Short range correlation of the erythrocyte membrane fluctuations

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Abstract. The erythrocyte membrane fluctuations analysis was performed for two suspension media, plasma and phosphate buffered saline (PBS) respectively. The investigation methods consist of detrended fluctuation analysis (DFA) and spectral analysis applied on data series formed by successive values of cellular area which were obtained by managing the sequential images set for each cell. We have shown that the suspension media influences significantly the membrane fluctuation characteristics. Detrended fluctuation analysis revealed two short range correlations both for cells suspended in their natural medium and artificial medium. Moreover, we found out the strength on interaction between terms in series by using spectral analysis and autoregressive modeling. The correlation between parameters obtained by the above mentioned methods was evidenced by theoretical models and certified by our experiments.

1. Introduction
The human red blood cells (RBC) are characterized by vibratory motions, referred to as flickering [1]. The first quantitative studies [2] revealed that flickering under normal conditions does not display any significant isolated frequency, but rather is made of a mixture of a large number of low frequencies varying from about 40 Hz to zero. The interest for this phenomenon appeared to be constant and significant [3]. The character of the cell fluctuation was basically done by spectral and fluctuation analysis respectively of the time series describing the fluctuation of various geometrical parameters of the cells.

The aim of this paper was to study the influence of the liquid (i.e. plasma or PBS) on RBCs membrane fluctuations. The reason is that the natural environment of the erythrocytes is the plasma which has a complex composition whereas all the in vitro experiments are performed in an artificial environment. This usually consists of isotonic buffered saline. Indeed we found significant differences in the flickering of the two different environments. Laboratory experiments confirmed that the mathematical model is suitable for describing the membrane flickering phenomenon for both suspension media.

2. Experimental
The fluctuations of the erythrocyte membrane were analyzed on diluted cell suspension, obtained from human blood. RBCs were prepared as described in [4]. Investigations were performed for a set of 110 cells. In our experiments we prepared 20 µl sample volumes, using Rosenthal counting cell with 0.2 mm optical depth. We recorded 1000 sequential images for each one RBCs. The images set were
captured at 0.2 s interval using a CCD camera (Optikam PRO3) mounted on an inversed optical microscope (OPTIKA XDS-2). The cell images were processed using Image J [5] software in order to obtain the area of red blood cells. This software reduces the red cell shape to a simple form and extracts the area values, returning a numerical data series.

3. Detrended fluctuation analysis
The method of detrended fluctuation analysis has proven useful in revealing the extent of long-range correlations in time series [6-8].

In our case the log-log plot is not linear and, instead, the relationship between $\log F(n)$ and $\log(n)$ appears to have two distinct linear segments, the slopes of both lines are calculated separately and termed $\alpha_1$ and $\alpha_2$, respectively. The value of $\alpha_1$ proved to be superior to $\alpha_2$ in terms of prognostic ability.

For erythrocytes suspended in plasma we have recorded closer values for $\alpha_1$ an $\alpha_2$. Performing Two Sample Independent t-Test we have obtained the $P$ value being 0.439. We could suppose that the phenomenon is governed by a 1/f power law while the median correlation exponent value is 1.02 ± 0.01.

![Figure 1. DFA slope for cellular area fluctuations when suspended in plasma (black) and PBS (red). Two slopes can be observed in PBS case (short range correlation) while plasma case could be approximate with 1/f slope.](image1)

![Figure 2. Gaussian fit for the first correlation segment $\alpha_{1}\text{plasma}$ (black) and $\alpha_{1}\text{PBS}$ (red) distributions.](image2)

We have considered $\alpha_1$ parameter (correlation between closer terms) both for erythrocytes suspended in plasma and PBS (figure 2) to evidence the different effect of the suspension media on the fluctuation characteristics. The $P$ value was 0.00896 ($\alpha_{1}\text{plasma} = 1.00 \pm 0.00$, $\alpha_{1}\text{PBS} = 0.96 \pm 0.01$).

4. Autoregressive model (AR1)
AR1 model may apply to temporal or spatial processes and the significance of interaction factor $\phi$ can be better understood when applied to such particular processes. For example in the case of a temporal process the parameter $\phi$ can be understood in terms of a relaxation time $\tau = -1/ \log (\phi)$ (for details see [9]).

