

# MOTILITY EVALUATION OF HUMAN SPERMATOZOA BY PHOTON CORRELATION SPECTROSCOPY

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**ABSTRACT** The application of photon correlation spectroscopy for the evaluation of motility parameters of undiluted human sperm is investigated. Measurements on semen samples, selected visually as good (i.e., fraction motile spermatozoa larger than 0.6 and a positive appreciation of the motion), gave estimates of the fraction motile spermatozoa, reproducible within 10%, and of the mean velocity of the motile cells, reproducible within 5%.

## INTRODUCTION

Following the work of Nossal and Chen (1-3), light-scattering techniques have been increasingly applied to the study of the motion of motile microorganisms. The progress in this field has been reviewed by Cummins (4).

The determination of the motility of human spermatozoa has been reported by a group of French investigators (5-7). These authors used a heterodyne mixing technique combined with data analysis in the frequency domain.

Application of photon correlation techniques has been reported by Cooke et al. (8) and Hallet et al. (9). They evaluate the motility of dilute bull sperm using a homodyne technique and measurements in the time domain.

In this paper we investigate the applicability of the latter method as a clinical tool for the evaluation of motility parameters of undiluted human sperm.

## EXPERIMENTAL

The light-scattering apparatus used is essentially the Malvern 4300 Photon Correlation Spectrometer, (Malvern Instruments Ltd., Malvern, Worcs., WR 14 1AL, England) including a 96 time-delay channels correlator. The light source is a 5-mW He-Ne laser (Spectra-Physics Inc., Mountain View, Calif., model SP 120). The scattering cell is a cylindrical glass vessel with  $\approx 3$  mm i.d. and  $< 0.1$  cm<sup>3</sup> vol. Data analysis was performed on-line with a Hewlett-Packard HP9825A calculator connected to the correlator with a BCD interface (HP98033A, Hewlett-Packard Co., Palo Alto, Calif.). A block diagram of the apparatus is given in Fig. 1. Scattering angles in the range of 10-20° were used because for these angles most of the scattered intensity comes from the spermatozoa.

## THEORY

Evidence has been given that light scattered by motile spermatozoa obeys Gaussian statistics (10), so that the (normalized) intensity autocorrelation function  $g_2(\tau)$  built up with previous apparatus can be related to the normalized electric field autocorrelation function  $g_1(\tau)$  by:

$$g_2(\tau) = 1 + C |g_1(\tau)|^2, \quad (1)$$

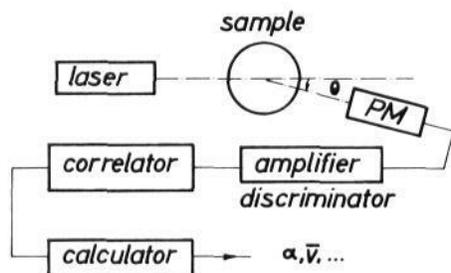


FIGURE 1 Block diagram of the experimental apparatus.

where  $\tau$  is a delay time and  $C$  is a complicated function of several factors depending on the experimental conditions (11). Our approach, however, is to consider  $C$  as an unknown parameter to be determined in the data-fitting procedure.

If we now assume that only two different kinds of scatterers, i.e., motile and nonmotile spermatozoa, contribute to the scattered intensity, we can write:

$$g_1(\tau) = \alpha g_{1,m}(\tau) + (1 - \alpha)g_{1,d}(\tau). \quad (2)$$

Here  $\alpha$  is the fraction of motile spermatozoa and  $g_{1,m}$  and  $g_{1,d}$  are the field autocorrelation functions of the motile and nonmotile cells, respectively.

For motile cells, assuming them to be point sources moving at constant velocities for times long compared to the decay times of the observed function, the simplest model (2, 3) has proven to be a good approximation for the motion of the spermatozoa (6, 9). Assuming an isotropic distribution of velocities, this model leads to (2, 3)

$$g_{1,m}(\tau) = \int_0^\infty \frac{\sin(qv\tau)}{qv\tau} P_s(v) dv, \quad (3)$$

where  $P_s(v)$  is the swimming speed distribution function and  $q$  is the modulus of scattering vector.

