Dynamics of a growing cell in an optical trap

Giovanni Volpe and Gajendra Pratap Singh
Institut de Ciències Fotòniques (ICFO), 08860 Castelldefels (Barcelona), Spain

Dmitri Petrov[a)]
Institut de Ciències Fotòniques (ICFO), 08860 Castelldefels (Barcelona), Spain
and Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010, Barcelona, Spain

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We analyze the forward scattered light from a single optically trapped cell during its growth. We show that the cell continues adjusting itself to the applied optical force because of the growth processes, and hence it keeps changing its orientation in the trap. We point out the importance of taking this variation into account in the interpretation of spectroscopic data. This method can also be used as a means for cell identification and cell sorting. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2213015]

Unlike homogeneous spheres, such as polystyrene beads commonly used in optical trapping experiments, living cells present a complex mixture of chemical constituents having different optical properties: high-refractive index granules of different sizes embedded in the cytoskeleton can be observed even under a bright-field microscope.[1] The focal spot of the optical trap is usually several times smaller than the cell size. The cellular constituents can move around the equilibrium position in the optical trapping potential. This movement is not only due to the Brownian motion, as for solid microspheres, but also due to the inherent biological motility. A rotation of the cell is also possible, since its optical inhomogeneity can induce a torque.[2,3] In living cells metabolic activity leads to a continuous change in their chemical content and physical properties, as well as in their shape and size. Hence the equilibrium position around which the trapped cell oscillates in solution can change over time. Unlike the thermal fluctuations, these fluctuations are affected by the biochemical processes and allow the cell to locally adjust its mechanical properties according to its needs.[4]

The understanding of the behavior of a growing cell in an optical trap is interesting in itself and can also have a major relevance for techniques utilizing optical tweezers. One of the most promising ways to study biochemical processes in single living cells that normally live in suspension is to combine optical tweezers[5,6] with optical techniques such as Raman spectroscopy,[7–14] fluorescence, and nonlinear wave generation.[15–19] In these techniques the same beam that traps the cell can also be used to excite the spectrum. Therefore, in all the spectroscopic techniques mentioned above only that part of the cell which is near the focus contributes to the measured spectra, especially for nonlinear effects or confocal geometries. Thus the interpretation of experimental spectra can often be ambiguous, particularly if the performed experiments span over the cell life cycle or if they involve the presence of variable environmental conditions that can invoke a biochemical response from the cell. In all these cases for a correct interpretation of the spectroscopic data it is essential to understand the behavior of the cell in the optical trap. The spectroscopic measurements usually collect either the backscattered or the forward scattered light from the trapped particle. If the scattered trapping light changes its direction, it is reasonable to suggest that measured spectroscopic data are affected as well.

In this letter we propose to measure the spatial distribution of the forward scattered light by the trapped cell, using a quadrant photodiode (QPD), to gain insights into the behavior of the cell in the trap. This technique can be helpful in avoiding the misinterpretation of spectroscopic data obtained from optically trapped living cells, and also in cell identification and sorting.

The combination of optical tweezers and a high-resolution photodiode position detector [photonic force microscope (PFM)] has been utilized to make quantitative measurements of nanometer displacements and piconewton forces with millisecond resolution.[20–23] In the usual scheme for biophysical experiments, a polystyrene bead chemically attached to the biomolecule or cell under investigation is optically trapped, and the study of its dynamics permits one to gain insights into the mechanics of the object it is attached with.[24,25] In our scheme, the living cell itself is used as a probe in a PFM geometry, detecting its forward scattered light.

