Eur Biophys J (1990) 18:85–91

European Biophysics Journal
© Springer-Verlag 1990

Biophysical models of ciliary activity: Gaussian frequency distributions *

P. Thyberg [1], **R. Rigler** [1], **K. Svartengren** [1,2], **and L. G. Wiman** [2]

[1] Department of Medical Biophysics and
[2] Department of Lung Medicine, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden

Received September 19, 1989/Accepted in revised form November 20, 1989

Abstract. A model of a freely rotating exended scatterer is proposed to describe light scattering from beating cilia. Gaussian rotation frequency distributions, characterized by a mean angular frequency and a standard deviation, are introduced in order to simulate intensity autocorrelation functions and to fit the model to experimental data. Thus the ciliary beats are characterized by a mean beat frequency and a standard deviation of the beat frequency distribution. The standard deviation influences the damping of the intensity autocorrelation function of light scattered from cilia. The calculated intensity autocorrelation function shows a more prominent oscillating behaviour the smaller the standard deviation of the beat frequency. The validity of the model is supported by experimental data in two ways: *1*) The model fits very well to experimental data in computer evaluations, *2*) Neither the model nor information obtained from measurements are dependent on the measuring angle.

Key words: Ciliary activity – Light scattering – Gaussian beat frequency distribution – Model fittings

Introduction

Cilia, mostly 5 to 20 μm long and about 0.2 μm in diameter, can be found associated with many mammalian cells. The main function for cilia in the respiratory tract, for instance, is to clear the airways of gases, particles and chemical irritants trapped or dissolved by the mucus layer covering the respiratory epithelium. By repeated pushes billions of cilia on the surface are continuously transporting the mucus, the presence of which is essential for the efficient clearance of particles. The flow depends on ciliary activity, the rheological properties of mucus and the amount of mucus to be cleared. We have focused our interest on the ciliary activity.

Respiratory cilia are moving in a two step cycle: An effective and a recovery stroke. In the effective stroke the cilia are fully extended and giving the mucus on the cilia tips a push forward. In the recovery stroke the cilia are bent and returning near to the cell surface to reach an initial position. The ciliary carpet moves in waves caused by the co-ordinated beats of cilia. The wavetops consist of extended cilia in the effective stroke and the valleys of bending cilia in the recovery stroke (Sleigh et al. 1988). Amazingly rapid changes in beat frequency, even in temperature controlled measurements, are observed in cilia from rabbit respiratory mucosa (Kennedy and Duckett 1981) and cilia from frog palate epithelium, which furthermore show a large variation of beat frequency in space (Spungin and Silberberg 1984).

The frequency of ciliary beats can be measured from the rhythmic intensity variations of the light reflected from or transmitted through the ciliated surface. The beat frequency can be obtained directly from the intensity recordings by counting the number of times the intensity oscillates per time unit (Dahlham 1962; Håkansson and Toremalm 1965; Yager et al. 1978; Bonnaire et al. 1980). However, the determination of the beat frequency can be improved by the use of an appropriate analysis method. Frequency analysis by a Fourier transform of the intensity is one method where the beat frequency spectrum is expressed directly from the frequency components of the intensity (Verdugo et al. 1980; Kennedy and Duckett 1981; Spungin and Silberberg 1984). The width of the beat frequency distribution is obtained from the standard deviation of the spectral line. The introduction of a parameter representing the standard deviation of the frequency distribution made it possible to estimate the dependence of spread in cilia beat frequency on temperature (Eshel and Priel 1986) and on the size of the measured area (Eshel and Priel 1986). The autocorrelation of the intensity provides another concise method for expressing the degree to which the intensity correlates with itself over a period of time. The beat frequency can be calculated from the inverse of the period of its oscillating part (Lee and Verdugo 1976; Hennessy et al. 1986;

* The contents were presented in part at the 9th International Biophysics Congress in Jerusalem, Israel, August 23–28, 1987
Offprint requests to: P. Thyberg

Svartengren et al. 1989). Very few attempts have been made to describe the recorded autocorrelation function in detail. In a previous study the oscillations in the autocorrelation function were said to originate from the wave motion upon the epithelial surface (Lee and Verdugo 1976). The oscillation frequency agreed with the ciliary beat frequency and the oscillations were damped exponentially with a damping coefficient indirectly related to the coherence of the ciliary movements. The model, however, was not fitted to experimental data. In the present study the cilia are modeled as rods freely rotating with a Gaussian distributed frequency. The model fits well with experiments and information regarding mean frequency and standard deviation of the frequency distribution is obtained from data evaluations. This is the first time, to our knowledge, that the standard deviation has been determined from the autocorrelation function. From model simulations the damping of the oscillations of the autocorrelation function is found to be dependent on the standard deviation of the beat frequency.

