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tion of $S_2 - S_1$ that is in phase with $A_1$, that is, in phase with $\sin(\omega t)$, and from Eq. 2 the slope will therefore be

$$\text{slope}_1 = D_2B_2\cos(\gamma)/B_1 - D_1. \quad (8)$$

Similarly, the slope of $S_2 - S_1$ vs. $A_2$ will be

$$\text{slope}_2 = -D_1B_1\cos(\gamma)/B_2 + D_2. \quad (9)$$

Therefore,

$$D_1 = -[\text{slope}_1 - \text{slope}_2 B_2\cos(\gamma)/B_1]/[1 - \cos^2(\gamma)], \quad (10)$$

$$D_2 = [\text{slope}_2 - \text{slope}_1 B_1\cos(\gamma)/B_2]/[1 - \cos^2(\gamma)], \quad (11)$$

$$D_2 - D_1 = [\text{slope}_1 + \text{slope}_2 - \cos(\gamma)(B_2\text{slope}_2/B_1 + B_1\text{slope}_1/B_2)]/[1 - \cos^2(\gamma)]. \quad (12)$$

For very small values of $\gamma$, $B_1 \rightarrow B_2$, and $D_2 - D_2 \rightarrow (\text{slope}_1 + \text{slope}_2)/(1 + \cos[\gamma]) \rightarrow (\text{slope}_1 + \text{slope}_2)/2$. However, the individual values for $D_1$ and $D_2$ depend on the small difference between the nearly equal values of $\text{slope}_1$ and $\text{slope}_2$, so that much greater precision is required in the measurements of the slopes to obtain individual values for $D_1$ and $D_2$, as opposed to the sum $D_2 - D_1$. When the phase difference, $\gamma$, approaches $\pi$ rad, the denominators in Eqs. 7 and 12 become small, and large errors in the results can be obtained if the data are imprecise. It is therefore prudent to limit the calculations to cases where the phase difference is not much greater than $\pi/2$ rad. Since the typical bending wave lengths for sea urchin spermatozoa are 30–40 $\mu$m, this means that the beads should not be separated by $>\sim 8$ $\mu$m.

After values for $D_2 - D_1$, $D_1$, and $D_2$ are obtained in this manner, the calculations can be repeated iteratively, replacing the approximate Eq. 3 with

$$A_1 = A_{10} + B_1\sin[\omega t - B_1D_1\gamma\sin(\omega t)/S_{210}]. \quad (13)$$

However, in all of the cases that were examined, the values of $D_2 - D_1$ were not significantly modified by this iterative recalculation (at most a change of 1 nm).

There are therefore two independent ways to fit the data and obtain the doublet separation, $D_2 - D_1$, one using $B_3$ and $\alpha$, the other using $\text{slope}_1$ and $\text{slope}_2$. Both methods have been used routinely to calculate doublet separation, and the mean value has been used. In any cases where the two values obtained for the doublet separation did not agree closely, the analysis has been carefully examined for errors caused by inaccuracies in determining $\gamma$ or $\alpha$, and in a few cases the limiting value for $\gamma = 0$ has been used instead. Both procedures can in principle also yield independent values for $D_1$ and $D_2$, but only if the data are very precise.

## *Calculation of Theoretical Distributions*

The doublet microtubules to which the measured beads are attached are not known. Interpretation of the doublet separations requires comparison of the distribution of measured doublet separations with the distribution expected from random attachment of beads to the outer doublet microtubules. In the simplest case, the expected distribution contains 81 values given by

$$\text{doublet separation/diameter} = \{\cos[2\pi(n - 1)/9] - \cos[2\pi(m - 1)/9]\}/2, \quad (14)$$

where $n$ and $m$ take on all integer values from 1 to 9 (Brokaw, 1989*a*). The diameter is the diameter of a circle passing through the neutral surface of each outer doublet, that is, the surface that undergoes no longitudinal compression or extension when the doublet bends. By measuring 81 values of doublet separation, the distribution of values given by Eq. 14 can be directly compared with the measured values (Brokaw, 1989*a*). Absolute values for both the theoretical and measured doublet separations are used in this comparison. If the number of measured values, $N$, is not equal to 81, an appropriate distribution can be obtained by a Monte Carlo simulation, in which $N$ values are chosen at random from the 81 values given by Eq. 14, and the average of a large number of simulated distributions is used for comparison with the measured values.

