Microthermometry of Laser-Trapped Chinese Hamster Ovary Cells and Sperm Cells

Yagang Liu †*, Gregory J. Sonek †*, Curtis F. Chapman *, Bruce J. Tromberg *,
Pasquale Patrizio ‡, Yona Tadir *, Michael W. Berns*

University of California, Department of Electrical and Computer Engineering †, and Beckman Laser Institute and Medical Clinic *, and Department of Obstetrics and Gynecology ‡, Irvine, CA 92717

ABSTRACT

Microthermometric measurements on optically-trapped Chinese Hamster Ovary (CHO) cells and sperms cells are reported, using a non-invasive microfluorometric detection technique. Within an optical tweezer system that has been outfitted with a spectral fluorescence excitation and detection capability, the changes in temperature induced by the process of sample confinement by a focused laser beam has been quantified over micron-sized spatial regions of both motile and immotile cells. Our measurement technique is based on the use of environmentally sensitive fluorophores that can be incorporated into the cell membrane and used to sense local changes in temperature when the cell membrane is perturbed optically or via other environmental stress factors. Using a cw 1.064 μm Nd:YAG laser for trapping CHO and human sperm cells, a temperature increase of ~1°C per 100 mW laser power was observed. At this infrared wavelength, cellular heating as result of laser confinement appears to be mainly due to radiation absorption by water.

Keywords: optical trapping, optical tweezers, local heating, microthermometry, temperature, CHO, sperm.

2. INTRODUCTION

Optical laser traps (optical tweezers) have become a powerful tool for the confinement and physiological study of biological cells and organelles. Since the first demonstration of the single-beam gradient force optical trap,[1] optical tweezers have been employed in a variety of biological applications,[2,3] including the manipulation and force measurement of viruses and bacteria,[4] cells and organelles,[5,6] sperm cells,[7,8] and chromosomes.[9,10] To date, most optical trapping has been performed using the infrared Nd:YAG laser wavelength of 1.064 μm, primarily chosen for biological cell trapping studies because this wavelength minimizes the extent of chromophore absorption and reduces the potential for cell damage during laser confinement and manipulation. Thusfar, optical trapping at 1.064 μm has been assumed to be a non-destructive and non-invasive process. However, several key issues have yet to be addressed in detail, including the extent of heating and temperature changes induced inside optically trapped samples, the wavelength dependence of trapping, and the corresponding physiological changes that can be directly attributed to the interaction of a highly focused laser beam and a cell. Herein, we provide experimental evidence for localized cell heating induced by infrared optical tweezers in two different cell species, including the immotile Chinese Hamster Ovary (CHO) cell and the highly motile human sperm cell.

The basis for the present study is a new microfluorometric technique that was recently developed for making thermal measurements on biological samples with spatial resolution on the order of microns.[11] The optical measurement technique makes use of temperature-dependent fluorescence emission spectra from dye-labeled bilayers in liposomes [12-14] to quantify thermal changes with an accuracy and resolution of ~0.1 °C. Herein, we have extended this basic technique to the measurement of temperature changes in single living cells. The extent of localized heating produced by a Nd:YAG trapping laser beam is assessed in the following manner. The cell species under study is impregnated with a fluorescence dye that primarily gets incorporated into the cell membrane. For a suitable dye probe that can respond to environmental changes within the cell membrane region, such as Laurdan (6-dodecanoyl-2-dimethyl aminonaphthalene), a change in cellular phase or membrane structure due to an external or environmental perturbation appears as a Stoke's shift in the dye probe's fluorescence emission spectrum from the cell membrane. That is to say, while an infrared (IR) laser beam creates a gradient force trap which confines the single cell sample, a UV beam is used to excite fluorescence from the cell membrane region. With the absorption of infrared radiation from the trapping beam by the cell core and membrane regions, the sample becomes heated and, at a specific transition temperature, the bilayer membrane of the sample undergoes a phase transition from a gel to a liquid crystalline state, with a corresponding change in the membrane permeability to water. This transition is accompanied by a large Stokes shift in the probe fluorescence emission spectrum. The emission from micron-sized regions of the membrane is then collected and used to directly quantify the change in localized temperature with fluorescence emission shifts

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as a function of applied laser trapping power or wavelength. From this data, the change in cellular temperature induced by optical trapping at 1.064 μm is derived.