Theory

Our model is based on a concept mostly used for describing the light scattering from cylindrically symmetric molecules (Berne and Pecora 1976). The incident light has an electric field

$$\boldsymbol{E}_i(\boldsymbol{r}, t) = \boldsymbol{n}_i E_o \exp \mathrm{i}\,(\boldsymbol{k}_i \cdot \boldsymbol{r} - \omega_i t) \tag{1}$$

with a form factor $\boldsymbol{n}_i E_0$, where $\boldsymbol{n}_i$ is a unit vector in the direction of the incident electric field, and a phase factor $\exp \mathrm{i}\,(\boldsymbol{k}_i \cdot \boldsymbol{r} - \omega_i t)$, where $\boldsymbol{k}_i$ is the propagation vector and ω_i is the angular frequency of the light. The scattered light depends on scattering properties of the scattering objects. For objects with statistically independent positions the autocorrelation of the scattered electric field, defined

$$G_{\text{field}}(\tau) = \int \boldsymbol{E}_s^*(t) \cdot \boldsymbol{E}_s(t+\tau)\, \mathrm{d}t \tag{2}$$

is shown to be the product of two terms, one concerning the form factor and one concerning the phase factor. We have neglected the contribution from the phase factor component which only contains information about translational motion of the scatterer and modeled the cilia as purely rotating rods. The form factor component depends on the time lapse of the polarizability tensor, and thus contains information about the rotational motion of the cilia. In a laboratory fixed coordinate system the polarizability tensor, which is an object fixed property, changes with the reorientation of the light scatterer. With the mentioned approximations the autocorrelation of the electric field is equivalent with the autocorrelation of polarizability tensor components.

For free rotors with statistically independent orientations the reorientation correlation is

$$C(\tau) = \int p(\omega)\, P_2(\cos \omega \tau)\, \mathrm{d}\omega \tag{3}$$

(Berne and Pecora 1976) which we have used to describe the electric field autocorrelation of light scattered by moving cilia. P_2 is the second-order Legendre polynomial and $p(\omega)$ is a function describing how the angular frequency, ω, of the rotation about an axis perpendicular to the axis of symmetry is distributed. With this interpretation the model corresponding to an intensity autocorrelation is expressed as

$$G_{\text{int}}(\tau) = (B + A \cdot C(\tau))^2 + D \tag{4}$$

where A, B and D are different types of amplitudes. In similar models for optically anisotropic molecules A and B are describing the isotropic part of the polarizability tensor, α by

$$B = \langle N \rangle \cdot \alpha^2 \tag{5}$$

and the optical anisotropy, β by

$$A = 4\, \langle N \rangle \cdot \beta^2/45 \tag{6}$$

where $\langle N \rangle$ is the average number of molecules in the measurement. D is just an amplitude necessary to relate the field autocorrelation to the experimental intensity autocorrelation. The intensity autocorrelation function is defined

$$G_{\text{int}}(\tau) = \int I(t) \cdot I(t+\tau)\, \mathrm{d}t \tag{7}$$

Expressing the angular frequency distribution by a Gaussian function

$$p(\omega) = 1/\sqrt{2\pi\, \sigma_\omega^2} \cdot \mathrm{e}^{-0.5((\omega - \langle\omega\rangle)/\sigma_\omega)^2} \tag{8}$$

Equation (3) can be calculated numerically. The mean angular frequency, $\langle\omega\rangle$, is directly related to the average ciliary beat frequency and σ_ω is related to the spread of beat frequencies during the measurement.

Experimental

Material

Bovine tracheas, taken from a slaughter house, were kept and transported for about one hour in nutrient medium (Parker 199) before use in the experiments. They were gently shaken to remove the mucus layer. Pieces of mucosa (1 cm^2) were dissected from the trachea and mounted in a glass cuvette for measurement.