Figure 1(a) presents a schematic representation of our experimental setup. A 785 nm Gaussian nonpolarized laser beam of a semiconductor laser coupled into a monomode optical fiber (Monocrom, Barcelona, Spain) is focused by a 100× objective [numerical aperture (NA)=1.25] to serve as

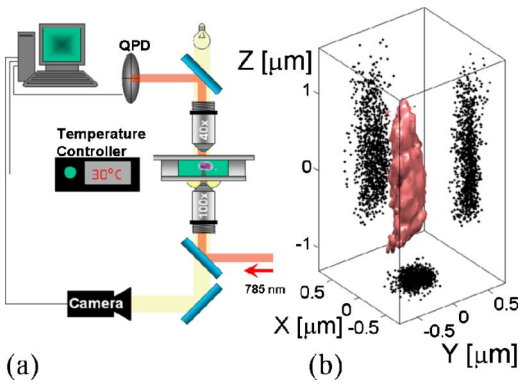

FIG. 1. (Color online) (a) Experimental setup. (b) Distribution of a 4.5 μm polystyrene bead position (120 s acquisition time and 1000 Hz sampling frequency).

[a)]Electronic mail: dmitri.petrov@icfo.es

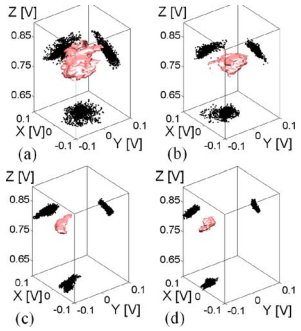

FIG. 2. (Color online) Forward scattered light distribution detected by the QPD at (a) 6, (b) 10, (c) 30, and (d) 45 min after trapping (120 s acquisition time and 1000 Hz sampling frequency).

a single beam optical trap. From our previous experiments,[14,28] we know that the laser power at the sample should be low (around 500 μW) to permit the cell to grow in the optical trap. The forward scattered light is collected by a 40× objective and detected by a QPD (Silicon Sensors, QP50–6-SD).[29] The resulting sum and differential signals are then transferred through an analog to digital conversion card to a computer for analysis.

To characterize our optical trap we perform a three-dimensional (3D) position fluctuation analysis applying Boltzmann statistics to the movement of an optically trapped 4.5 μm polystyrene bead.[23] The size of the bead is similar to that of the cells we used in our experiments. In Fig. 1(b), we show the surface that encloses a volume where the bead can be found with 90% probability.[22] The surface reveals the characteristic ellipsoidal shape due to the difference in the stiffness coefficients along the beam axis and perpendicular to it. The measured stiffness coefficients are 0.03 pN/μm for the on-axis stiffness and 0.08 pN/μm for the off-axis stiffness. No considerable variation is observed over the time of the experiment (up to 60 min).

For the experiments with living cells, we use yeast *Saccharomyces cerevisiae* because it is an excellent model organism for research in cellular and molecular biology as many fundamental cellular processes are conserved from yeast to human cells.[26] Moreover, it is nontoxic and easy to grow, and it completes its cell cycle in about 2 h under optimum conditions [yeast peptone dextrose (YPD) nutrient medium, 30 °C].[27] The cell grows and then separates in two through a process called budding in which a small bud appears on the mother cell, continues to increase in size, and finally gets separated as a daughter cell.

A single yeast cell which does not have a bud is trapped and, after the alignment of the QPD, data are acquired continuously until the cell shows a bud of an appreciable size. The entire process lasts between 45 and 60 min depending on the individual cell. Due to the complex content and shape of the cell, the 3D position analysis cannot be applied. However, the resulting sum (Z) and differential (X and Y) signals of the QPD can be monitored, and 3D surfaces can be constructed in a similar way as for the 3D position surfaces of the polystyrene sphere. Such surfaces provide information on the preferred propagation direction of the forward scattered light. Figure 2 shows results of this experiment. During the first 10–12 min after the cell is trapped the distribution of the forward scattered light shrinks, as we can see comparing Figs 2(a) and 2(b). This can be interpreted as a consequence of the adjustment of the membranes, organelles, and granules