3. MATERIALS AND METHODS

3.1. Sample Preparation

Chinese Hamster Ovary (CHO) cells were maintained in culture using standard procedures.[15] For suspension measurements the cells were labeled while attached in 25 cm^2 tissue culture flasks. For Laurdan, a $4x10^{-4}$ M solution of laurdan in ethanol was added to the cell-containing medium (GIBCO's minimum essential medium supplemented with 10% vol./vol. fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin) such that the ethanol concentration was 3 per cent. The cells were incubated at 37 °C for 35 minutes. The cells were then treated with 0.25% trypsin to cause the cells to detach, followed by fresh medium to deactivate the enzyme. The medium with detached cells was placed in a culture tube and centrifuged for 5 minutes at 1000 rpm. The supernatant was removed and the cell pellet resuspended in PBS. The typical size of the cell is from 8 μm - 15 μm.

Human sperm samples were placed in a sterile container and diluted in Hepes buffered human tubal fluid (HTF-h). The samples were centrifuged once at 200x g for 10 minutes and resulting pellet was resuspended in HTF-h. For dye incubation, a $4x10^{-4}$ M solution of ethanol was directly added into the HTF-h suspended sperm solution, by controlling the ethanol concentration was from 0.5 to 2 percent. After 30 minutes, the sample was diluted in PBS and then injected into the temperature-controlled microchamber. Before and after incubation of Laurdan, the swimming force of sperms were measured by using optical trapping method.[7,16] No significant swimming force difference was found between sperm with and without Laurdan incubation. Therefore, the damage of the Laurdan incubation on the sperm cells were neglectable.

