and require special, often invasive, preparation procedures, such as micro-bead insertion inside cells.

In this work, we propose and demonstrate a simple, fast and reliable method to monitor the actin-cytoskeleton structural transformations in optically trapped yeast cells (*Saccharomyces cerevisiae*) by tracking the forward scattered light via a quadrant photodiode. This technique is similar to the photonic force microscope [12, 13] and has been applied for living cells in [7, 14-16]. The non invasive character of Optical Tweezers allows the observation of cellular response to stress without interference due to non physiological handling [17-21], which might lead to measurement artefacts. In addition, since this technique relies only on the inherent properties of the optical trap, without requiring external markers or biochemical sensitive spectroscopic techniques, it can be readily combined with existing optical tweezers set-ups.

## 2. Experimental setup

Figure 1 illustrates the main components of our experimental setup. The trapping beam is given by a semiconductor laser (785 nm, Monocrom, Barcelona, Spain) with a monomode fiber output that generates a Gaussian beam and emits a maximum power of 15 mW. It is tightly focused into the sample by an Olympus oil-immersion infinity corrected objective lens (100X, 1.25 N.A.). A telescope, formed by lenses  $L_1$  ( $f_1 = 10\text{ cm}$ ) and  $L_2$  ( $f_2 = 30\text{ cm}$ ), assures a collimated beam overfilling at the objective input pupil. The forward scattered light from the trapped object is collected by a second objective lens (40X, 0.75 N.A.) positioned over the sample and projected onto a quadrant photodiode (QPD), to track the Brownian motion of the trapped cell organelles. The resulting signals are, then, transferred through an analog to digital conversion card to a computer for the analysis. The light from a LED, focused on the sample by the same 40X objective, was used to illuminate the sample; an image of the trapped cell was obtained by using a CCD camera, coupled to the microscope. The cells are suspended in physiological solution and contained in an home-made sample holder, constituted by two sandwiched 80  $\mu\text{m}$  coverslips separated by a 100  $\mu\text{m}$  spacer and sealed with water-insoluble silicone vacuum grease to prevent sample evaporation.

For this experiment, we use a kind of yeast, *Saccharomyces cerevisiae*, 3 – 5  $\mu\text{m}$  diameter. It is an excellent model organism for research in cellular and molecular biology as many

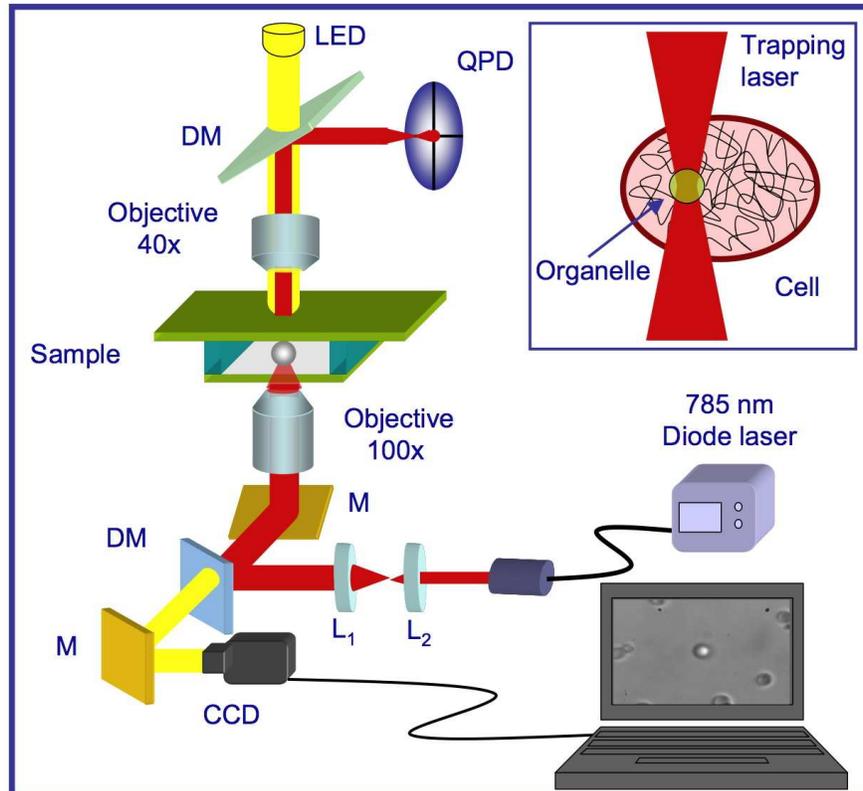


Fig. 1. Experimental setup: L, lens; M, mirror; DM, dichroic mirror; QPD, quadrant photodiode.