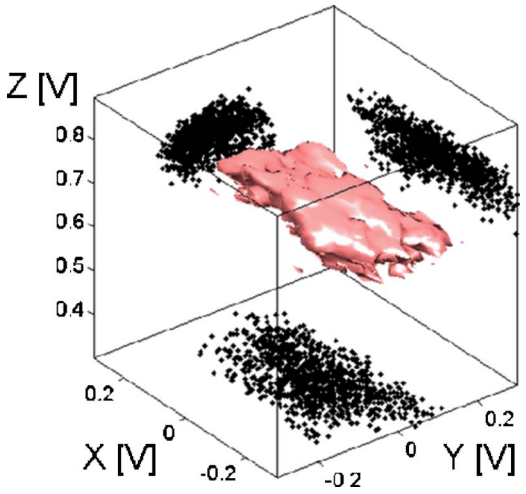

FIG. 3. (Color online) Forward scattered light distribution for a trapped heat-treated yeast cell (120 s acquisition time and 1000 Hz sampling frequency).

inside the cell reacting to the applied optical force: organelles and small granules get attracted to the laser focus due to their higher refractive index.[1] This process can last for several minutes at our laser intensity and leads to a concentration of the scattered light around the optical axis of the system. As soon as the cell starts budding, the direction of the forward scattered light moves off axis [Fig. 2(c) and 2(d)]. Probably the conformational changes in the budding cell cause a variation in its equilibrium position and affect the propagation direction of the scattered light. The angular distribution of the scattered light remains narrow, and it can imply that the organelles and vesicles inside the cell stay near the focus.

This experiment shows that while combining optical tweezers with spectroscopic techniques to study living cells, it is crucial to take into account the dynamics of the equilibrium position of the cell in the trap. When the cells do not appreciably change their geometrical shape, our results confirm that the changes in the spectra are mainly due to the biochemical processes occurring inside the cells themselves, and not due to the change in the equilibrium position of the cells. In particular, the spectroscopic data changes obtained before the budding process starts can mainly be assigned to the biochemical processes occurring inside the cell. The start of the budding process induces the off-axis movement of the scattered light. This observation can also be used as a means to detect the initiation of the budding process itself.

Figure 3 shows results of a control experiment performed on a heat-treated cell (80 °C for 20 min). This cell, which has a bud, is trapped and its forward scattered light is analyzed for about 60 min. The heating process makes the cell more opaque, producing an increase in the differential signals compared to the ones for a living cell. There is no shrinking of the scattered light distribution during the first 15 min of the experiment; this can be due to the fact that either there is no shrinking at all because the heating processes denature the proteins, or the shrinking happens in the very first minutes of the experiment before the data acquisition can be started. The shape of the scattered light distribution does not change appreciably over time. Thus we can conclude that in the case of live budding cells the displacement of the scattered light distribution can be attributed to

the change of its orientation in the trap due to its changes in size and shape. The clear asymmetry observed is due to the orientation of the budding cell in the trap. This fact can be used to distinguish a budding cell from a nonbudding one. Such an orientation of the cell can also happen in the case of a living cell [Figs. 2(c) and 2(d)], but this varies over time due to the growth process.

In conclusion, we experimentally confirmed that the distribution of the scattered trapping light from a living cell can change during the cell cycle. These effects are particularly relevant when the cell is not symmetric, as for budding yeast cells, or if it changes its size and shape appreciably, as for living and growing cells. A possible approach to better control the trapping forces acting on the cell could be a multibeam tweezer setup;[30] however, this technique is better suited to space-resolved than to time-resolved spectroscopy.

These effects should be taken into account in the interpretation of data obtained by spectroscopic techniques such as Raman spectroscopy, fluorescence spectroscopy, or nonlinear wave generation when combined with optical tweezers to work with single cells. We propose that time-resolved spectroscopic data should be compared only when the cell is stable inside the trap, and not when abrupt changes in the Rayleigh scattering are observed.

These experiments also show that the analysis of the scattered light can be a means to probe the physiological and morphological states of the cell, distinguishing between living and dead cells and identifying the cell-cycle stage. In this way forward scattered light can offer a new and cheap imaging system for cell identification and sorting, especially in integrated devices (Lab-on-a-Chip).

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