3.2. Experimental System for Optical Trapping and Fluorescence Detection

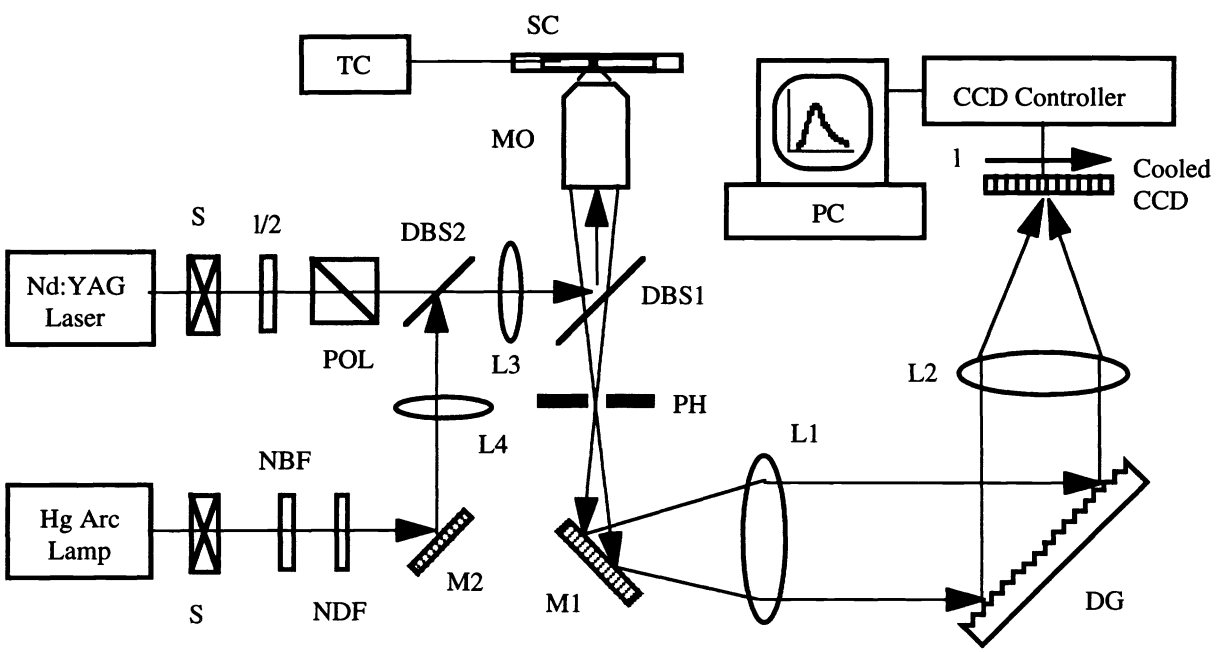

Fig.1. Experimental system for implementing fluorescence excitation and detection within an optical tweezer. System components are: S: shutter; l/2: half-wave plate; POL: polarizer; NBF: narrow band filter; NDF: neutral density filter; DBS: dichroic beam-splitter; L: lens; MO: microscope objective; SC: sample chamber; TC: temperature controller; PH: pinhole aperture; M: mirror; DG: diffraction grating; CCD: charge-coupled detector; and PC: personal computer.

An experimental system was developed for the simultaneous implementation of optical trapping and fluorescence spectroscopy, as shown in Fig.1. The optical trapping beam was derived from a Nd:YAG laser that could emit up to 1 Watt CW in the TEM_{00} mode at a wavelength of 1.064 μm. The infrared (IR) beam is then directed into a high magnification (100 X) and large numerical aperture (1.2 N.A.) water immersion microscope objective to form the basic optical trap. A 365-nm ultraviolet (UV) excitation beam is derived from a 200 W Hg arc lamp that is used in conjunction with a 365-nm narrow band filter. This beam is made collinear with the IR trapping beam, and also deflected into the focusing objective via a UV/IR dichroic beam splitter. An electronic shutter is used to control the fluorescence excitation time. The fluorescence emission from the optically trapped sample is collected by the same objective lens, and passed through an adjustable pinhole located at the image plane of the microscope objective. A 0.1 - 1 mm diameter pinhole effectively converts the optical system into a confocal trapping microscope that has micron spatial resolution. A 300 g/mm diffraction grating is then used to disperse the light, which is subsequently focused onto a electric-cooled CCD array. Spectral data, over a 400 nm bandwidth, is acquired and analyzed using a personal computer. We note that since microscope objective immersion oil can, in fact, have its own autofluorescence signal that overlaps with that of the Laurdan dye probe, a water immersion objective (Leitz, 100x, 1.2 N.A.) was used for optical trapping and fluorescence collection. To reduce the potential for photobleaching and possible stress effects created by UV light exposure, both the UV intensity and pulse duration was controlled to produce a minimum S/N ratio of $>10^2$:1. In this geometry, suspended CHO cells could be trapped with less than 10 mW of laser power, while sperm cells typically required much larger trapping powers, typically from 30 mW up to several hundreds milliwatts.

3.3. Sensing and Measurement of Sample Temperature

A customized sample chamber was constructed for containing the cell suspensions, and for sensing and controlling the cell solution temperature, as shown in Fig.2. The chamber consisted of two attached microchambers, one on top of the other, formed by 170-μm thick coverslips which formed the outer chamber walls, and separated by a single coverslip in-between. The chamber sidewalls were formed with 3 mm thick silicone gaskets. In the upper microchamber, filled with de-ion water, an electrically controlled heating coil was introduced through the silicone gasket for heating up the medium temperature. In the lower chamber, a thermocouple was placed at the center of the microchamber in order to monitor the background solvent solution temperature. The tip of thermocouple is placed against the surface of the bottom coverslip where the sample is located. The cell under study was also very close to the tip of thermocouple (within 20 - 100 μm lateral distance). Therefore the error of temperature calibration induced by the possible existing temperature gradients inside the chamber was minimized. The structure of the separated double microchamber provides not only provides a sterile environment for the cells, but also a stable temperature (better than 0.1°C) environment by effectively blocking the heating turbulence from the upper microchamber. By controlling the heating coil current provided by current power supply, the temperature in the bottom layer of lower microchamber can be controlled from room temperature (~20 °C) up to 60 °C. Optically, the chambers could still provide clear access for trapping, illumination, and fluorescence excitation beams.