Measurements

Temperature (ranging from 20 °C to 44 °C) as well as humidity were regulated by a water circuit thermostat cell specially made for this application (Fig. 1). An attenuated light beam from an argon ion laser (Spectra Physics, model 165) operating with a power output of 120 mW, was focused to a spot size of 20 μm in diameter on the tissue preparation (Fig. 2). The power of the attenuated light ranged between 0.3 and 100 mW for the different measurements. The scattered light was detected by a photomultiplier tube (RCA 8850) operating in a single photon counting mode (Rigler and Thyberg 1984) and analyzed in real time by a Langley-Ford 1096 digital correlator. The correlator calculated a 72 point autocorrela-

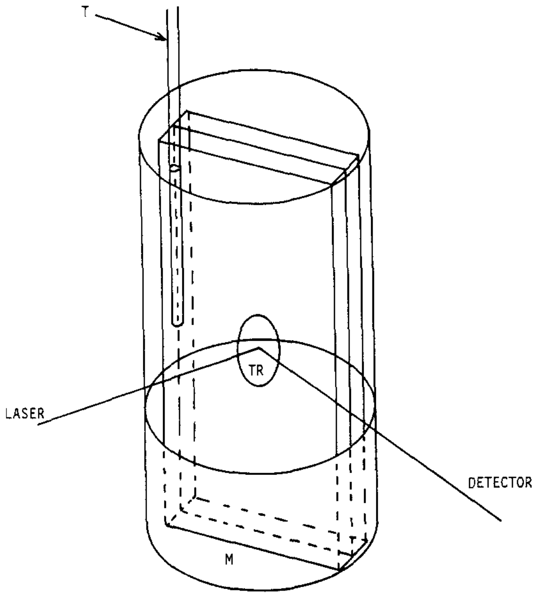

Fig. 1. Thermostatted cuvette with tracheal preparation (TR). Thermistor (T) for temperature control. The bottom part of the closed cuvette was filled with Parker's medium (M) for control of humidity conditions

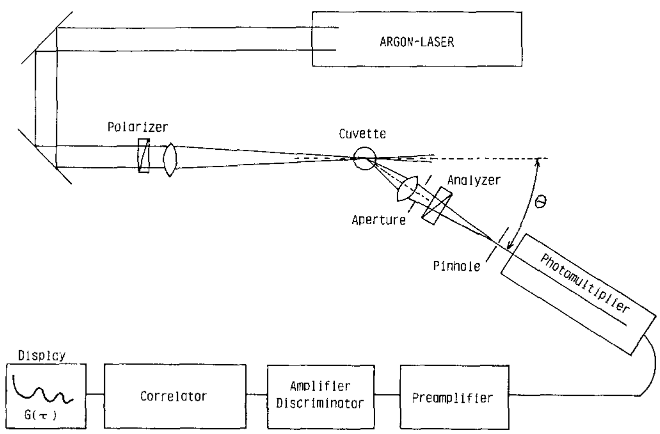

Fig. 2. Set-up for in vitro measurements of ciliary activity by laser light scattering using an argon-ion laser source, a thermostatted cuvette, a photomultiplier tube and a signal processor (correlator). Measuring angle $= \theta$

tion function with 36 bit precision directly from the intensity.

Data evaluation and fitting

A computer algorithm (Marquardt 1963) was used in order to minimize the least squares difference between the experimental data and the model (4) by means of the 5 parameters: A, B, D, σ_ω and $\langle\omega\rangle$. The goodness of the fit was expressed by the residual root mean square (RRMS) and the error limits of the parameters by standard deviations from the computer evaluation. Ciliary beat frequencies (Tables 1–4) corresponding to the estimated angular frequencies were calculated by dividing the ω-values by π.

Table 1. Parameter values of model fitted to data of a light scattering measurement of 5 s duration

	Estimated value	Beat frequency
$\langle\omega\rangle$	76.78 $\pm$0.08	24.44$\pm$0.02
σ_ω	4.8 $\pm$0.3	1.51$\pm$0.09
A	0.224$\pm$0.008	
B	0.45 $\pm$0.02	
D	0.75 $\pm$0.02	
RRMS	0.005	

Table 2. Parameter values of model fitted to data of a light scattering measurement of 30 s duration

	Estimated value	Beat frequency
$\langle\omega\rangle$	77.2 $\pm$0.3	24.56$\pm$0.09
σ_ω	12.6 $\pm$0.3	4.00$\pm$0.09
A	0.54 $\pm$0.01	
B	0.137$\pm$0.009	
D	1.028$\pm$0.003	
RRMS	0.01	

Results

Simulations

The autocorrelation function calculated from (2) showed oscillating behaviour. The frequency of the oscillations was dependent on the mean angular frequency $\langle\omega\rangle$ in (8) and the damping of the oscillations was dependent on the standard deviation of angular frequencies (σ_ω). The dependences were investigated for σ_ω ranging from 1 Hz to 50 Hz (Fig. 3) and for $\langle\omega\rangle$ ranging from 75.4 Hz to 157.1 Hz. The numerical calculations of the autocorrelation showed less prominent oscillating behaviour the higher the σ_ω-values. In other words, larger standard deviations of the frequency distribution lead to more damped autocorrelation functions. The frequency of the oscillating part in the autocorrelation function was proportional to $\langle\omega\rangle$, with a proportionality factor of $1/\pi$.

