AUTOREGRESSIVE ANALYSIS OF THE ERYTHROCYTE FLICKERING

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Abstract. We developed a non-invasive method to analyze the membrane fluctuation in order to characterize the flickering phenomena from a quantitative point of view. To analyze the red blood cell (RBCs) membrane undulation, we considered the variation of the diameter series of the cell. This RBC parameter was obtained both for cells suspended in plasma and buffered serum, pH = 7.4. Frequency spectra of the erythrocyte membrane flickering were fitted with a first order of autoregressive model (AR1). We found that most RBC diameter series are fitted in a good manner using the AR1 model. The interaction factor for the cell diameters series was considerably higher for the erythrocytes suspended in plasma than for those suspended in buffered serum.

Key words: erythrocyte flickering, autoregressive model, interaction factor.

INTRODUCTION

The flickering process consists in spontaneous cell membrane fluctuation performed at low frequency (0.3–30 Hz) and reflects the membrane bending deformability. These membrane fluctuations were reported for the first time for red blood cells (erythrocytes) in 1975 by Brochard and Lennon [1]. It was also observed in different nucleated cells including monocytes, lymphocytes, 3T6 fibroblasts, cardiomyocytes [4] and murine lymphoma B and T cell lines [6]. The mechanism is not completely understood. It was shown to depend on Mg ATP-ase [5] which is involved in the phosphorylation of cytoskeleton proteins. This process is followed by the thermal energy delivery that governs the membrane undulation. Later, Tuvia and coworkers [9] have shown that the flickering process is driven by metabolic energy, in addition to thermal energy. The metabolic driving force was shown to be associated with actin ATP-ase [10].

The first quantitative measurements of the flickering phenomena were performed by Fricke *et al.* [3] using phase-contrast and reflection-interference microscopy. Single cell membrane undulation measurements became possible after

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the development of point dark field microscopy [8]. This method consists on the temporal light scattering measurements of a small area along the edge of the cell fixed on a glass substrate.

We propose a new method that involves the investigation of the cells in free-floating state. All previous studies have been performed on fixed cells. On the other hand, the anchoring of the cells on the glass support may alter the frequency and amplitude of undulations. Further in our investigation sequential images of the erythrocyte have been recorded. Instead of using the cell thickness as a parameter in the dark point field microscopy, we used the diameter of the cell to characterize the membrane fluctuation. This parameter can be obtained by processing the cell images.

The previous studies presupposed that the flickering phenomenon is fractal that is along-range memory process. However, in this work we interpret the flickering data as a short-range memory process which can be modeled by an autoregressive process.

THE AUTOREGRESSIVE PROCESS

The first order autoregressive processes, AR1, have a structure similar to random walks:

$$X(t) = \varphi X(t-1) + \varepsilon(t) \tag{1}$$

where $\varepsilon(t)$ is assumed to be independent identically-distributed random variables sampled from a normal distribution with zero mean [12]. The coefficient φ represents the strength of the interaction between successive terms of the series and t represents the time. If AR1 model is applied to a spatial process, the φ term can be understood as the interaction factor between a term and the previous one.

In the special case when φ equals one, AR1 reduces to a random walk. If φ is greater than one, the process is also dynamic. For a value of φ positive and less than one, there is a positive dependence on the past but the process is stationary (stochastic). If φ equals zero, we have a process of order zero (white noise) [2].

A stationary, or stochastic process, $X(t)$, has characteristics that are unchanged in the course of time t, so it is invariant relative to translations in time:

$$t \to t + \alpha, \qquad X(t) \to X(t + \alpha) \tag{2}$$

for any fixed value of the variable α.

MATERIALS AND METHODS

The fluctuations of the erythrocyte membrane were analyzed on diluted cell suspension, obtained from human blood collected on anticoagulant (sodium citrate

3.8%). The blood was centrifuged 10 minutes at 3000 rot/min. The plasma was isolated and ultra-centrifuged 5 minutes at 7000 rot/min in order to remove the remaining blood platelets. For the experiment in which we have used integral blood, the erythrocytes were re-suspended in plasma. For the experiment where phosphate buffered serum (PBS pH 7.4) was used, the RBCs were washed three times with PBS and re-suspended in PBS. To avoid the erythrocytes aggregation and to analyze single cells we used cells suspensions at high dilution.

Experiments were performed on 20 μl sample volumes, using Rosenthal counting cell with 0.2 mm optical depth. The images set (500 images) were captured sequentially at 0.2 sec interval using a CCD camera (PixelLINK A-741. CMOS sensor) mounted on an optical microscope equipped with a 40× objective.

RESULTS AND DISCUSSIONS

The cell images were processed using ImageJ [7] software in order to obtain the diameters of red blood cells. This software reduced the RBC shape to a simple form (Fig. 1). ImageJ extracted the diameter values, returning a numerical data series.

(a) (b) (c)

Fig. 1. The red cell image (a) threshold (b) and the simple form of the cell (c).

A free-floating cell may also move out of the focal plane of the objective, thereby affecting the apparent diameter. All these movements overlap on membrane flickering and the time series may contain non-stationary components (trends) that must be removed. The cell flickering occurs at low frequency (0.3–30 Hz), while up and down or out-of-plane movements have a higher frequency. To extract the trend, we approximated it with a 10^{th} order polynomial. The time series obtained after the trend is removed represents the flickering fluctuations.

The trend removal and the modeling with AR1 model were done using software of our own, edited in Matlab 7.0 [11].