Dubois et al. (6) have empirically observed that  $P_s(v)$  of human spermatozoa can be described to a good approximation by

$$P_s(v) = (4v/\bar{v}^2) \exp(-2v/\bar{v}), \quad (4)$$

where  $\bar{v}$  is the mean speed of the distribution. This yields

$$g_{1,m} = \left[ 1 + \left( \frac{q\bar{v}\tau}{2} \right)^2 \right]^{-1}. \quad (5)$$

The function  $g_{1,d}(\tau)$  falls off slowly with time compared to the decay of  $g_{1,m}(\tau)$ . If one assumes that the nonmotile cells behave in a first approximation as identical Brownian scatterers,  $g_{1,d}$  can be represented by a single exponential:

$$g_{1,d}(\tau) = \exp(-\tau/\tau_d), \quad (6)$$

where  $\tau_d$  is a characteristic decay time. So finally Eq. 1 becomes

$$g_2(\tau) = 1 + C \{ \alpha [1 + (\tau/\tau_m)^2]^{-1} + (1 - \alpha) \exp(-\tau/\tau_d) \}^2 \quad (7)$$

with

$$\tau_m = 2/q\bar{v}.$$

Stock (12) proposed a similar equation, except that Brownian motion is included both for the motile and nonmotile scatterers. Under this assumption Eq. 7 takes the form

$$g_2(\tau) = 1 + C \exp(-2\tau/\tau_d) \{ \alpha [1 + (\tau/\tau_m)^2]^{-1} + (1 - \alpha) \}^2. \quad (8)$$

## DATA ANALYSIS

The set of the 96 experimental estimates of  $g_2(\tau)$  were fitted with Eqs. 7 and 8 using a nonlinear least squares procedure (13)<sup>1</sup> yielding estimates of the following parameters: (1) the fraction motile spermatozoa  $\alpha$ ; (2) the mean speed  $\bar{v}$ ; (3) the value of  $C$ ; and (4) the decay time  $\tau_d$  of the nonmotile cells. The variance-covariance matrix of these parameters was also estimated. This provides estimates of the errors on the parameters and the correlation between them. From the correlation coefficients between the different parameters it appeared that as a rule of thumb a good choice of the sample time  $T$  of the correlator is  $T = 1/10 \tau_m$  (for a 96 channel correlator). Smaller sample times lead to appreciable correlation between the fraction motile spermatozoa,  $\alpha$ , and their mean speed,  $\bar{v}$ .

Note that Eq. 1 holds in fact only in special cases, such as for Gaussian-Lorentzian light. The temporal integration in the post detection signal processing will, however, distort the functional dependence on  $\tau$  (14). This is especially so when motile scatterers are present. The effect of this distortion was evaluated by computer simulation for typical experimental conditions. From this it appeared that the distortions due to temporal integration did not affect  $\alpha$  and  $\bar{v}$  by more than 0.1%, so we considered it to be negligible.

To obtain some indications of the uniqueness of the fitting procedure, sets of data, including noise, were generated with Eq. 7 for typical values of the parameters. In all cases of practical interest the two clinically important factors,  $\alpha$  and  $\bar{v}$ , were recovered within  $\pm 5\%$  for  $\alpha$  and  $\pm 2\%$  for  $\bar{v}$ .

After having obtained a first set of fitting parameters, their values were altered at random by a variable amount of maximum  $\pm 30\%$ . Starting with the values thus obtained, the same set of fitting parameters was recovered within the calculated estimates of the errors on these parameters.

## RESULTS

A set of 20 semen samples, selected as good when observed visually by microscope (i.e.,  $\alpha > 0.6$  and a positive appreciation of the motion of the spermatozoa) was examined. When comparing the results of the data analysis with Eq. 7 and 8, it turned out that the major

<sup>1</sup>For minimizing the sum of squares, the method of M. J. D. Powell was used.

difference was that the fractions of motile spermatozoa obtained with Eq. 8 were 10–20% higher than the results obtained with Eq. 7. For the further discussion we use the results of the data analysis with Eq. 7.

