# DETERMINATION OF ACTIVITY OF SPERM BY THE LIGHT SCATTERING METHOD

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The possibility of determining the activity of sperm by means of spectra of scattered light was studied. A homodyne laser optical mixing spectrometer was used for the measurements. Solutions of bovine sperm were used as the test object. The activity of the sperm, determined from the motility of the spermatozoa, may be determined from the half-width of the spectrum of scattered light.

KEY WORDS: activity of sperm; optical mixing spectroscopy; laser.

The activity of sperm is determined by the motility of the spermatozoa. A method of analysis of the activity of sperm under the microscope is known [2-4], which includes sampling the sperm, dilution, application of a drop of sperm on a supporting film, followed by visual analysis under the microscope using a 10-point system. The operator counts the number of motile spermatozoa in every 10 cells. This known method has certain important disadvantages. The quantity of sperm which can be tested is insufficient to allow the objective analysis of activity. To obtain reproducible results the drop of sperm must be of a strictly definite volume. The method of applying the drop of sperm itself causes death of some of the spermatozoa and this introduces additional error into the measurement. When the method is put into use, difficulties arise in the stabilization or controlled change of the conditions under which the spermatozoa are kept (temperature, pH of the medium, pressure, and so on). Assessment by a point system is subjective and requires the use of highly trained operators.

The possibility of determining the activity of sperm by the use of spectra of laser light scattered in it was studied in the investigation described below. Scattering of light in solutions of spermatozoa has been investigated previously both experimentally [6] and theoretically [7]. However the measurements in [6] were qualitative in character and the results were not compared with the usual method of measurement of sperm activity.

## EXPERIMENTAL METHOD

If living spermatozoa in physiological saline are examined under the microscope they can be seen to perform movements in a straight line at constant velocity. The time of the trajectory until a change in the direction of movement is of the order of 1 sec. This time is long compared with the characteristic time of decay of fluctuations in the concentration of spermatozoa because of ordinary diffusion; for that reason, monochromatic laser light, scattered on these moving particles, will undergo a Doppler frequency shift equal to

$$f = \frac{1}{2\pi} v_q q, \tag{1}$$

where q is the vector of scatter; $v_q$ the projection of velocity on this vector, when $q = \frac{4\pi n}{\lambda} \sin\frac{\theta}{2}$, n the refractive index of the sperm, $\gamma$ the wavelength of the laser light, and $\theta$ the angle of scatter. The solution contains dead (nonmotile) and living (motile) spermatozoa; the living spermatozoa have a certain distribution by velocity. The spectrum of light scattered in such a system lies in the region of undisplaced frequency and usually has a maximum at an undisplaced frequency. The width of the spectrum is determined by the mean velocity of movement by the spermatozoa. The more numerous the motile spermatozoa and the higher their velocity, the wider the spectrum.

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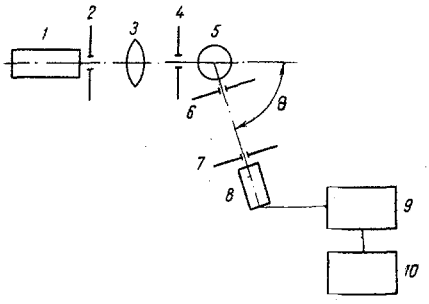


Fig. 1. Block diagram of apparatus: 1) LG-38 laser; 2) diaphragm 3 mm in diameter; 3) lens, focal length 150 mm; 4) diaphragm 2.5 mm in diameter; 5) cuvette with sperm solution; 6, 7) diaphragms 0.5 mm in diameter; 8) FÉU-79 photoelectronic multiplier; 9) S4-44 spectral analyzer; 10) N-110 automatic writer.

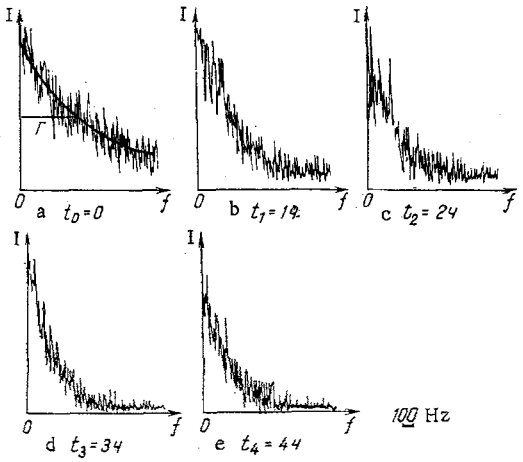


Fig. 2. Spectra of photocurrent induced by light scattered in sample of sperm, recorded at successive intervals of 1 h.

The width of the spectrum can be estimated by equation (1). When $\lambda = 6328$ Å, $h = 1.33$, $\theta = 30°$ and $v_q = 6$ mm/min, we have $f = 260$ Hz. Spectra of this width can be measured by the method of optical mixing spectroscopy [5].

The apparatus shown schematically in Fig. 1, giving measurements in the region of the spectrum between 20 and 20,000 Hz, was built for the measurements. The apparatus can be used to undertake various microbiological investigations and was described in detail previously [1].

Measurements were made on bovine sperm frozen in granules at −196°C. The granules were thawed at 40°C in a warm 2.9% solution of trisodium citrate (+ $5H_2O$). The following solution was used as diluting agent: 100 ml distilled water, 11.5 g lactose, 20 ml hens' egg yolk and 5 ml glycerol. Activity of the spermatozoa after thawing of the frozen sperm was assessed visually at 5 points. The solution of sperm was poured into a 2-ml cuvette at 39 ± 0.5°C. The emission power of the laser used was chosen so as not to cause a change in the activity of the sperm during the experiment, as confirmed by visual point assessment of the motility of the spermatozoa before and after irradiation. Spectra were obtained with an angle of scatter theta = 30°.