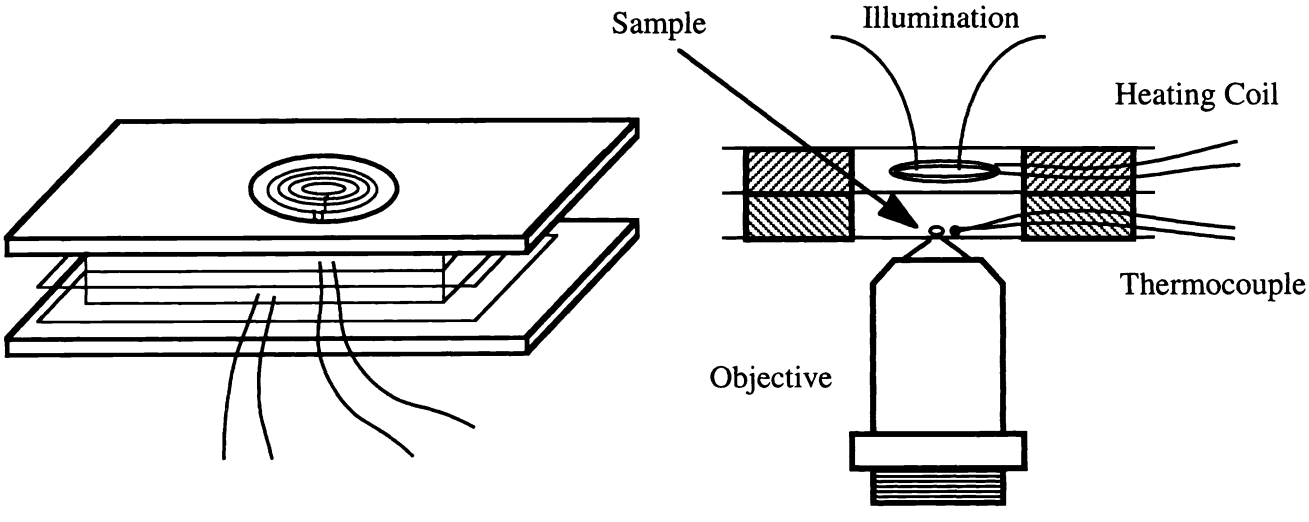

Fig.2. Schematic diagram of a customized sample chamber with temperature sensing and controlling.

3.4. Optical Measurement of Cellular Temperature

The basis for making localized temperature measurements on optically confined cells is the measurement of Stokes-shifted fluorescence emission as a function of sample temperature. This emission spectrum shift can be quantified by generalized polarization (GP). GP was first described by Parasassi et al.[12,17] in studies of the relaxation dynamics and temperature sensitivity of Laurdan in phospholipid vesicles. GP is defined as the ratio $(I_g - I_l)/(I_g + I_l)$, where I_g and I_l are the fluorescence intensities measured at the maximum emission wavelengths of the Laurdan dye, when the membrane bilayer is in its pure gel and liquid-crystalline states, respectively. As the bilayer transitions between these two states with an increase in temperature, the sample fluorescence emission undergoes a red shift, and the magnitude of GP decreases as I_g decreases and I_l increases. Since this process is reversible, a decrease in sample temperature would correspond to a decrease in I_l, an increase in I_g, and an increase in the magnitude of GP.

4. RESULTS AND DISCUSSION

The effects of optical trapping on sample heating were studied for two classes of samples, including non-motile Chinese Hamster Ovary (CHO) cells and motile human sperm cells. Our experiments consisted of bringing the cell into the field-of-view of the microscope, exposing the cell to continuous ultraviolet (UV) radiation, and then monitoring the generalized polarization, before and after switching on the laser trapping beam, as functions of incident laser power. A UV power density of less than 100 mW/cm^2 was used to prevent photobleaching of the membrane probes. After microscope objective, laser power levels were adjustable from 0 - 700 mW.