The experimental data collected for scattering angles above roughly  $20^\circ$  cannot be retained for analysis with Eq. 7 or 8 since the contribution to the scattered intensity due to the seminal liquid becomes nonnegligible. It turns out that the fraction motile spermatozoa is, within the experimental reproducibility, independent of the scattering angle in the range used ( $10\text{--}20^\circ$ ). However, the values of the mean speed decrease systematically with increasing scattering angles. This may be due to multiple scattering, which may indeed occur since the concentration of spermatozoa is rather high in the investigated undiluted semen sample. Instead of measuring at one fixed value of the scattering vector  $q$ , determined by the scattering angle  $\theta$ , the observed intensity, in the case of multiple scattering, corresponds to a spread of different scattering vectors. The result is that the observed intensity autocorrelation function is less dependent on the scattering angle  $\theta$  than Eq. 7 or 8 would predict. So the analysis with these equations, in the case that multiple scattering occurs, will lead to an apparent decrease of  $\bar{v}$  with the scattering angle  $\theta$ . Another cause for the apparent decrease may be interference from distributed scattering centers (15, 16). Further studies are necessary to distinguish between these two possible explanations for the observed deviations from  $q\tau$  scaling. Nevertheless the estimates of  $\bar{v}$  obtained at one fixed angle  $\theta$  are reproducible, thus allowing one to compare samples.

Fig. 2 shows an intensity autocorrelation function measured about half an hour after ejaculation. The accumulation time was 200 s and data analysis was performed in about 5 min so that we could evaluate  $\alpha$  and  $\bar{v}$  of this sample in less than 10 min. In Fig. 3 a histogram for  $\bar{v}$  for the 20 semen samples is given. The average of the speed  $\bar{v}$ ,  $\langle \bar{v} \rangle$  is  $130 \mu\text{m}/\text{second}$  with a mean square deviation of  $52 \mu\text{m}/\text{second}$ . Jouannet et al. (7) report for a set of 91 samples

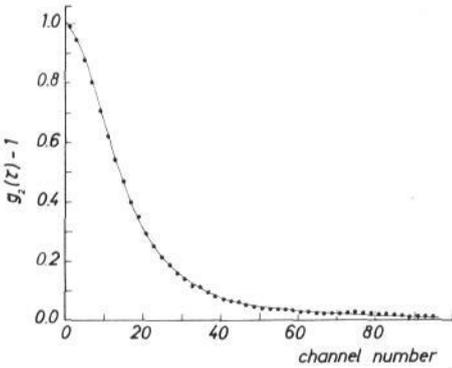


FIGURE 2

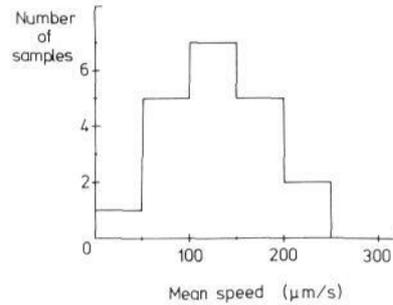


FIGURE 3

FIGURE 2 A typical intensity autocorrelation function (normalized) for human spermatozoa. Dots are experimental points (one out of two) and the full line was obtained by a least squares fit with Eq. 7. The experimental conditions were: scattering angle  $\theta$ ,  $10^\circ$ ; delay time per channel,  $200 \mu\text{s}$ ; clip level, 2; and temperature,  $33^\circ\text{C}$ . Estimated values of the motility parameters were: fraction motile,  $0.80 \pm 0.04$ ; and mean velocity,  $180 \pm 2 \text{ m}/\text{second}$ .

FIGURE 3 Distribution of the mean speed  $\bar{v}$  for 20 semen.

taken at random ( $\langle \bar{v} \rangle$  ( $= 2 \langle vc \rangle$  in their notation)  $= 96 \mu\text{m}/\text{second}$ ). This value holds, however, for a set of semen samples including several of poor motility.

Measurements on different samples of an ejaculate and repeated measurements on the same sample yielded the following: (a) the fraction motile was reproducible within 10%, and (b) the mean velocity  $\bar{v}$  was reproducible within 5%. To obtain information about the absolute accuracy of the method, a systematic comparison with the much slower and more cumbersome microcinematographic analysis will be undertaken. Nevertheless, at the present stage photon correlation spectroscopy yields relative values of motility parameters of human spermatozoa that are meaningful in that they allow one to compare samples.

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