LASER LIGHT-SCATTERING SPECTROSCOPY

A New Application in the Study of Ciliary Activity

WYLIE I. LEE *and* PEDRO VERDUGO

From the Center for Bioengineering and Department of Biological Structure, University of Washington, Seattle, Washington 98195

ABSTRACT A uniquely precise and simple method to study ciliary activity by laser light-scattering spectroscopy has been developed and validated. A concurrent study of the effect of Ca^{2+} on ciliary activity in vitro by laser scattering spectroscopy and high speed cinematography has demonstrated that this new method is simpler and as accurate and reproducible as the high speed film technique.

Despite the critical function that the ciliary epithelium performs in both the respiratory and reproductive organs, a simple and objective method to study ciliary activity has not been developed. In fact, high speed cinematography combined with phase microscopy has been the only way to assess reliably the frequency of ciliary beat. However, this method is rather expensive to implement and extremely time consuming for data reduction. Although other techniques like photometric monitoring (1) and stroboscopic observation (2) have also been used to measure ciliary activity, there exists evidence that demonstrates these methods are unreliable when a precise assessment of the frequency of ciliary beat is required (2, 3). An objective and simple technique that can be employed to study ciliary activity directly and quantitatively has been urgently needed.

We have found that laser light-scattering spectroscopy provides an improved, precise and simple method to study ciliary activity. This communication reports the application and validation of this method to measure frequency of ciliary beat in vivo. Laser light-scattering spectroscopy has been used extensively in recent years to study the diffusional motions of macromolecules (4), sperm motility (5), and bacterial movement (6). The method is based on spectral analysis of the scattered light from moving objects. When moving particles are illuminated by a laser beam, the spectral structure of scattered light depends on both the type and magnitude of movement of the scattering particles and the angle of collection of the scattered light.

The essential requirement for this particular application is a safe low power source of coherent light with a very narrow bandwidth of frequencies as is produced by a laser. The light from a laser beam is directed at a ciliated surface and due to the Doppler effect the scattered light returning from moving cilia has an altered frequency and phase induced by the movement of the reflecting surfaces of the cilia. The effect of this altered frequency and phase is to produce fluctuations in the intensity of the scattered

light. The spectral structure of these intensity fluctuations can be demonstrated to be dependent upon the frequency of ciliary beat and the homogeneity of the ciliary movement. The scattered light can be detected with a photomultiplier tube and the spectral structure of its intensity fluctuations can be analyzed to provide quantitative information regarding the frequency and synchrony of the ciliary beat.

When a culture chamber containing ciliated cells is illuminated with the laser beam, two fields of scattered light are mixed on the cathode of the photomultiplier tube: (*a*) the local oscillator field, E_{Lo}, which is produced by the scattering at the air-glass interface and which has a frequency equal to the frequency of the laser light, and (*b*) the scattered field produced by the ciliated epithelium, E_c. The photocurrent of the photomultiplier resulting from this optical mixing, known as heterodyne mixing mode, may be expressed by (7)

$$i(t) = \beta[E_{Lo}(t) + E_c(t)]^* \cdot [E_{Lo}(t) + E_c(t)], \tag{1}$$

where β is an instrumental constant which includes the quantum efficiency and the collection efficiency of the photomultiplier tube, and the asterisk identifies the complex conjugate character of the first term in this expression.

The spectral structure of the scattered light intensity fluctuations can be expressed either by standard spectrum analysis or by the autocorrelation function of the detected photocurrents. The autocorrelation function establishes the relation between a signal and a time-shifted version of itself. Of immediate utility as a means of detecting periodic signals in noise, autocorrelation can also serve as the intermediate process in spectrum analysis (power density spectrum from autocorrelation function by Fourier transformation). This is of particular interest in low frequency work, where spectrum analysis is very difficult by direct methods. The autocorrelation function of this photocurrent can be shown as[1]

$$C(\tau) = ei_{Lo}\delta(\tau) + i_{Lo}^2[1 + (2i_c/i_{Lo})\,\epsilon^2 \exp(-\tau/\tau_R)\cos\omega_c\tau], \tag{2}$$

where ω_c is the frequency of ciliary beat, $\delta(\tau)$ is the Dirac delta function, i.e., $\delta(\tau) = 0$ if $\tau \neq 0$ and $\int_{-\infty}^{\infty} \delta(\tau)\,d\tau = 1$. The currents i_c and i_{Lo} are the average photocurrents produced by the light scattered by the cilia and the local oscillator, respectively, and $i_{Lo} \gg i_c$ is assumed. The heterodyne mixing efficiency, ϵ, represents the degree to which the local oscillator and the cilia scattered wavefront have matched phase fronts over the detector's surface. The decay constant τ_R characterizes the roughness of the epithelial surface and can be used as an indirect index of the coherency of ciliary movements. Therefore, the photocurrent autocorrelation function consists of a short noise term, a DC term, and a third term, which is a damped cosine function whose frequency equals the frequency of ciliary beat, ω_c, and whose damping coefficient, τ_R, relates indirectly the coherence of ciliary movements. In our experiments the autocorrelation function was computed on line by a Saicor Correlation and Probability Analyzer (Honeywell, Hauppauge, N.Y.) and plotted on an *x-y* recorder. Fig. 1 illus-

[1] Details of theory and other studies will be published separately by the authors.

