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A thermal study of cellular motility by optical time-resolved correlation

F. J. Sierra-Valdez,[1] A. J. Cisneros-Mejorado,[2] D. P. Sánchez Herrera,[1] and J. C. Ruiz-Suárez[1,a)]

[1]*Cinvestav-Monterrey, Vía del Conocimiento 201, PIIT, Autopista al Aeropuerto, Km. 9.5, Apodaca NL 66600, Mexico*

[2]*Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, 44340, México.*

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The study of motor properties of cells under appropriate physical-chemical conditions is a significant problem nowadays. The standard techniques presently used do not allow to evaluate neither large samples nor to control their thermodynamic conditions. In this work, we report a cell motility sensor based on an optical technique with a time-resolved correlation, adapted in a system able to study several samples simultaneously. Image correlation analysis is used to follow their temporal behavior. A wide variety of motile cells, such as archaea, bacteria, spermatozoa, and even contractile cells, can be studied using this technique. Here, we tested our technique with the study of sperm motility. In particular, both the sperm motility and its prevalence are studied under a temperature range from 0 to 37 °C. We found that incubation at 10 °C presents the lengthiest prevalence in motility and observed, for the first time, an interesting thermal reversibility behavior. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.3700248]

I. INTRODUCTION

There is in the scientific literature a good deal of methods to study the motility of spermatozoa in a semen sample.[1–16] Such methods have become increasingly sophisticated with improvements in image analysis and computing. Indeed, from simple techniques such as "Passage Counting,"[1] which involves microscopic observation of the number of spermatozoa crossing a defined line or area on the field of view in a given period, to more elaborated methods such as photon correlation spectroscopy,[15,16] where swimming speed distribution is reconstructed from the correlation data by Stocks method of splines, ingenious techniques have been proposed to evaluate the spermatozoa kinematics.

Despite the great amount of available methods, here we revisit the field aiming to develop a simple technique to study samples of sufficiently large populations while controlling their thermodynamic conditions. Our cell motility sensor is based on a time-resolved correlation,[17] and the cell motility parameter (CMP) is indirectly estimated from a temporal autocorrelation analysis of images. Our technique allows us to study several samples simultaneously, even in contact with different drugs or doses of the same type. In this work, we focused on a thermal study of the sperm motility from mice and their prevalence through a wide temperature range from 0 to 37 °C. We found that the lengthiest prevalence in motility is approximately at 10 °C. Furthermore, we discovered that cells that reduced their motility after being at 37 °C for 1.5 h are recovered by lowering the temperature to 10 °C.

II. EXPERIMENTAL SETUP

Our experimental setup is shown in Fig. 1. A beam laser (Helium-Neon Gas, 633 nm, 1 mW) is projected over a mirror that reflects it onto the bottom of an aluminum chamber where a sample holder is deposited. The sample holder is a stainless steel cylinder with six concentric holes and two transparent acrylic covers made to encapsulate the liquid samples. The laser beam illuminates the six holes and light passes through them. The resulting scattering pattern, produced by each sample, is recorded by a digital camera (PixeLINK, 1024 × 768 pixels). Each hole has a maximum capacity of 300 μl. We used an optical table to avoid vibration noise. The temperature of the samples is controlled by a water re-circulating system (Temperature controller, PolyScience) connected to the aluminum chamber.

III. MATERIALS

The sperms were recovered from male mice CD1 aged 3–5 months. The epididymes were dissected and rinsed in 2 ml of a Whitten-Hepes (WH) medium which comprises (in mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 HEPES, 10 glucose, and 1 sodium pyruvate at pH 7.3 (NaOH). The WH medium keeps the physiological conditions for sperm incubation.[18] Several incisions were made in the epididymis in the solution and a swim up method was used to separate sperms with greater than 90% of motility.[19] Finally, the upper part of the solution which contains the more motile sperms is isolated in another container in order to avoid contamination of epididymal tissues, thus, getting a final sperm suspension.