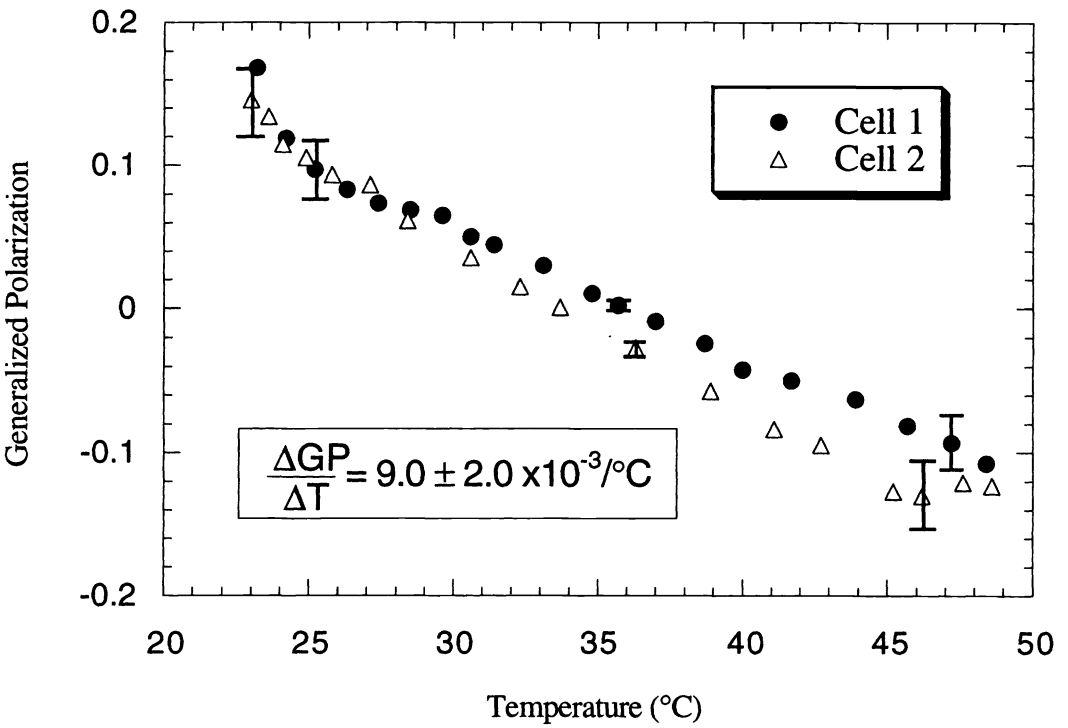

Fig.3. Generalized polarization (GP), plotted as a function of cell temperature for a Chinese hamster ovary (CHO) cell, when the cell is heated from 23°C (•) and cooled from 49°C (▲).

Prior to optical trapping, a generalized polarization plot was generated to serve as a calibration curve for sample temperature. By slowly increasing temperature of sample chamber by means of a heating coil embedded within the upper chamber, a set of fluorescence emission spectra from a free suspended liposome were taken, and than transferred into GP. The

results of calibrated temperature measurements made on 10 μm diameter PBS-suspended spherical CHO cells (Fig. 3) show that the GP decreases in nearly a monotonic fashion with increasing temperature at a rate of $\Delta GP/\Delta T \sim 9.0 \times 10^{-3}$/°C. As a result, GP display a greater linear dynamic range and can be used to quantify the extent of localized heating over a large temperature range. The shift in CHO fluorescence spectral response and decrease in GP is reversible when the cell temperature is decreased. With the application of a trapping laser beam, the CHO cell temperature was also observed to increase. For example, at 200 mW, the change in GP and temperature were 0.021 and 2.2 °C, respectively. For multiple measurements made on five different CHO cells having approximately the same diameter, the average laser-induced temperature changes were found to vary between ~1.9 and 2.5°C (Table I.), with a standard deviation of ~0.5°C. Similarly, over the range of laser powers examined, the CHO cells displayed a heating rate of ~1.1±0.2 °C/100mW. These variations may be due to intrasample variability arising during the culturing process, or the fact that the CHO cells may have a different absorption coefficient due to their heterogeneous structure, or a thicker cell membrane wall.

Table I. CHO Heating By IR Optical Tweezers (P_0=200mW)

Cell No.	1	2	3	4	5
ΔT (°C)	2.05[a] ± 0.44[b]	2.47 ± 0.49	2.46 ± 0.46	2.22 ± 0.49	1.90 ± 0.50

a Average temperature for each cell measured 10 times.
b Standard deviation of measurement.