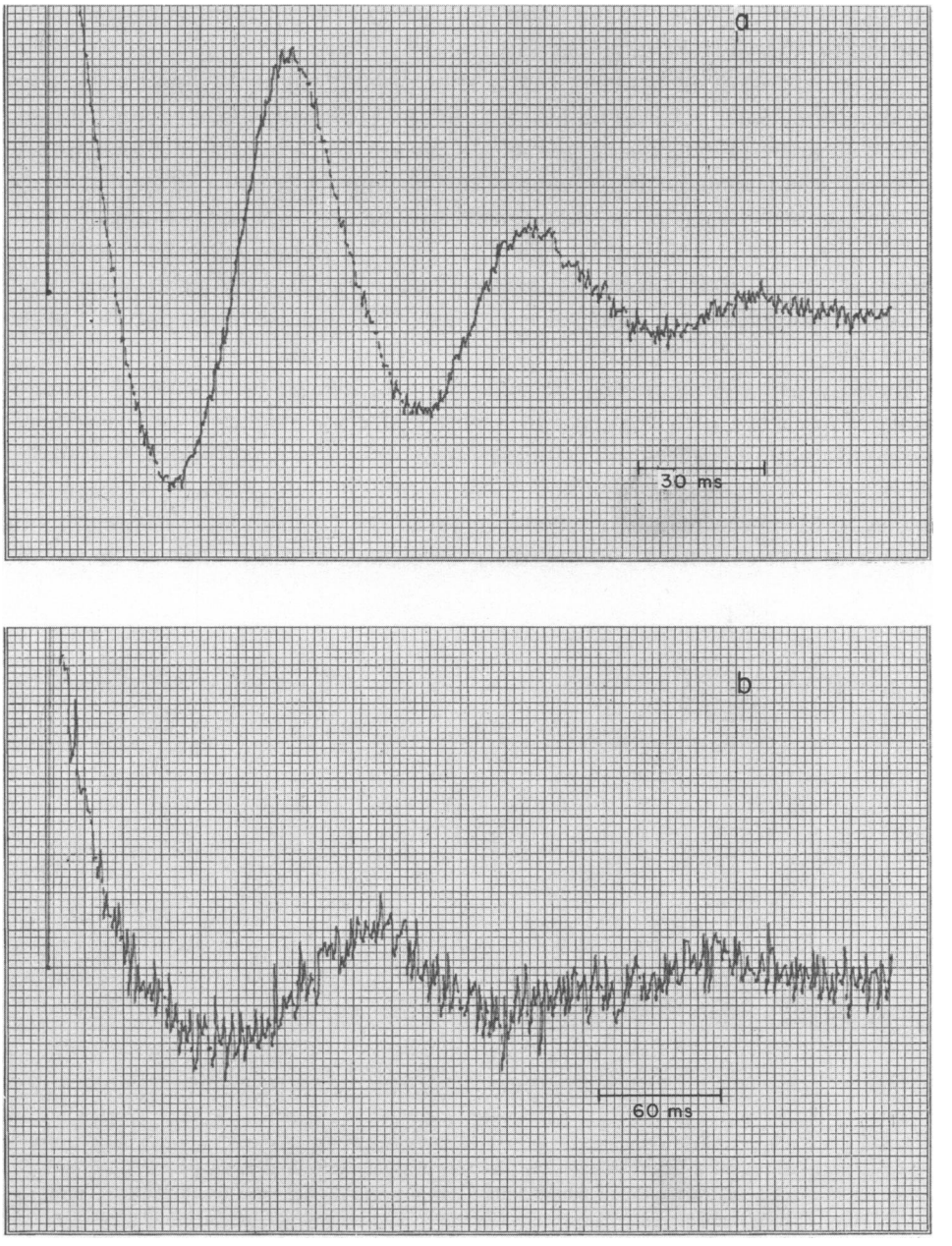

FIGURE 1 Typical autocorrelation functions of light scattered from ciliated cells of the oviduct. The strong, decaying, periodic components in the autocorrelation function are caused by the frequency and coherency of the periodic movements of the cilia. The frequency of ciliary beat can be computed as the reciprocal value of the period of the signals measured in this record. 1 *a* and 1 *b* correspond to frequency of ciliary beat of 17.5 and 6.5 Hz, respectively.

trates the autocorrelation function from two clusters of cilia beating at dominant frequencies of 18 Hz (Fig. 1*a*) and 7 Hz (Fig. 1*b*).