fundamental cellular processes are conserved from yeast to human cells. Moreover, they are non-toxic, easily available and easy to grow. To examine the effects of the actin cytoskeleton depolymerization we have treated the cell with latrunculin A (LAT-A), a monomeric actin sequestering drug that depolymerizes the actin cytoskeleton in numerous cell type, including yeast cell [22, 23]. The cells were grown to exponential phase, which was checked by measuring the Optical Density (OD) of the cell culture (1.4 at 600nm). LAT-A was added from a 10mM dimethyl sulfoxide (DMSO) stock to a final concentration of 200 $\mu$ M, at a temperature of 25°C. After the alignment of the QPD, a single yeast cell, i.e. which does not have a bud, is trapped and the sum (z) and differential (x and y) signals from the QPD are monitored. This technique is extremely sensitive to the distance of the trap from the coverslip, which was always set to 15 $\mu$ m for the reproducibility of the experiment. By studying Brownian motion of granules inside them we distinguished healthy cells (in DMSO) from cells treated with LAT-A.

### 3. Results and discussion

Single yeast cells can be trapped by using moderate laser power ( $P \simeq 1.5$  mW on the sample). At this power level, the heating and photodamage can be considered negligible. Indeed, for an aqueous sample irradiated by a focused laser beam ( $\lambda = 1064$  nm), the heating rate is about 10°C/W [24, 25] and yeast cells have been shown to progress in their cell-cycle under such trapping conditions [26].

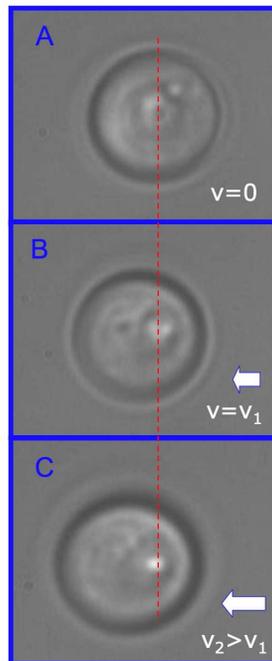


Fig. 2. (A), yeast cell under zero-dragging condition ( $v = 0$ ): the trapped organelle is in the cell center. (B), yeast cell under dragging condition ( $v = v_1$ ) the whole cell is translated while the organelle remains fixed. (C), increasing the dragging force ( $v_2 > v_1$ ) trapped organelle is almost in contact with the cell membrane.

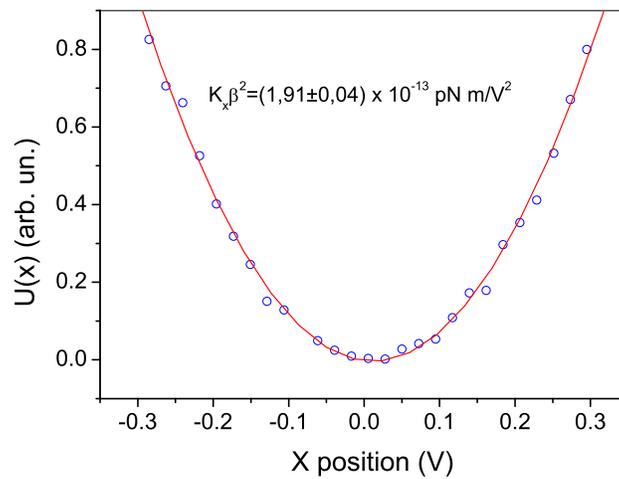


Fig. 3. The optical potential well  $U(x)$  for a healthy yeast cell. The solid line represent the best fit performed with a parabolic function.