Measurements

The standard deviation of the angular frequency (σ_ω) was found to increase with prolonged time of the measurement. σ_ω of 4.8 Hz (Table 1, Fig. 4) and 12.6 Hz (Table 2, Fig. 5) were obtained from model fittings of measurements carried out with various time intervals at 34.7$\pm$0.5 °C. The ciliary beat frequency showed a clear temperature dependence. The mean angular frequency ($\langle\omega\rangle$) was 33.11 Hz (Table 3, Fig. 6) at 23.3$\pm$0.5 °C and 84.4 Hz (Table 4, Fig. 7) at 36.0$\pm$0.5 °C. The measurements were taken at a measuring angle of 90°.

In order to test the angle dependence, $\langle\omega\rangle$ and σ_ω were determined at various measuring angles (θ) ranging from 25° to 110°. Both $\langle\omega\rangle$ and σ_ω showed insignificant dependence on the measuring angle. A slope of -0.007 Hz/° with a correlation coefficient of 0.84 was

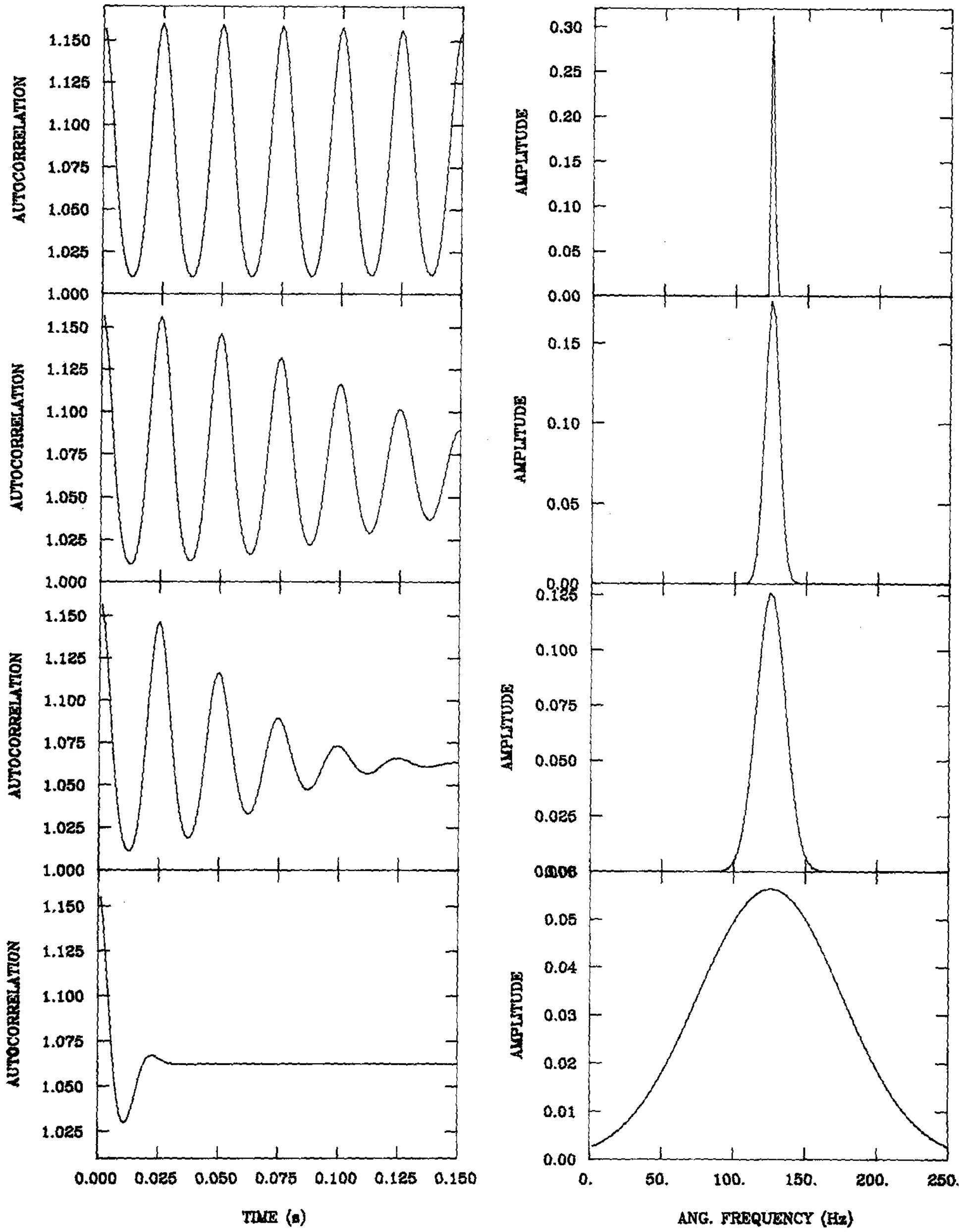

