LETTERS TO THE EDITOR

Cell Surface Heating during Fluorescence Photobleaching Recovery Experiments

Dear Sir:

Two papers have recently appeared describing the experimental arrangement (1) and data analysis methods (2) used in the fluorescence photobleaching recovery (FPR) technique to measure lateral motion of fluorescence-labeled molecules on cell surfaces and artificial bilayer membranes. In this technique, the fluorescence in a $\sim 10\ \mu m^2$ area on the surface of a living cell is photobleached by a brief flash of intense, focused laser light of Gaussian intensity profile. Subsequent motion of surrounding unbleached fluorophore into the bleached area is detected by measuring the recovery of fluorescence excited by the same, although much attenuated, laser beam. It is important to know whether the bright bleaching flash or the subsequent attenuated observation flashes induce a significant temperature rise on the cell surface, which could conceivably damage the cell surface or distort the fluorescence recovery curve. We show here that this is not usually the case: under typical conditions of an experiment measuring lateral motion of a labeled surface protein, the local temperature rise on the cell surface is no more than 0.03°C.

The rapid conduction of heat away from the laser-illuminated "source" into the intra- and extracellular aqueous medium limits the local temperature increase. We assume in the following calculation that all of the heating results from light absorption only by the extrinsic fluorophore on the cell surface and not by intrinsic cellular biomolecules. This assumption is reasonable at usual labeling levels if the fluorescence excitation wavelength is in the visible range. Therefore, we consider the heat evolution from a finite two-dimensional heat source in a three-dimensional homogeneous aqueous medium.

Heat is produced by absorption of laser light of power P focused on the cell surface with a radial intensity profile $I(r)$ at the focal plane, given in polar coordinates by

$$I(r) = (2P/\pi w^2)\exp(-2r^2/w^2) \tag{1}$$

The local rate of heat production $Q(\mathbf{r}, t)$ on the membrane surface is proportional to $I(r)$ and is expressed in cylindrical coordinates with the z-axis normal to the membrane, as follows:

$$Q(\mathbf{r}, t) \equiv \begin{cases} 0 & \text{for } t < 0 \text{ and } t > T \\ (q/\rho c)\exp(-2r^2/w^2)\delta(z) & \text{for } 0 \leq t \geq T \end{cases} \tag{2}$$

where q = rate of heat production per unit area at the $r = 0$ position of the source, in cal s^{-1} cm^{-2}; ρ = mass density of the medium; c = specific heat of the medium; and T = time duration of the bleaching flash beginning at $t = 0$.

The heat conduction equation is

$$\kappa \nabla^2 T(\mathbf{r}, t) - \frac{\partial T(\mathbf{r}, t)}{\partial t} = Q(\mathbf{r}, t) \tag{3}$$

where $T(\mathbf{r}, t)$ ≡ increase of temperature over the ambient temperature; and κ ≡ thermal diffusivity of the medium.

The solution of Eq. 3 can be obtained from its well-known Green's function $G(\mathbf{r} - \mathbf{r}', t - t')$:

$$T(\mathbf{r}, t) = \int dt' \int d^3\mathbf{r}' G(\mathbf{r} - \mathbf{r}', t - t') Q(\mathbf{r}', t') \tag{4}$$

where

$$G(\mathbf{r} - \mathbf{r}', t - t') = \begin{cases} [4\pi\kappa(t - t')]^{-3/2} \exp[\,|\,\mathbf{r} - \mathbf{r}'\,|\,^2/4\kappa(t - t')] & \text{for } t > t' \\ 0 \qquad \text{for } t \leq t'. \end{cases} \tag{5}$$

Physically, the Green's function corresponds to the temperature evolution from an instantaneous point source of unit strength; this solution is then integrated over the spatial and temporal extent of the actual source.

We are primarily interested in the maximum local temperature rise, which occurs at $\mathbf{r} = 0$. Setting $\mathbf{r} = 0$ and also making the variable change,

$$\beta \equiv (t - t')^{-1/2}, \tag{6}$$

Eq. 4 combined with Eq. 5 becomes

$$T(0, t) = (q/\rho c)(4\pi)^{-1/2}\kappa^{-3/2} \int_{t^{-1/2}}^{\infty} d\beta \int_0^{\infty} dr' r' \times \exp\{-r^2[(\beta^2/4\kappa) + (2/w^2)]\}. \tag{7}$$

The r' and β integrations can be readily performed to give

$$T(0, t) = (qw/\rho c\kappa)(8\pi)^{-1/2}\{(\pi/2) - \arctan[w/(2\kappa t)^{1/2}]\}. \tag{8}$$

Eq. 8 shows that $T(0, t)$ approaches a steady state T_s:

$$T_s = (\pi/2)^{1/2} qw/4\rho c\kappa = (\pi/2)^{1/2} qw/4K \tag{9}$$

for times much longer than a critical time, t_c, where

$$t_c \equiv w^2/2\kappa, \tag{10}$$

and $K = \kappa\rho c$ is the thermal conductivity of the medium.

The rate of local heat production equals the power density of that part of the incident light that is absorbed without reradiation. To estimate the maximum possible local heating, we will assume that virtually all the absorbed light is converted into heat; i.e. that the fluorescence quantum efficiency is near zero. In this case, at $\mathbf{r} = 0$.

$$q = (2P/\pi w^2)(\epsilon n/N)[10^{11} \ln(10)/4.2]$$

where ϵ = extinction coefficient of the fluorophore at the bleaching wavelength (liter mole^{-1} cm^{-1}); N = Avogadro's number; n = number of fluorophores per μm^2 of cell surface; and P = total laser power in watts.

We now estimate t_c and T_s for a "typical" FPR lateral motion measurement. We have $\kappa = 0.0014\ cm^2\ s^{-1}$ (for water) and, typically,

$$w = 1 \times 10^{-4}\ cm.$$

Then $t_c = 3\ \mu s$. Therefore, the steady-state temperature is reached in a time much

shorter than the duration of a typical bleaching flash, which ranges from several seconds for some labeled cell surface protein motion experiments to several milliseconds for labeled lipid probe experiments.

In addition, for typical experiments on cell surface proteins labeled with tetramethyl rhodamine, $P = 1 \times 10^{-3}$ W, $\epsilon = 5 \times 10^4$ liter mole^{-1} cm^{-1}, $n = 5 \times 10^3$ μm^{-2}, $\rho = 1$ g cm^{-3}, and $c = 1$ cal deg^{-1} g^{-1}, so that

$$T_s = 0.03°\text{C}.$$

Thus, for experiments on cell surface proteins, the increase in surface temperature during bleaching is entirely negligible.

However, for lipid probe experiments where the relatively high mobility of the lipids demands that the bleaching flash must be made considerably briefer than for protein experiments, the laser power must be increased by two or more orders of magnitude to induce sufficient bleaching. In addition, the surface density n of lipid probe fluorophore may be several times higher than that of a typical labeled cell surface protein. In this case, the temperature during the brief bleaching flash could conceivably rise by 10°C or more. However, this problem can be completely avoided by increasing w, thereby lengthening the permissable bleaching and characteristic recovery times, and/or signal averaging recoveries from successive bleachings of lower power.

The local heat generated during bleaching is conducted away in a time much shorter than the characteristic fluorescence recovery time. Also, fluorescence recovery is always observed with a greatly ($\sim \times 10^3$) attenuated excitation light intensity that causes negligible heating during recovery. Therefore, even in the most unfavorable conditions, the cell surface is at essentially ambient temperature during the fluorescence recovery phase of the FPR experiment.

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