Living cells present a complex mixture of chemical constituents having different optical properties. We monitored the motion of small endogenous particles present inside the cytoplasm of a yeast cell like lipid granules [7] and/or vacuoles. Vacuoles contain a mixture of salty water, proteins and lipids and their size ranges between 1 and 2 microns. These organelles have the highest refractive index comparing with other cell organelles, almost spherical, and filled with lipids [27]. Previously this was shown by a staining with the lipophilic marker FM4-64 [28], and it was demonstrated that it is the vacuole or some endosomal compartment. Also a staining with DAPI showed nuclear staining and mitochondria [29]. In light microscope, these organelles appear as spheres (see Fig. 2) that move randomly near their equilibrium positions. Sacconi et al.[30] have demonstrated the possibility to trap them to exert a pushing force on the nucleus. These endogenous particles can be also used as a probe embedded in the polymeric network of the cytoskeleton. The links between the mitochondria, the vacuole and the nucleus with the actin cytoskeleton was proved before in, for example, [31, 32].

In our experiment, by tracking Brownian motion of the cellular organelles, we monitor the structural evolution of the cytoskeleton. It is important to underly that the beam waist at the focal plane is of the order of the wavelength in the medium ( $\lambda \simeq 0.6 \mu m$  in our case) and is much less than the cell size. In these conditions, while a single organelle is trapped, all the other cellular components continue to move freely. To illustrate this, we applied a drag force to the trapped cell. That was achieved by translating the stage with the sample at a given velocity. The frame A of Fig.2 corresponds to zero-dragging condition ( $v = 0$ ): it can be seen that the granules are confined in the optical trap. When the drag was on ( $v = v_1$ ), the whole cell except the trapped granules was translated (see frame B). Increasing the dragging ( $v = v_2 > v_1$ ), the cell displaced even more so that the trapped granules are almost in contact with the cell membrane (see frame C).

The fact that the granules are the trapped part of the cell represents a big advantage because that allows to study the mechanical response of the cytoskeleton. That was done by tracking the Brownian motion of the trapped granules by means of the forward scattering light and a position detector. The position of the trapped granule was measured in terms of the signal provided by the QPD ( $V_x$ , expressed in Volts) and for small displacement from the laser focus the optical potential well  $U(V_x)$  formed by the optical beam along the axis  $x$  perpendicular to the laser propagation axis can be written as:

$$U(V_x) = \frac{1}{2} k_x \beta^2 V_x^2 \quad (1)$$

where  $k_x$  is the trap stiffness  $\beta = x/V_x$  is the voltage-to-displacement conversion coefficient. In Fig. 3 we show a typical potential well obtained by trapping a healthy yeast cell. As it can be seen, although we deal with a non-spherical particle, for small displacements the potential shape approximates quite well a parabola. The shape of the potential along the axis  $y$  looks quite similar. In some cases, we found a strong deviation of the potential well from the parabolic behavior; this occurs, in particular, when more than one particle was trapped. In these cases, the measurement was rejected. From the fit of the experimental data we could estimate the term  $k_x \beta^2$  in Eq. 1. As it will be better clarified later, for the purpose of our experiment it is sufficient to express the stiffness not in absolute units but in terms of QPD calibration factor  $\beta$ . We have repeated the same procedure trapping different healthy yeast cells ( $N \simeq 30$ ) and calculated the average of the values  $k_x \beta^2$ :

$$\langle k_x \beta^2 \rangle = (2.9 \pm 0.8) \cdot 10^{-13} pN \cdot m/V^2 \quad (2)$$

Afterwards we studied cells treated with LAT-A.