Fig. 3. Intensity autocorrelations, to the left, and angular frequency distributions, to the right, calculated from the model using $\langle\omega\rangle = 125.7$ Hz, $A = 0.2$, $B = 0.2$, $D = 1$ and various σ_ω-values. From the top to the bottom $\sigma_\omega = 1$ Hz, 5 Hz, 10 Hz, 50 Hz

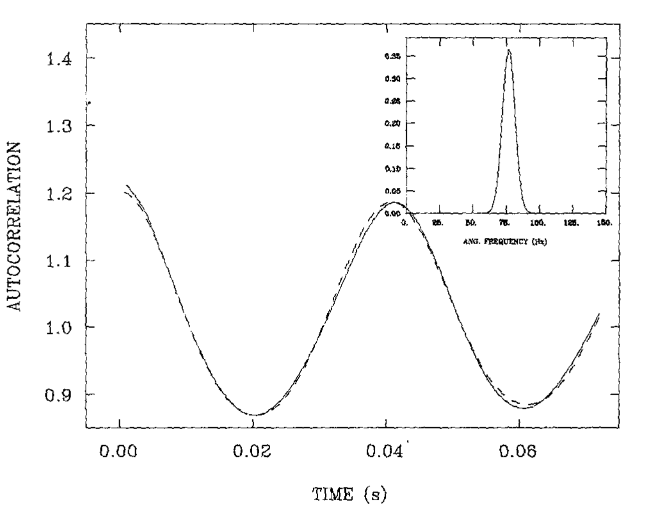

Fig. 4. Intensity autocorrelation function obtained from: An experiment of 5 s duration (continuous line), least square fit of model to the experiment (dashed line). Insert: Angular frequency distribution

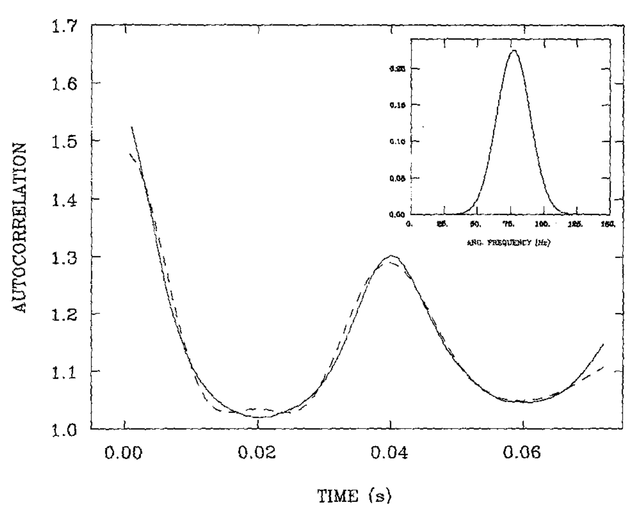

Fig. 5. Intensity autocorrelation function obtained from: An experiment of 30 s duration (continuous line), least square fit of model to the experiment (dashed line). Insert: Angular frequency distribution

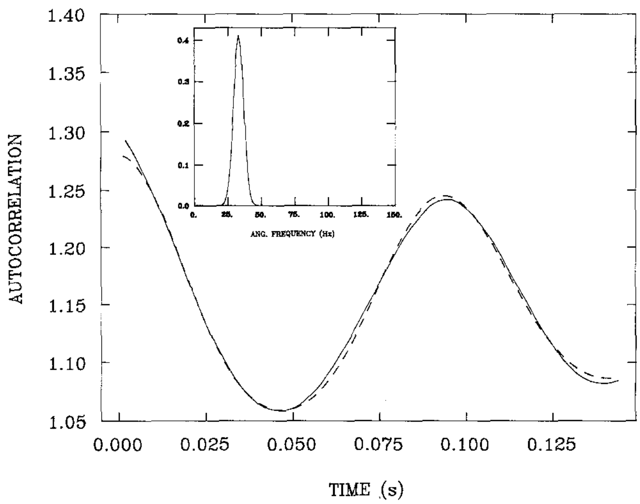

Fig. 6. Intensity autocorrelation function obtained from: An experiment at 23.3 °C (continuous line), least square fit of model to the experiment (dashed line). Insert: Angular frequency distribution