IV. EXPERIMENTAL PROCEDURE

The final sperm suspension was kept at 15 °C during 1–2 h previous to the measurements. Thereafter, 200 ml of this suspension is deposited in each hole of the sample holder. Further, to avoid bacterial growth, penicillin and streptomycin are used as antibiotics, in a concentration of 1% for each

a)Electronic mail: jcrs.mty@gmail.com.

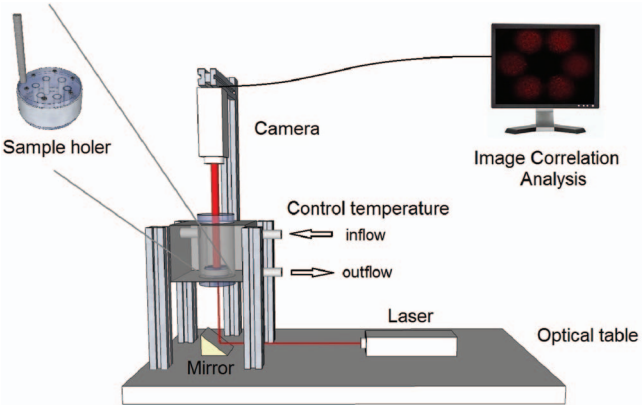

FIG. 1. Experimental setup. A beam of a He-Ne laser is optically driven to a chamber that contains a sample holder. This sample holder has six small holes that can be filled with the sample. Light passes through each one of these receptacles. The scattering pattern is recorded by a digital camera for image correlation analysis. The sample temperature is controlled.

sample. A careful study was performed to ensure that antibiotics do not affect sperm motility at this concentration. Finally, once the sample holder is deposited into the chamber, we wait around 15 min to achieve thermal equilibrium. As previously mentioned, we analyzed the motility of the sperm samples at different temperatures: 0, 10, 20, 25, 30, and 37 °C. Therefore, the procedure above described is implemented for each temperature. It is important to note that the experiments at each one of these temperatures were carried out using three different mice, in order to perform a statistical analysis. The error bars are obtained simply by the arithmetical average of the measurements.

V. IMAGE PROCESSING

The images were taken by a lapse time of 3 min each hour during 6 h (see Fig. 2). These temporal parameters are adequate to explore both the instantaneous sperm motility as well as their prevalence. The CMP is estimated using a temporal autocorrelation of the images. We implemented a semi-automated program to process the acquired images as follows: At each measurement, a section of the first frame, taken at t_0, was compared with similar sections of the successive frames taken at t_n, as shown in Fig. 3. The frame sections are squares of 100 × 100 pixels (corresponding to 1 mm^2), producing an intensity matrix I(X,Y,t) in gray scale (see Fig. 3(b). It is important to note that these frame sections are the largest ones that can be circumscribed inside the speckle's circle. Larger frames may have regions with no speckles affecting the correlation analysis. The temporal autocorrelation model used in this work is given by the following expression:

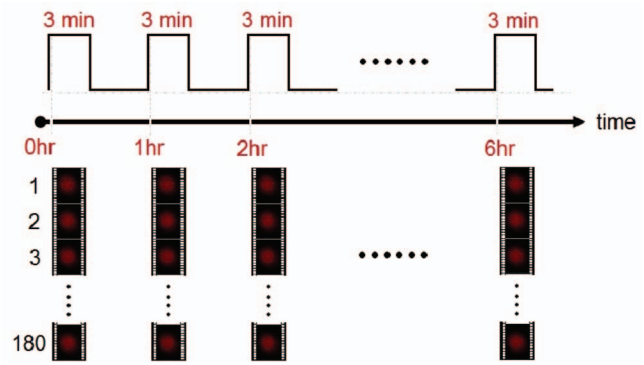

FIG. 2. Schematic drawing to show the procedure of obtaining the frames. Note that all measurements were taken in a constant rate of one frame per second.