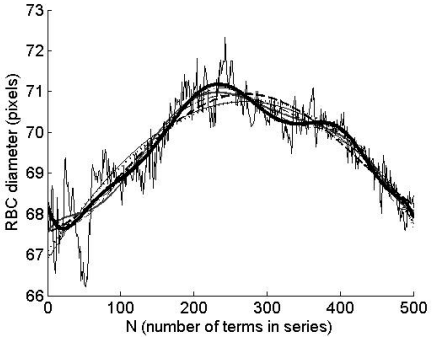

Fig. 2. The time series of diameters of RCBs (thin line). The trend arising from movements of the floating cells was approximated by 1st to 10th order polynomial (thick line).

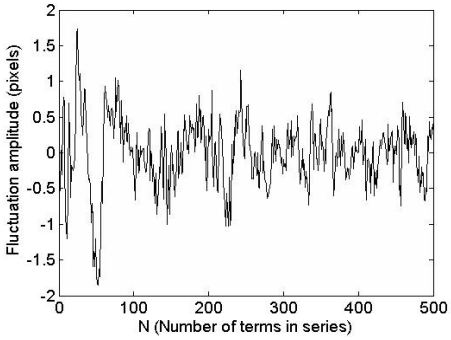

Fig. 3. The time series of RCB diameter fluctuations obtained from the data shown in Fig 2 by removing the trend (10th order polynomial).

To model the diameters series with the autoregressive model, we had to average the series. The interaction factor takes different values for different number of averaged terms. Between the 18th and 28th number of averaged terms appears a plateau. We preferred the 21th values for averaging all the series used in this experiment. Also the trend is approximated with one to ten order polynomials. In order to standardize the method, we considered the 10th order polynomial. Figure 4 represents the calculated interaction factor φ versus the number of averaged terms for a RBC diameter series in which the trend that was approximated with different order polynomial is removed.

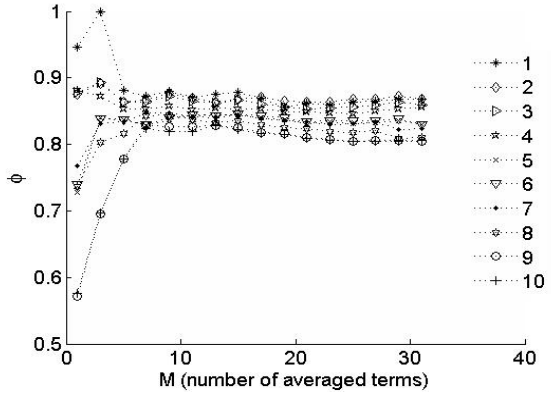

Fig. 4. Values of the interaction factor, φ, for a RBC diameter series, plotted versus the number of averaged terms. Distinct markers represent the order of polynomial that approximates the trend.

After the number of averaged terms and the polynomial order were assigned, we modeled the diameter of the red cell flickering series with AR1 (Figs. 5, 6).

Some series deviate from this model, but they can be described by higher order AR models. We can see that the AR1 approximation is quite good.

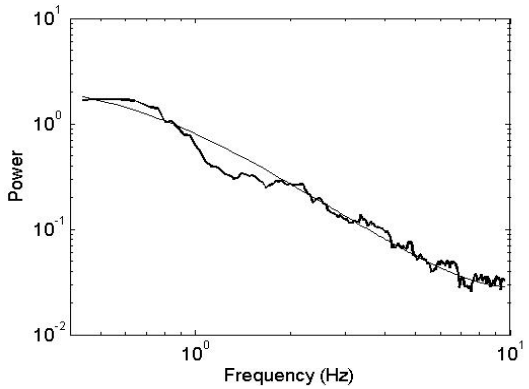

Fig. 5. AR1 modeling of the flickering phenomenon for the erythrocyte suspended in the plasma. M is the number of averaged terms.

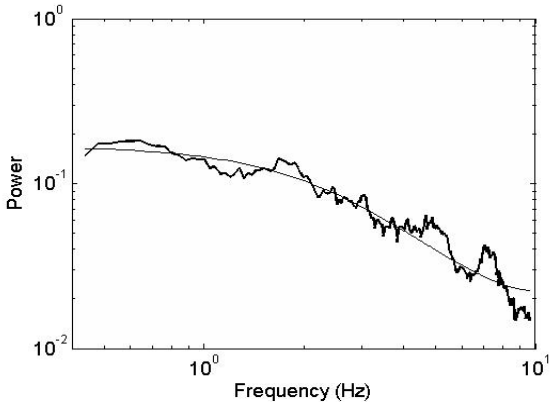

Fig. 6. AR1 modeling of the flickering phenomenon for the erythrocyte suspended in phosphate buffered serum. M is the number of averaged terms.

We found that the interaction factor φ depends strongly on the medium in which the red cell is suspended. For cells suspended in the plasma, $\varphi = 0.84 \pm 0.02$, considerably higher than for cells suspended in the buffered serum $\varphi = 0.59 \pm 0.02$.

CONCLUSIONS

We have done a comparative analysis of red blood cell flickering phenomena in plasma and buffered serum suspension. A significantly higher value of the interaction factor indicates a better correlation between the flickering processes of

red blood cells suspended in their own plasma than for those suspended in buffered serum.

The AR1 model approximates quite well both flickering kind of series, allowing us to extract the interaction factor. We proved that a first order autoregressive model offers a reasonable approximation of the flickering phenomena.

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