For these cells, we tracked the Brownian motion at different incubation time. Data were collected between 15 min and 2 h after the application of the LAT-A, when the effect of this

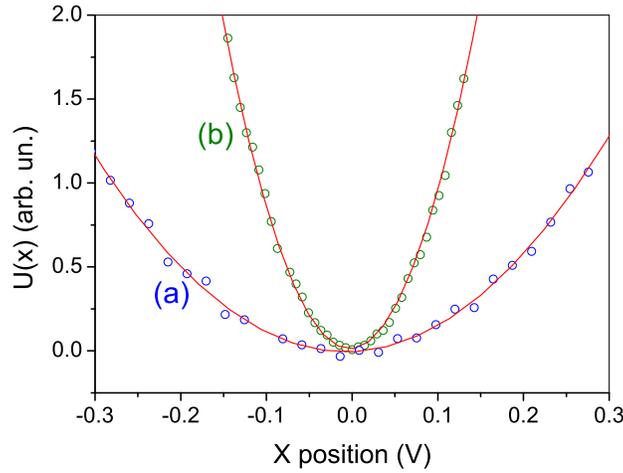


Fig. 4. The optical potential well for a LAT-A treated cell after a incubation time of 15 min (a) and 2 h (b).

drug reached its saturation. In Fig. 4 we report the optical potential at the beginning and at the end of this process. From a parabolic fit we found  $k_x\beta^2 = (2.5 \pm 0.2) \cdot 10^{-13} pN \cdot m/V^2$  at the beginning of the LAT-A application. This value is consistent with the average value obtained from the statistic analysis of healthy cells shown above. The final effect of LAT-A is quite evident if we look at the potential well 2 h after the application of the drug (see Fig.4).

The time evolution of  $k_x\beta^2$  during the LAT-A action is shown in Fig.5. In particular, after 2 h from the application of LAT-A  $k_x\beta^2 = (19.2 \pm 0.4) \cdot 10^{-13} pN \cdot m/V^2$ , i.e. a value rather different from that of healthy yeast cells. Therefore, if we assume that the calibration factor  $\beta$  is independent on the status of the cell (i. e. LAT-A does not affect the granules), we can conclude that the trap stiffness increases for the yeast cells treated by LAT-A.

In order to obtain more specific information concerning the medium inside the cell we subsequently analyzed the stochastic signal derived from the granules Brownian motion. According to the solution of the Langevin equation for each cartesian coordinate the theoretical Power Spectral Density (PSD) of the Brownian motion for a trapped particle is given by the function [12, 13]:

$$P(f) = \frac{k_B T}{\pi^2 \gamma} \frac{1}{(f_c^2 + f^2)} \quad (3)$$

where  $T$  is the absolute temperature of the sample,  $k_B$  the Boltzmann constant and  $f$  the frequency. The frequency  $f_c$  is the corner frequency which is related to the trap stiffness by the relation:

$$f_c = \frac{k_i}{2\pi\gamma} \quad (4)$$

where  $k_i$  is the trap stiffness constant along the  $i^{th}$  direction,  $\gamma = 6\pi\eta a$  is the hydrodynamic coefficient,  $\eta$  the fluid viscosity and  $a$  the radius of optically trapped particle. To test our apparatus we trapped first a spherical dielectric particle in water. In Fig. 6 trace (a), we show the PSD of a 4.5  $\mu m$  diameter polystyrene bead in water; the experimental data fit with a Lorentzian profile.

Our analysis of the PSD obtained from the yeast cells started from healthy cells. Measurements were performed keeping the trapped cell under observation for about two hours. In Fig.6 trace (b), we show a typical PSD signal for a healthy yeast cell. As it is possible to see, although the Langevin equation which governs the Brownian motion of a over-damped rigid sphere in a harmonic potential well does not strictly apply to the granules, the experimental data fit sufficiently well with the function of Eq. 3. From the fitting of the experimental PSD we could estimate the corner frequency. In Fig. 7, trace (a) we report the corner frequency value for a single healthy cell as function of observation time. The  $f_c^x$  and  $f_c^y$  values resulted consistent and uniformly distributed around their mean value. The error on the single determination was estimated from the fitting procedure. It is important to point out that no detectable transformation was induced by the presence of the trap, in accordance with reference [26]. The average value for  $f_c$  calculated on  $N = 30$  repeated measurements is reported in Tab.1.