Similar trapping experiments were also performed on sperm cells. For the sperm cells, we separated them into two groups: one consisting of very low motility sperm ("dead"), and the other with normal (actively swimming) motility. For calibration of the temperature dependent fluorescence emission of dead sperm cells, we used the same method as on CHO cells. The calibration curve is shown as Fig.4. For the calibration of motile sperm cells, we uses a relatively weak trapping

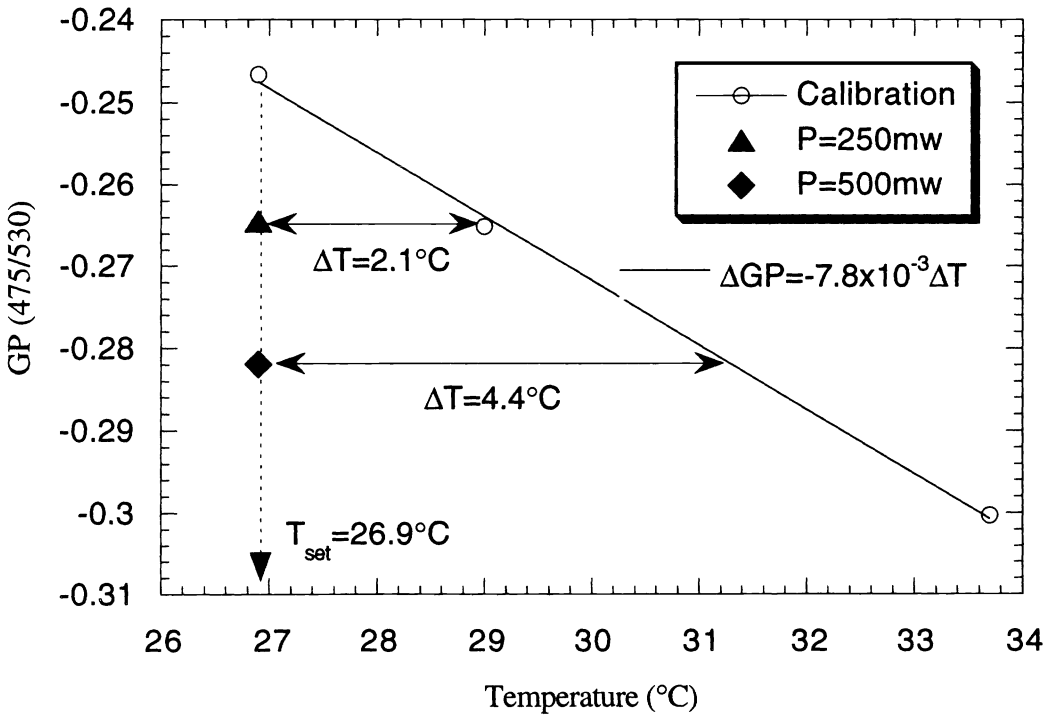

Fig.4. GP calibration with temperature for dead sperm cells. Temperature was set 26.9 °C while 250 mW and 500 mW trapping laser were applied on the samples.