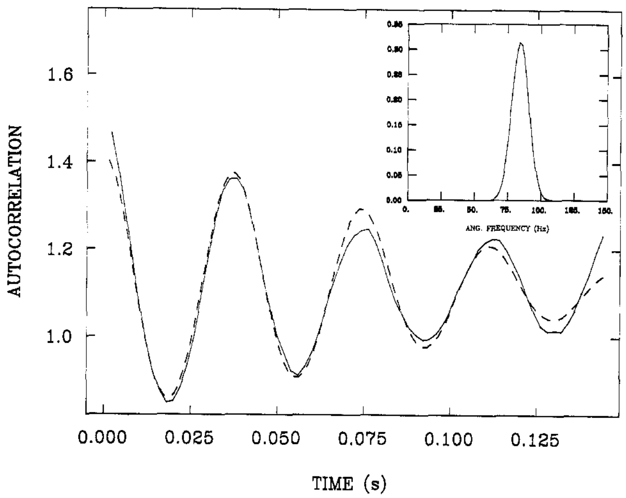

Fig. 7. Intensity autocorrelation function obtained from: An experiment at 36.0 °C (continuous line), least square fit of model to the experiment (dashed line). Insert: Angular frequency distribution

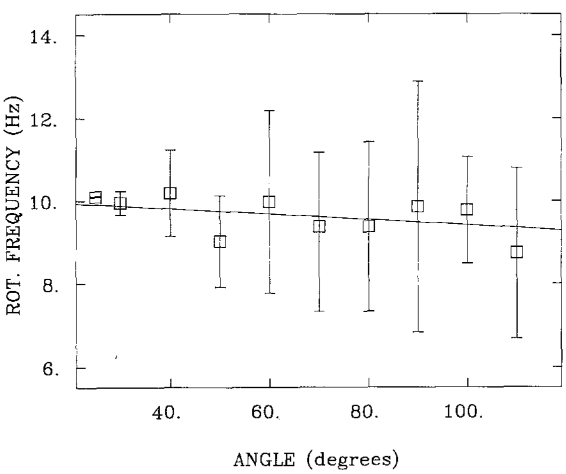

Fig. 8. Mean rotation frequency of cilia modeled as free rotors compiled from measurements at various measuring angles. Average value (□) and standard deviation (error bars) are indicated for each separate angle

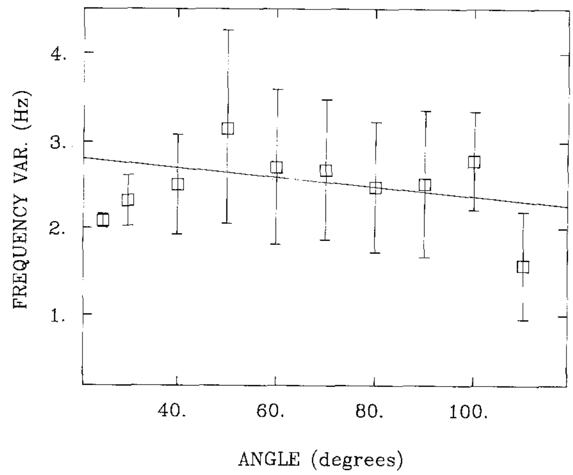

Fig. 9. Standard deviation of rotation frequencies of cilia modeled as free rotors compiled from measurements at various measuring angles. Average value (□) and standard deviation (error bars) are indicated for each separate angle

Table 3. Parameter values of model fitted to data of a light scattering measurement at 23.3 °C

	Estimated value	Beat frequency
$\langle\omega\rangle$	33.11 ±0.05	10.54±0.02
σ_ω	3.76 ±0.08	1.20±0.03
A	0.211±0.007	
B	0.30 ±0.01	
D	1.016±0.008	
RRMS	0.004	

Table 4. Parameter values of model fitted to data of a light scattering measurement at 36.0 °C

	Estimated value	Beat frequency
$\langle\omega\rangle$	84.4 ±0.2	26.86±0.07
σ_ω	6.5 ±0.3	2.06±0.08
A	0.21±0.05	
B	0.84±0.22	
D	0.31±0.38	
RRMS	0.03	

found for $\langle\omega\rangle$ as a function of θ (Fig. 8) and a slope of -0.006 Hz/° with a correlation coefficient of 0.77 was found for σ_ω as a function of θ (Fig. 9).