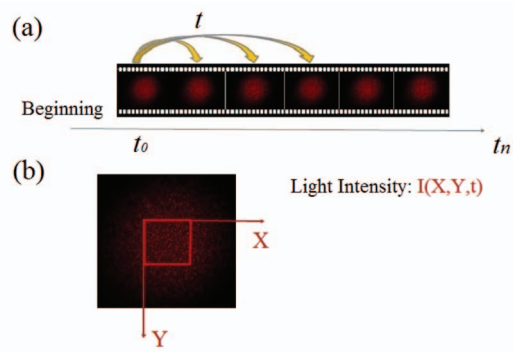

FIG. 3. A schematic example of the image processing. (a) The first frame at time t_0 is compared with each consecutive frame taken at time t_i (i goes from 1 to 180 s). (b) A square of 100 × 100 pixels is taken from the center of each frame as an intensity matrix to be processed by Eq. (1).

$$c_I(t_0, t) = \frac{1}{\beta}\left[\frac{\langle I(t_0)I(t_0+t)\rangle_{X,Y}}{\langle I(t_0)\rangle_{X,Y}\,\langle I(t_0+t)\rangle_{X,Y}} - 1\right], \tag{1}$$

where the average intensity at time t_0, $I(t_0)$, stands for the sum of all the elements of the matrix I(X,Y,t$_0$) averaged over N pixels. β is a normalizing factor to restrict the function exactly between zero and one. This factor is needed because, in practice, the images recorded by the CCD are affected by a pixel and time-dependent dark (or electronic) noise and, possibly, by the non-uniform illumination of the detector.

Note that in Fig. 3 we show only a simple speckle pattern to describe the procedure, but in this work we have implemented six speckle patterns as we said in the experimental description.

Due to the fact that the autocorrelation of the images shows an exponential decay, we used a stretched exponential to get the motility information:

$$c_I(t_0, t) = \exp(-t/\tau)^{\alpha}, \tag{2}$$

where α takes values between 0.05 to 0.3 and τ is taken as the CMP. In fact, since c_I is calculated every hour, τ gives the temporal behavior of cell motility.

Figure 4 shows a characterization of the maximum and minimum motility boundaries for a given sperm sample, where CMP is in the order of 10^4 and 10^{-13}, respectively. The maximum motility stands for the average sperm motility measured about 1.5 h after extraction and the minimum motility for the total lack of sperm motion achieved from using sodium azide.

Once the CMP is obtained to show the temporal behavior of the samples, in some cases we implemented the use of the

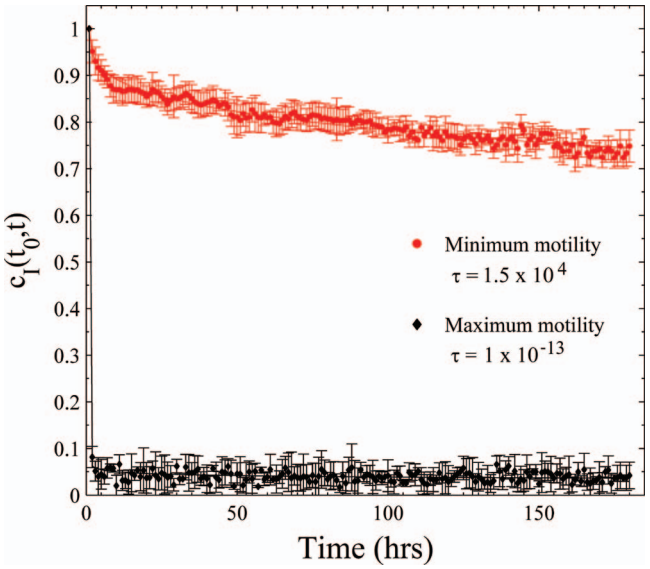

FIG. 4. Autocorrelation curves showing motility boundaries. A stretched exponential is fitted to obtain the CMP by Eq. (2).