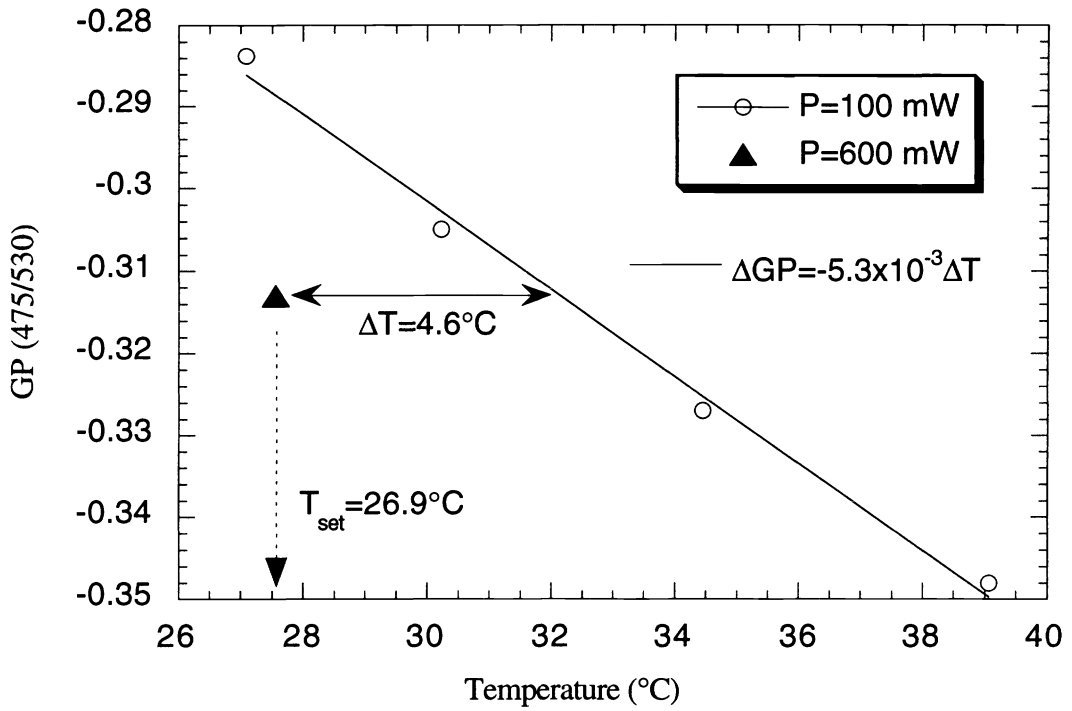

Fig.5. GP calibration with temperature for living sperm cells. A 100 mW trapping laser was used for holding the sample in the measurement. Temperature was set at 27.6°C while 600 mW trapping laser was applied on the sample.

power to catch the sperm and hold it for spectral measurements at different setting temperatures. The calibration curve of living sperm, held by 100 mW trapping laser beam, is shown as Fig.5. The GP nearly decreases with increasing temperature at a rate of $\Delta GP/\Delta T \sim -7.8 \times 10^{-3}$/°C for dead sperm and $\sim -5.3 \times 10^{-3}$/°C for living sperm. The "dead" sperm were then trapped by the trapping laser beam, a setpoint temperature of 26.9 °C was established, and then a fluorescence spectrum taken again. The GP changes were found to be about -0.016 for 250 mW trapping laser power, and -0.034 for 500 mW trapping laser power. These GP changes correspond to about 2.1 and 4.4 °C temperatures increases, according to the calibration curve in Fig.4. While at 27.6 °C, the "living" sperm was trapped by 600 mW laser beam, which is 500 mW more power than that used for calibration. This resulted in a 0.024 GP decrease, corresponding a temperature increase of about 4.6 °C. The average temperature increase per 100 mW of IR trapping laser power is about 0.9±0.7 °C.

In comparison to the CHO cells, sperm cells exhibit more of a variation in their fluorescence spectra than those of CHO cells. This might be due to the fact that the CHO cells are obtained from a well-defined cell culturing process, from which the intrasample variation of structure and cell membrane composition is more uniform than that of the sperm cells. The above results indicate that, while the basic microfluorometric technique is applicable to the measurement of temperature changes in living CHO and sperm cells, the senstivity of the technique can vary depending on the cell species under study. Cells having more complex membrane structures may not be amenable to dye probe incorporation, nor show a sensitivity to optically monitored environmental factors. This work, however, should form the basis for further studies into the heating effects induced by optical tweezer beams in both motile and nonmotile cell systems.

5. CONCLUSION

The results of non-invasive local temperature measurements have shown that infrared optical tweezers induce, to a certain extent, cell heating and temperature increases of several degrees centigrade in CHO and human sperm cells for laser powers that vary from tens to hundreds of milliwatts. Hence, for trapping powers of < 100 mW, a ΔT of < 1.5 °C at 1.064 μm is

consistently obtained for liposomes, CHO cells, and sperm cells that are surrounded by a fluid suspension medium. Even larger temperature changes might be expected for other cell species having different absorption coefficients, or when the optical trapping process is performed at shorter wavelengths where chromophore absorption might be more significant. These factors may be of primary concern in, for example, the study of motile cells where several hundred milliwatts may be required to confine a cell in the trap. In the case of sperm cell trapping, temperature increases of several °C have the potential to alter motility, impact fertilization capability, or cause damage to the genetic material contained within the sperm head. By contrast, in non-motile cell manipulation, where relatively small trapping powers are typically used (<100 mW), the present results suggest that direct thermal damage mechanisms may not be important for these samples. The present results should provide insight into the relative importance of thermal effects in optical trapping, provide new strategies for minimizing temperature increases, and allow us to assess their impact on cellular physiology and biology.

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