Discussion

Within the error limits both the mean frequency and the standard deviation (SD) of the frequency were independent of the measuring angle because of the flat slopes (Figs. 8 and 9) and the low values of the correlation coefficient. The validity of the present model, obviously also independent of the measuring angle, was therefore supported by the measurements. In contrast to models containing translational motions which always are dependent on the measuring angle, the present model contained only a rotational reorientation. The very simple model of cilia as free rotors with Gaussian distributed angular frequencies opened the possibility to determine important physiological properties.

In a previous study the damping of the oscillations in the intensity autocorrelation was thought to relate indirectly to the coherence of ciliary motion (Lee and Verdugo 1977). The oscillations were said to be damped exponentially with a damping coefficient $\tau_f/k^2\sigma^2$, where τ_f and σ are both parameters describing the roughness of the epithelial surface and k is the wavevector of the light. Since the two parameters τ_f and σ occur in the same term of the model it is impossible to estimate either of them from experimental data without prior knowledge of the other. The model in the previous study is not shown to fit to any experimental data. The model in the present study, in contrast, has parameters that can be determined in data evaluations and it fitted well to experimental data. Model fittings provided information regarding mean ciliary beat frequency and SD of the beat frequency distribution.

The oscillations of the autocorrelation function were clearly shown to be dependent on the width of the frequency distribution (Fig. 3) and were more damped the broader the frequency distribution function. Examples were shown in experiments where the ciliary beat frequencies were of different stability. The frequency distribution obtained in the 30 s measurement (Fig. 5) was broader than the one obtained during 5 s (Fig. 4), probably as a consequence of the increased chance for a frequency change the longer the recording time. In view of the rapid changes in ciliary beat frequency observed in rabbit (Håkansson and Toremalm 1965; Kennedy and Duckett 1981; Eshel et al. 1985), beat frequency changes in bovine trachea were very probable over 30 s durations. From previous investigations the SD of the frequency distribution obtained from the Fourier transform of the intensity was found to increase with increased temperature (Eshel and Priel 1986). In the present study the SD of the frequency distribution, σ_ω, was bigger and the autocorrelation function more damped at 36 °C (Table 4, Fig. 7) than the ones obtained at 23.3 °C (Table 3, Fig. 6) which is in agreement with previous findings. Thus the present study suggests that the degree of damping in the oscillations of the intensity autocorrelation function depends on σ_ω, which was measured in Hz. A verification of our findings concerning the dependence of the SD of the frequency on time and temperature would, however, require analysis of a larger amount of data or simultaneous recordings of the ciliary motion with high speed cinematography.

The SD of the beat frequency distribution depends on the size of the measured spot (Eshel and Priel 1986). In the previous study various spot sizes between 1.25 µm and 12.5 µm in diameter were investigated for tissues from the palate and lungs of frog at 23 °C. The smallest SD in the frequency distribution of 1.34 Hz was obtained for a spot size of 2.5 µm in diameter. For spots larger than 2.5 µm the SD increases with increasing spot size because of a decrease in homogeneity or larger distribution of ciliary beat phases in the metachronal wave. At similar temperature in the present study we found a SD of beat frequency of 1.2 Hz (Table 3 and Fig. 6), which was much smaller than one would expect from a measurement with a spot size as large as 20 µm in diameter. One is tempted to believe that the cilia in bovine trachea are beating more homogeneously or having metachronal waves with longer wavelengths than the frog palate cilia. The difference in SD between the two studies can also be due to the difference in the time lapse of the measurement, 5 s in the present study compared with 10 s in the previous. In accordance with the discussion above the shorter measurements should give smaller SD.

We found an increase in oscillation frequency of the intensity autocorrelation proportional to the increase in mean angular frequency, $\langle\omega\rangle$, which was natural. At this point one can ask why the frequency of the autocorrelation oscillations was twice the rotation frequency corresponding to the $\langle\omega\rangle$ of the model. A rod, rotating around an axis perpendicular to the axis of symmetry, causes two intensity cycles because the free rotor takes equivalent orientations two times for each rotation cycle it undergoes. Thus the intensity autocorrelation should oscillate twice as fast as the rod rotates. The ciliary beat frequency must therefore be interpreted as the model rotation frequency multiplied by two. With this interpretation ciliary beat frequencies equal the inverse of the period time of the oscillations in the autocorrelation function. A more detailed study of the relation between ciliary beats and intensity oscillations is taking place in our laboratory, investigating simulations of light scattering from more complex models of cilia in motion (data to be published).