For the purpose of validating the application of laser light-scattering spectroscopy to measure frequency of ciliary beat in vitro, several experiments were conducted using tissue cultures of ciliated cells of the rabbit fimbria. The cultures were grown in Rose chambers in a modified Eagle's solution culture medium. After 48 hr of incubation the cultures were positioned in the spectrometer, maintaining their temperature constant at 37°C. The ciliated cells were then illuminated with an attenuated beam of an He-Ne laser which was focused down to 10 μm in diameter. The scattered light was collected by a photomultiplier tube positioned at an angle of 120° relative to the direction of the laser beam.

The quantitative evaluation of the method was conducted by comparing measurements of frequency of ciliary beat by high speed cinematography and laser light-scattering spectroscopy. Seven experiments were performed in which the frequency of ciliary beat was changed by equilibrating the cultures in Ca-free Hanks' solution containing 2 mM ethylenediaminetetraacetic acid (EDTA). Measurements of frequency of ciliary beat were conducted by using the two methods at timed intervals during the

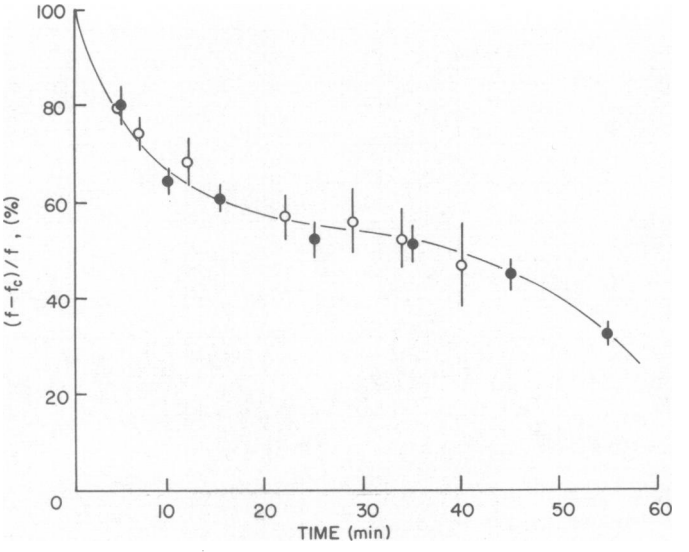

FIGURE 2 Time course of the changes of frequency of ciliary beat induced by perfusing the cultures of ciliated cells of the rabbit fimbria with a calcium-free Hanks' solution containing 2 mM EDTA. *f* is the frequency of ciliary beat observed in culture equilibrated in standard Hanks' solution containing 1.25 mM calcium. *fc* is the frequency of ciliary beat measured at different times after starting equilibration in calcium-free Hank's solution containing 2 mM EDTA. Data points obtained by high speed cinematography correspond to the average ± SD of about 120 samples of frequency of ciliary beat. Measurements were performed from the films, in fields of the culture containing approximately 25 ciliated cells each. Measurements conducted with the laser scattering spectroscope were obtained by scanning the culture with the laser beam and sampling an equivalent number of data points. Filled circled correspond to data obtained by high speed cinematography and unfilled circles to data obtained by laser scattering spectroscopy.

equilibration period. As illustrated in Fig. 2, there is good correlation between the frequency of ciliary beat as measured by laser light-scattering spectroscopy and high speed cinematography.

In this experiment we observed that a decrease in extracellular concentration of divalent cations consistently produces an inhibition in frequency of ciliary beat and also induces a remarkable asynchronism of the cilia beating, probably due to calcium depletion of the ciliated cells. Under these circumstances the frequency ω_c of the autocorrelation function follows very closely the frequency of ciliary beat as measured by high speed filming, and also the damping of the autocorrelation function is observed to increase (see Fig. 1 *b*) as predicted to occur when asynchronic modes of ciliary beat are developed.

The evidence presented here substantiates the idea that laser light-scattering spectroscopy is a reliable technique which presents several and important advantages over methods currently used to measure the frequency of ciliary beat. It combines the precision and reproducibility of high speed cinematography and simplicity and on-line access to the data of the photometric monitoring method. However, it does not present any of the unreliabilities of either the stroboscopic or the photometric monitoring techniques. An additional advantage of this method is that since the laser beam can be transmitted through fiber optics (8), laser scattering spectroscopy can be developed using an optical fiber and eventually applied in situ to measure ciliary activity on the walls of the oviduct or the respiratory tract.

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