Finally, we have applied this method to determine the effect of LAT-A on actin cytoskeleton in yeast cells. Again we started our observation after 15 min from the LAT-A application while the duration was of about 2 hours. A comparison of the  $f_c$  at different incubation time of LAT-A treated cells is shown in Fig. 7, trace (b): the frequency corner increases with time and reaches a plateau value ( $f_c \simeq 3.5 Hz$ ) after two hours. For completeness the measured  $f_c$  at the beginning ( $t = 15 min$ ) and after 2h from the LAT-A application are reported in Tab.1.

Table 1. A comparison of the trap stiffness, corner frequency and their ratio  $R$  for different conditions of yeast cell.

Cell type	$k_x \beta^2 (x10^{-13})$ ( $pN \cdot m/V^2$ )	$f_c^x$ (Hz)	$R(x10^{-13})$ ( $pN \cdot m/V^2 Hz$ )
Healthy yeast cell	$2.9 \pm 0.8$	$2.1 \pm 0.5$	$1.38 \pm 0.5$
LAT-A treated cell ( $t=15 min$ )	$2.5 \pm 0.2$	$2.2 \pm 0.2$	$1.14 \pm 0.2$
LAT-A treated cell ( $t=2 h$ )	$19.2 \pm 0.4$	$3.4 \pm 0.3$	$5.65 \pm 0.12$

Cytoskeleton depolymerization, induced by LAT-A, leads to a change of both the trap stiffness ( $k_x \beta^2$ ) and corner frequency ( $f_c$ ). The former change can be ascribed to a variation of the refractive index of the depolymerized actin network. Indeed, the trap stiffness depends on the ratio between the refractive index of the trapped object and the surrounding medium. Less direct is the interpretation of the latter change. The corner frequency  $f_c^x = k_x / (2\pi\gamma)$  depends both on the stiffness and on the viscosity. Therefore, it seems convenient to calculate their ratio  $R$ :

$$R = \frac{k_x \beta^2}{f_c^x} \propto \eta. \quad (5)$$

In this way, we can obtain direct information about the viscosity. These data are plotted in Fig. 8 as function of the LAT-A action time, while the values at beginning and at the end of this process are listed in Tab. 1. As it can be seen in Fig. 8, it seems that the depolymerization of the Actin-cytoskeleton network increases the viscous character of the intracellular environment.

It is important to emphasize that the goal of this investigation is not to study cellular viscoelasticity. This kind of measurements would require the determination of the complex shear modulus  $G^*(f)$ , which is the physical quantity commonly employed to quantify the viscoelasticity of a medium [33]. This approach requires a more sophisticated data analysis which is beyond the purpose of this work. On the contrary, our approach provides a fast and reliable method to sort cells in different physiological states, otherwise undistinguishable by using an optical microscope.

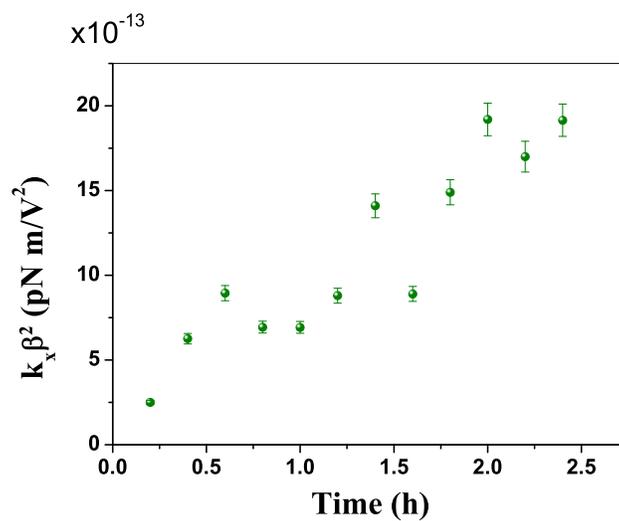


Fig. 5. Behavior of  $k_x \beta^2$  for a LAT-A treated cell versus the observation time.