Parameter $\phi$ can alternatively be regarded as the strength of interaction among the terms. Obviously the more distant the terms of the series, the lower is the correlation. Regardless of a temporal or spatial process, the parameter $\phi$ can be understood as the scale of short range memory of the system.

In order to perform the spectral analysis, we have detrended the series by substrating a 10th order polynomial which approximate the local trend. Moreover, the spectrum was averaged at 21 terms to
indentify the spectrum appearance [10]. An immediate consequence of the averaging procedure is that deviation from a power-law description of the spectra may be better disclosed. Sometimes, such a deviation can be obvious even if the spectrum is not averaged.

The differences for spectrum appearance are evidenced in figure 3, where shown the averaged spectrum for two RBCs, suspended in plasma and PBS. It could be observed that none of those spectra can be fitted by a straight line. They are fitted better with a first order autoregressive model. The Gaussians for interaction factors computed with AR1 (figure 4) confirms results obtained with DFA, while medians are $\varphi_{\text{plasma}} = 0.78 \pm 0.00$ and $\varphi_{\text{PBS}} = 0.73 \pm 0.01$. P value is 0.056.

![Figure 3](image1.png)

**Figure 3.** Averaged spectrum for RBC’s area when suspended in plasma (black) and PBS (red). None of them can be fitted linear.

![Figure 4](image2.png)

**Figure 4.** Gaussian fit for the interaction factor $\varphi_{\text{plasma}}$ (black) and $\varphi_{\text{PBS}}$ (red).

5. Theoretical model
We computed DFA on series governed by autoregressive law generated by using a software developed by us in MATLAB [11], with $\varphi \in [0.1, 0.9]$ [12]. Two slopes for the DFA plot were observed. We represented $\alpha_1$ function of $\varphi$ (figure 5) and found out an exponential dependency.

Experimental data are in good agreement with the theoretical model. The black line (figure 5) is for median value (Gaussian distribution) for $\alpha_{1\text{plasma}}$ and $\varphi_{\text{plasma}}$ and the red line for $\alpha_{1\text{PBS}}$ and $\varphi_{\text{PBS}}$. We can consider that the law which governs the fluctuation phenomena in plasma case only seeming 1/f. When phenomenon is rigorously verified, it appears to be short range correlation, and moreover, being fitted proper by a first order autoregressive model.
6. Conclusions
The suspension media influences the characteristics of the membrane fluctuations. Both DFA and spectral analysis evidenced the stress hereupon the cell is submitted when is removed by its natural suspension medium (plasma) and resuspended into an artificial medium (PBS).

The mathematical methods used in our study to investigate the RBCs membrane fluctuations are complementary and returns information about how strong is the correlation among terms in series and about the mathematical law that governs such fluctuations. It has been shown that those phenomena present short range correlations, even if the RBCs are suspended in plasma or PBS. The differences between suspension media are revealed both by DFA and spectral analyses. Moreover, the DFA parameters are strongly correlated with AR1 parameters, the theoretical model confirming the experimental results.

References
[1] Blowers R, Clarkson E M and Maizels M 1951 J. Physiol. 113 228-39
[2] Brochard F and Lennon J F 1975 J. Phys. 36 1035-47
[3] Costa M, Ghiran I, Peng C K, Nicholson-Weller A and Goldberger A L 2008 Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys. 78 020901
[4] Buimaga-Iarinca L 2008 Rom. J. Biophys. 18 67-72
[5] Rasband W S 1997-2006, ImageJ, U S National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij
[6] Peng C K, Buldyrev S V, Havlin S, Simons M, Stanley H E and Goldberger A L 1994 Phys. Rev. E 49 1685-9.
[7] Hu K, Ivanov P Ch, Chen Z, Carpena P and Stanley H E 2001 Phys. Rev. E 64 011114-1
[8] Chen Z, Ivanov P C, Hu K and Stanley H E 2002 Phys. Rev. E 65 041107-1
[9] Vamos C, Soltuz S M and Craciun M 2007 Rev. d’Analyse Numerique et de Theorie de l’Approximation 36 201-16
[10] Mandelbrot B B 1998 Multifractals and 1/f noise (New York: Springer)
[11] MATLAB R2008a © 1994-2009 The MathWorks, Inc.
[12] Morariu V V, Buimaga-Iarinca L, Vamos C and Soltuz S M 2007 Fluctuations and Noise Lett. 7 249-55.