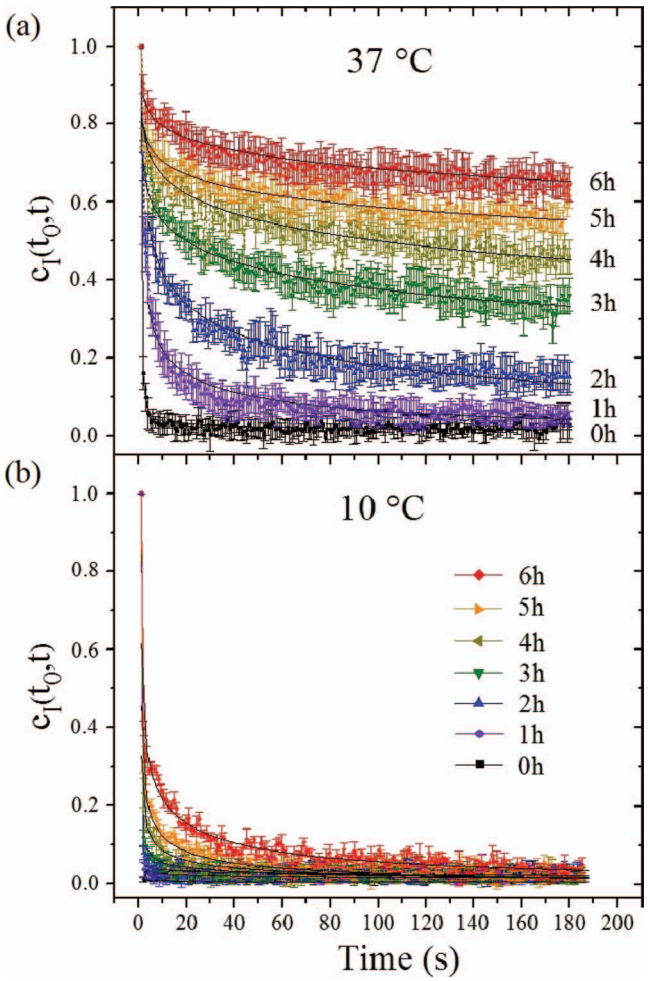

FIG. 5. Temporal development of the autocorrelation showed by sperm sampled during 6 h. Comparison between (a) 37 °C and (b) 10 °C.

inverse of the area under the curve (1/AUC), which gives us sharper information about sperm motility.

VI. RESULTS AND DISCUSSION

In Fig. 5, we show a comparison of the temporal autocorrelation between 37 and 10 °C. At the beginning of the experiment ($t = 0$), the lowest autocorrelation curve stands for the maximum motility. Thus, the increase in the autocorrelation function describes the loss of sperm motility. It is noted in Fig. 5(b) that 10 °C shows a longer prevalence in sperm motility.

In Fig. 6, we depict the temporal behavior of cell motility for various temperatures. It is interesting to note that the faster the curve grows, the more is the motility reduction. From this, it is observed that 37 °C is the temperature that causes a faster mortality of the sample. At higher temperatures the sperm mortality is even worst.Due to the fact that lower temperatures might freeze the sample solution, 0 °C was used as an endpoint temperature. From Fig. 6(b), the inverse of the area under the curve shows that the temperature presenting the lengthiest prevalence in motility was 10 °C.