Sometimes 'peaky' oscillations in the autocorrelation function, i.e., narrow maxima and broad minima, were observed. Some measurements even had small extra peaks in between two big maxima (such as in the fit in Fig. 5). According to our model, this should happen when the isotropic part of the polarizability tensor (B in (4)) is small as compared to the optical anisotropy (A). With a finite distribution width in angular frequency, $C(\tau)$ (3) can have negative parts since $P_2(\cos\omega\tau)=(3\cos^2\omega\tau-1)/2$ is partly negative. If B is too small to compensate for the negative parts of $A\cdot C(\tau)$ they will appear as positive maxima when squared in (4). We cannot explain why light scattering from cilia seems to be more anisotropic in some measurements than in others. However, the fact that 'peaky' oscillations are seen only in some measurements but not in others with the same experimental conditions (temperature, detection spot, detection angle etc.) indicates spontaneous changes in the ciliary beat pattern.

The observation of rapid changes in beat frequency in tissue preparations from different animals in vitro raises interesting questions for future studies: What is the functional meaning of varying the beat frequency? How stable is the beat frequency in vivo? And how stable is the beat frequency in man? We have here presented a method to estimate the variance of the ciliary beat frequency, which might be an important tool to answer these questions.

In conclusion, free rotors rotating with gaussian distributed frequencies describe the beating of cilia very well. The mean frequency as well as the width of its distribution were obtained from model fittings to experimental light scattering data.

Acknowledgements. We thank Ove Kristensen for expert technical assistance and Roza Jakabffy for preparing illustrations. This study was supported by grants from the Swedish National Association against Heart and Lung Diseases and from the foundations of Johan and Lisa Grönberg and Knut and Alice Wallenberg.

References

Berne BJ, Pecora R (1976) Dynamic light scattering with applications to chemistry, biology, and physics. Wiley, New York

Bonnaire Y, Debreuil A, Bouley G, Boudène C (1980) An improved device for frequency recording of tracheal ciliary beat. Microsc Acta 83:221–227

Dahlhamn T (1962) Frequency of ciliary beat measured with a photo-sensitive cell. Nature (London) 196:592–593

Eshel D, Priel Z (1986) Spectral characterization of ciliary beating: Biological meaning of the spectral linewidth. Biophys Chem 23:261–265

Eshel D, Priel Z (1986) Spectral characterization of ciliary beating: Temperature dependence of spectral parameters. Biophys Chem 25:215–222

Eshel D, Grossman Y, Priel Z (1985) Spectral characterization of ciliary beating: Variations of frequency in time. Am J Physiol 249:C160–C165

Håkansson CH, Toremalm NG (1965) Studies on the physiology of trachea. I. Ciliary activity indirectly recorded by a new "light beam reflex" method. Ann Otol Rhinol Laryngol (Stockh) 74:118

Hennesey SH, Wong LB, Yeates DB, Miller IF (1986) Automated measurements of ciliary beat frequency. J Appl Physiol 60: 2109–2113

Kennedy JR, Duckett KE (1981) The study of ciliary frequencies with an optical spectrum analysis system. Exp Cell Res 135:147–156

Lee WI, Verdugo P (1976) Laser light spectroscopy: A new application in the study of ciliary activity. Biophys J 16:1115–1119

Lee WI, Verdugo P (1977) Ciliary activity by laser light-scattering spectroscopy. Ann Biomed Eng 5:248–249

Marquardt DW (1963) An algorithm for least-squares estimation of nonlinear parameters. J Soc Indust Appl Math 11:431–441

Rigler R, Thyberg P (1984) Rotational and translational swimming of human spermatozoa: A dynamic laser light scattering study. Cytometry 5:327–332

Sleigh MA, Blake JR, Liron N (1988) The propulsion of mucus by cilia. Am Rev Respirat Dis 137:726–741

Spungin B, Silberberg A (1984) Stimulation of mucus secretion, ciliary activity in frog palete epithelium. Am J Physiol 247 (Cell Physiol 16):C299–C308

Svartengren K, Wiman L-G, Thyberg P, Rigler R (1989) Laser light scattering spectroscopy. A new method to measure tracheobronchial mucociliary activity. Thorax 44:539–547

Verdugo P, Johnson NT, Tam PY (1980) β-Adrenergic stimulation of respiratory ciliary activity. J Appl Physiol: Respirat Environ Exercise Physiol 48:868–871

Yager J, Chen T-M, Dulfano MJ (1978) Measurements of frequency of ciliary beats of human respiratory epithelium. Chest 73:627–633