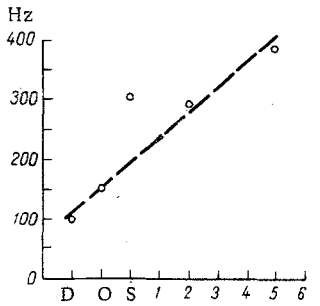


Fig. 3. Graph of correspondence between half-width of spectra and results of visual 10-point analysis of activity. Abscissa, points (1-5) of activity, D) dead, nonmotile spermatozoa, O) nonmotile spermatozoa with oscillatory movement, S) single spermatozoa with forward linear motion; ordinate, half-width H (in Hz).

## EXPERIMENTAL RESULTS

Spectra of the photocurrent induced by light scattered in the sample of sperm and recorded at successive intervals of 1 h are shown in Fig. 2. Clearly the spectrum became narrower with time. The spectra were characterized by their half-width (H), equal to the width of the noise-averaged spectrum at half the maximal intensity (see Fig. 2a). The value of the half-width at $t = 0$ was about 400 Hz. With the course of time it diminished, so that after 4 h it was about 100 Hz, thereafter remaining unchanged. Parallel observations under the microscope showed that this time interval corresponded to complete loss of motility of the spermatozoa. It follows from these results that the change in activity of the sperm during time can be determined from the spectrum of scatter.

Further experiments were carried out to study matching between the half-width of the spectra and activity estimated visually in points. The results are shown in Fig. 3, from which it is clear that higher values of the half-width correspond to higher point ratings of activity. The scatter of the points for S may have been due to the fact that the true motility of the spermatozoa in the volume of scatter was higher than in the drop under the microscope.

On the whole the results show that the activity of sperm can be determined from the half-width of the spectrum in Hz.

The method of analysis of sperm activity based on spectra of scatter possesses several advantages over the ordinary visual method:

1. More accurate information can be obtained by recording the spectra of scatter, for in the present case about $10^4$ spermatozoa were contained in the scattering volume, whereas in the ordinary method the estimation of activity is based on 10 spermatozoa.
2. Measurement of the half-width of the spectrum H facilitates the quantitative and objective analysis of sperm activity.
3. The cuvette contains 1-2 ml of diluted sperm, and the spermatozoa within the scattering volume tested are not exposed to the action of any marginal effects modifying the true activity of the sperm.
4. By means of this method sperm can be analyzed under different conditions (temperature, pressure, pH of medium, and so on) and these conditions can be stabilized with great accuracy.

The distributions of the spermatozoa by velocity can be obtained from the spectra of scatter. Exact measurements of velocity require improvement of the theoretical model and of the technique used to prepare the specimens of sperm for analysis, so that they correspond to simpler physical models. The development of this new method makes automation of the analysis of sperm activity possible. The method of determining activity of spermatozoa as described above must play a positive role in the diagnosis of infertility and improvement of the efficiency of artificial insemination.

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# MODIFIED METHOD OF DIFFERENTIAL STAINING OF SISTER CHROMATIDS


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A modified method of obtaining differential staining of sister chromatids is described. It is simple, quick, and highly reproducible, but at the same time is cheap and readily accessible, for the reagents used are widely available. When 5-bromodeoxyuridine was added to a Chinese hamster cell culture 24 h before fixation the proportion of metaphases with differential staining of chromatids was 95-98%, but if the substance was added 28 h before fixation to a culture of human lymphocytes the proportion varied between 75 and 90% depending on the individual. The mean number of sister chromatid exchanges in human lymphocytes was found to be independent of the fixation time.



KEY WORDS: differential staining; sister chromatids; chromatid exchanges.


The effect of 5-bromodeoxyuridine on differential staining of regions of the chromosomes was first demonstrated by Zakharov and Egolina [4]. Using this property of 5-bromodeoxyuridine, Latt [2] developed a method of differential staining of sister chromatids, which was improved by Perry and Wolff [3]. The method was based on the use of preliminary staining of chromosome preparations with the dye Hoechst 33258. Later, Korenberg and Freedlander [1] developed a method of differential staining of sister chromatids in which the preparations were heated in alkaline solutions. The final stage in these methods was staining by the Giemsa method in buffered solution. If the method of differential staining of sister chromatids in cytogenetics is to be widely used, it must be simple, cheap, and highly reproducible. A method satisfying these conditions and tested on cultures of human lymphocytes and Chinese hamster cells is described below.

## EXPERIMENTAL METHOD

A transplantable culture of Chinese hamster cells of clone $237_{2a}$ with 18 chromosomes in the karyotype and a short-term culture of human peripheral blood lymphocytes were used. 5-Bromodeoxyuridine in a concentration of 10 $\mu$g/ml was added 24 h before fixation to the culture of Chinese hamster cells and 28 h before fixation to the lymphocyte culture. The lymphocyte culture was fixed 72 and 96 h after addition of phytohemagglutinin. Colchicine was added to the cultures 2 h before fixation in a concentration of 0.5 $\mu$g/ml. Hypotonicity was produced when Chinese hamster cells were used by means of 1% sodium citrate (20 min) and when human lymphocytes were used, by means of a 0.55% potassium chloride solution (10 min) at 37°C. The cultures were fixed in a mixture of methanol and acetic acid (3:1). The specimens were prepared by applying the cell suspension to cold wet slides which were then dried in the air. After staining, the preparations were kept in a thermostat (37°C) for not less than 24 h.


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