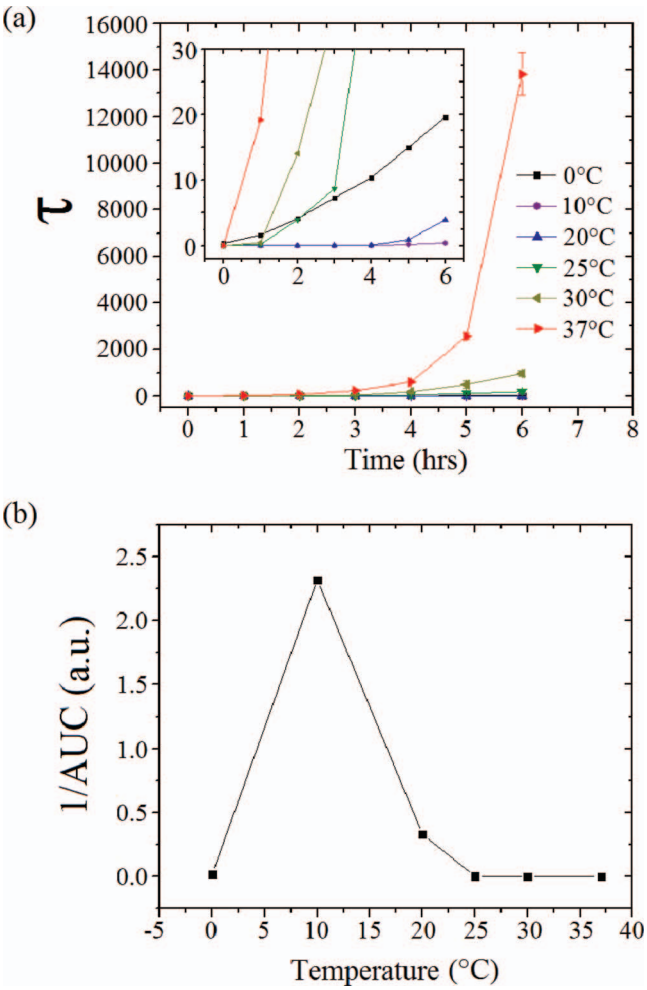

FIG. 6. (a) Temporal development of the CMP showed for different temperatures. The inset shows a zoom in order to appreciate the lower temperatures. (b) Prevalence of sperm motility depending on temperature, represented by the inverse of the area under the curve of the temporal development of CMP.

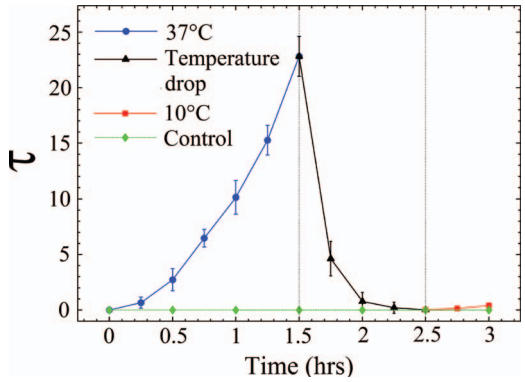

FIG. 7. A thermal reversibility behavior in sperm motility is observed when performing a temperature drop from 37 °C to 10 °C. The achieved motility is compared with the thermal behavior of the control sample at 10 °C obtained from previous analysis.

One of the main advantages of our technique is that we are able to study very large cell populations *in situ*. For instance, we can modify the temperature to see changes in motility. In doing so, we were able to find an unexpected reversible thermal behavior not observed before (see Fig. 7). As previously mentioned, 37 °C is the temperature where the sperm suffers the fastest decay in motility, but when the temperature is lowered to 10 °C, the motility of the sample is recovered. This certainly means that 10 °C, under WH conditions, gives to sperm the ideal thermodynamic conditions for the lengthiest prevalence of their motility, and further, that it is a reversible process.

The advantages that the present technique offers are the following: (1) the simultaneous analysis of several samples can be used to perform a statistical analysis, a reduction in the number of experiments, and the ability to perform a local comparison when the chemical conditions between each of the samples are different. (2) The control of the thermodynamic conditions in an isolated system allows us to ensure a constant thermodynamic state of the sample. Finally, (3) the use of an optical technique to obtain indirectly cell motility offers us the opportunity to study relatively large samples. The main advantage of an isolated system is that it offers the opportunity to measure cell motility without altering such system. Our technique is not able to obtain direct parameters about cell motility, but it is capable of giving information about phenomenological aspects under conditions that cannot be reproduced by common methods. It is important to consider that our technique can be complemented with the conventional methods to study some other direct parameters about cell motility with greater accuracy.

VII. CONCLUSIONS

Many other available techniques that usually obtain common motility parameters (average speed, viscosity, amount of motile sperms, and an assortment of options under microscope) often sacrifice the opportunity to discover a variety of thermal behaviors that cells cannot show easily. Based on a time-resolved autocorrelation method, we present here an optical technique for the indirect study of cell motility. Its main advantage is the possibility of controlling the thermodynamic conditions in an isolated system evaluating several samples simultaneously.

We tested our technique with mouse sperm and found that, at the WH medium the ideal temperature in which sperm acquires the longest survival time is at 10 °C. Finally, we have discovered an unseen thermal reversibility behavior in sperm motility that hints towards a strong dependence in the thermodynamic state of the cell.

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