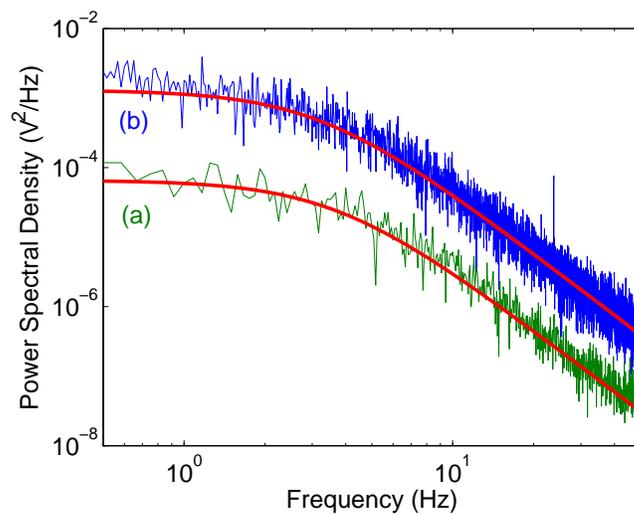


Fig. 6. Experimental PSD from the x signal for a trapped polystyrene bead (trace a) and for a trapped normal yeast cell (trace b). The Lorentzian fitting curves are also shown.

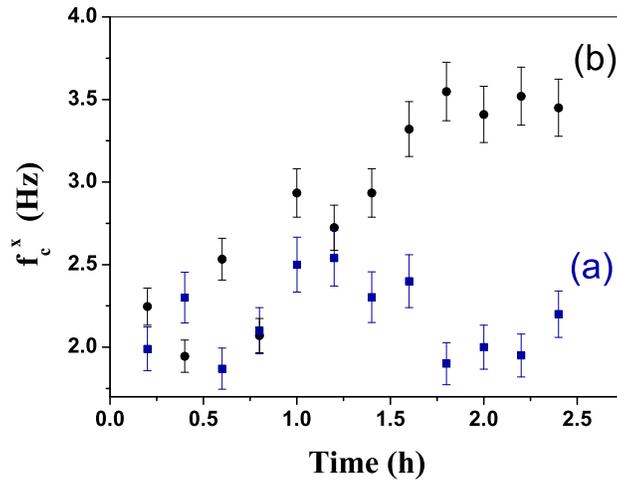


Fig. 7. Measured frequency corner for a living cell (a) and a LAT-A treated cell (b) during 2 hours. In the second case, the corner frequency increases with time.

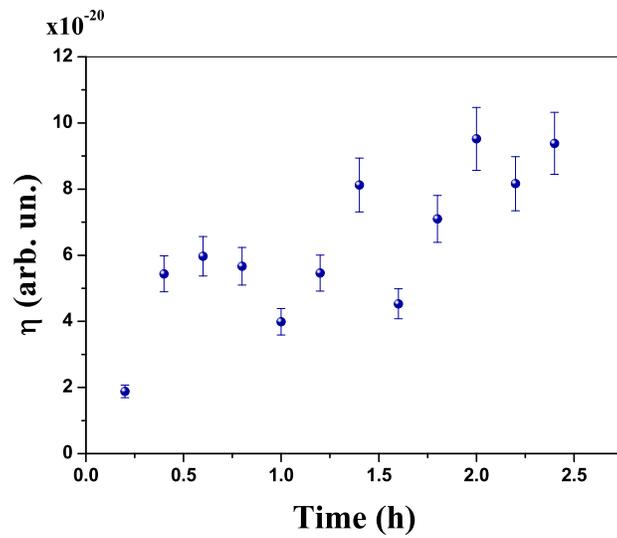


Fig. 8. Behavior of  $\eta$  for a LAT-A treated cell under 2 h.

#### **4. Conclusion**

The results shown demonstrate the possibility of monitoring the cytoskeleton structural transformations in optically trapped cells by tracking the forward scattered light via a quadrant photodiode. The analysis of its PSD allows us to distinguish healthy yeast cells from the ones whose cytoskeleton is depolymerized and it also permits us to track the depolymerization process in real-time. In particular, the F-actin cytoskeleton depolymerization, induced by treatment with LAT-A, results in a progressive increased corner frequency by a factor of 1.7. Since F-actin plays an important role in cellular mechanics, these changes alter the Brownian motion of the cellular organelles; therefore, this can be seen as an inherent cell marker that offers an alternative to traditional techniques and opens the door to a new and cheap tool for cell characterization.

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