Equation (14) is too simple, because it does not take into account several factors that will tend to smooth the distribution. For the analyses in this paper, theoretical distributions have been obtained by Monte Carlo simulations, averaging 400 distributions obtained by selecting $N$ values at random using the following equation:

$$\text{doublet separation/diameter} = (\{1 + FV_1\}\{\cos[2\pi(n - 1)/9 + V_2] - \cos[2\pi(m - 1)/9 + V_2]\} + V_1)/2. \quad (15)$$

To obtain each value of doublet separation, a random variable is used to select an integer from the range 1 to 81, and values of $n$ and $m$ are then found such that the selected integer is equal to $9n + m$. $V_1$ is a random variable chosen from a Gaussian curve for a normal distribution. Its purpose is to incorporate into the theoretical distribution the effects of errors in measurement of the values of doublet separation and random variations in diameter in the population sampled. The standard deviation for this distribution, and the factor $F$, were determined empirically. $F$ was determined by examining the relationship between the standard errors calculated for the slopes of the linear regressions of bead separation on shear angle and the values of the slopes. The values chosen were 0.25 for the *Ciona* sperm sample and 0.5 for the *Lytechinus* sperm sample. Values used for the standard deviation of $V_1$ were 0.08 for the *Ciona* sperm sample and 0.1 for the *Lytechinus* sperm sample. $V_2$ is a random variable chosen from a uniform distribution between 0 and $\pi/9$ rad. A value of $V_2 = 0$ corresponds to the assumptions that each bead is attached to just one outer doublet microtubule and the bending plane passes through doublet 1 and between doublets 5 and 6. A value of $V_2 = \pi/9$ corresponds to the assumption that each bead attaches to two adjacent microtubules, with the same bending plane. The uniform distribution allows for the uncertainty in how beads attach to the doublets and uncertainty in the exact position of the bending plane. Both $V_1$ and $V_2$ smooth the distribution. $V_2$ has very little effect on the shape of the distribution, while $V_1$ extends the tail of the distribution at high values of doublet separation.

After calculating the distribution, the value of diameter can be adjusted to give the minimum root mean square (RMS)[1] difference between the distribution and the measured values. This is done by sorting each distribution into increasing order of absolute values, and comparing the nth largest measured value with the nth largest calculated value, etc. Since this sorting of $N$ values removes $N-1$ degrees of freedom from the distribution, the residual RMS difference between the measured and calculated values is interpreted as a standard error for the estimate of the diameter.

# *Results*

## *Examples of Analyses of Bead Pairs on Individual Spermatozoa*

One *Ciona* spermatozoon was chosen as an example to illustrate the methods used for analysis of bead movements and the character of the data that were obtained. Fig. 3 shows examples of computer monitor displays of two images of this spermatozoon, chosen to show the extremes of bead movement between the second and third beads on the flagellum (counting from the sperm head). In principle, the motion of each bead could be analyzed individually, using the measurements of its position relative to the sperm head. In practice, this has not been sufficiently accurate, and better information has been obtained by analyzing the relative motion of two beads separated by distances of no more than 8 $\mu$m. The eight beads that were measured on the flagellum shown in Fig. 3 provide five pairs of beads that can be analyzed for relative sliding motions, with mean separations between the beads of each pair ranging from 1.1 to 3.5 $\mu$m. Five multiple-flash photographs of this spermatozoon, each containing $\sim$50 images, were available for analysis. In the first three photographs, the flagellum was beating with a stable frequency of 26.2 Hz. Between the third and fourth photographs, the frequency dropped to 24.8 Hz, and the fourth bead disappeared from the flagellum. The combined results for analysis of this spermatozoon are summarized in Table I, with standard deviations for the sample of five photographs. For bead pair 4–5, only the results from the first three photographs are given. Bead pair 2–3 of this spermatozoon showed the largest amplitude of oscillation of bead separation ($B_3$ = 289 nm) found in the *Ciona* sample. Results of the analysis of this bead pair, from one of the photographs, are shown in Figs. 4 and 5.

1. *Abbreviation used in this paper*: RMS, root mean square.