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Using Monte-Carlo based randomisation for stabilisation of data fitting in bioimpendance spectroscopy: Proof-of-concept

N.A. Boitsova [1] ✉, A.A. Abelit [1], N.A. Verlov [2], D.D. Stupin [1]

[1] Alferov University, St. Petersburg, Russia;

[2] Petersburg Nuclear Physics Institute named by B.P. Konstantinov of NRC "Kurchatov Institute", St. Petersburg, Russia

✉ natab2002@yandex.ru

Abstract. Nowadays, a lot of bioimpedance devices are proposed and signal processing for them is still under intensive development. In this study, we improved the complex non-linear least squares (CNLS) protocol for non-stationary bioimpedance data analysis. To be specific, we tested different Monte-Carlo applications for choosing starting point for CNLS approximation – an essential step for every non-linear problem. As a result, we have proved that symbiosis approach with usage of the CNLS-solution of the previous spectra as starting point and global Monte-Carlo searching of the starting point is the promising powerful combination for such a task.

Keywords: bioimpedance, CNLS, Monte-Carlo

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Материалы конференции
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Использование рандомизации Монте-Карло для стабилизации обработки данных в биоимпедансной спектроскопии: пилотное исследование

Н.А. Бойцова [1] ✉, А.А. Абелит [1], Н.А. Верлов [2], Д.Д. Ступин [1]

[1] Академический университет им. Ж.И. Алфёрова РАН, Санкт-Петербург, Россия;

[2] Петербургский институт ядерной физики им. Б.П. Константинова НИЦ «Курчатовский институт», Санкт-Петербург, Россия

✉ natab2002@yandex.ru

Аннотация. Сегодня активно создаются все новые биоимпедансные устройства и совершенствуются методы получаемых с их помощью данных. В этом исследовании мы улучшили комплексный нелинейный метод наименьших квадратов (CNLS) для анализа нестационарных биоимпедансных спектров. В частности, мы протестировали различные приложения метода Монте-Карло для выбора начальной точки CNLS-аппроксимации – важного шага для решения любой нелинейной задачи. В результате мы показали, что симбиоз использования результатов CNLS-обработки предыдущих спектров в качестве

начальной точки и применения глобального поиска начальной точки методом Монте-Карло является перспективной комбинацией для таких аппроксимаций.

Ключевые слова: биоимпеданс, CNLS, Монте-Карло

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Introduction

Today, bioelectronics is one of the most quickly expanding science fields [1, 2]. A lot of interest from researchers aimed at developing multipurpose bioelectronic devices for the need of healthcare and practical biology, for example, bioimpedance cytosensors. Although hardware for such apparatus is well-developed, signal processing still is in need of improvement [1]. In particular, almost all commercially available bioimpedance sensors use the Cell Index measure for the estimating cells state [3]. This approach is intuitive and easy in programming implementation, yet it cannot be used for reliable studying of the various biophysical phenomena due to the indirect relationship between such effects and Cell Index. An alternative approach for impedance analysis is to match impedance spectra with some theoretical models like equivalent schemes by using complex non-linear least squares technique (CNLS) [4]. Contrary to Cell Index, this approach allows us to separate different biophysical effects, although it requires an addition of mathematical processing. Namely, like most of the non-linear problems CNLS solution is obtained in an iterative manner, so choosing the starting point for the first iteration is also relevant for it. For non-stationary impedance spectra data, which is commonly used in cell research, this issue has one obvious solution: using the result of the previous spectra approximation as starting point for the new one. At the same time, this strategy has one significant drawback: if approximation for one spectrum fails, then risk of incorrect CNLS-processing of the next spectra will be higher [5].

In this paper, we aimed to overcome this issue by using Monte-Carlo methods (MC), which helps an iterative algorithm with jumping out from the failed approximation point. We have proposed two strategies for it, namely, local Monte-Carlo, which is based on the strategy of choosing the starting point randomly nearly the previous CNLS-approximation result, and global Monte-Carlo, which chooses the starting point randomly in the CNLS parameters' reasonable space. As a result, we discover that in the experiments with living cells the global Monte-Carlo approach provides the more stable inline CNLS-approximation.

Materials and Methods

In order to obtain bioimpendance data, we have used a multielectrode array 60StimMEA200/30 (MultiChannels Systems, Germany), which contained living cells. To perform FFT-based impedance measurement a setup was used, described in Ref. [1], Fig. 6. The spectra were collected in the frequency range from 20 Hz to 40 kHz with 2-Hz step.

As to validate our MC approach, we used HeLa cells, obtained from Institute of cytology of RAS. The cells were grown in multielectrode array in DMEM medium (Biolot, Russia) at 37 °C and 5% CO_2. Before bioimpedance measurements, the cells medium in multielectrode array was replaced by phosphate buffered saline (PBS, Biolot, Russia). The rhodamine 6G based home-made dye ABDS (cells membrane staining, green pseudo-color) was used to visualise cells. Microscopic photographs were made by Leica 4000 DM B fluorescence microscope (Leica, Germany). As biological stimulus during experiment we have used trypsin-Versene solution (1:4, Biolot, Russia), which decrease cell/electrode impedance due to cells de-adhesion process.

The impedance data was analyzed in the MatLab package by using NELM software (available by request). This algorithm is based on CNLS method of impedance approximation, namely

$$\sum_n |Y_m(\omega_n, \vec{p}) - Y(\omega_n)|^2 = \min, \quad (1)$$

where Y_m is model admittance, Y is experimental admittance, ω is angular frequency, and $\vec{p}$ is the vector of the scheme tuning parameters (resistance, capacitance, *etc.*). In the current study, we have used the series RL-CPE circuit as a model admittance Y_m. Consequently, the parameters vector $\vec{p} = [R, L, W, \alpha]$, where R is resistance, L is inductance (Op-Amp artifact [2]), W and α are parameters of the CPE element, which admittance is $W(i\omega)^{\alpha}$. In order to achieve a minimum of (1), we used simplex Nelder-Mead method [6].

To stabilize CNLS-approximation, in this paper two types of Monte-Carlo randomization were used: local, that generate starting point near the previous approximation of spectra $\vec{p}_{last}$, and global, which generate starting point near "universal" origin point $\vec{p}_{glb}$ given before calculations by the user of the program. Thus, the three strategies for getting initial $\vec{p}$ – value were examined: (i) no MC, $\vec{p} = \vec{p}_{last}$; (ii) Local MC, $\vec{p} = \vec{p}_{last} + \vec{r}$; (iii) Global MC, $\vec{p} = \vec{p}_{glb} + \vec{r}$. Here $\vec{r}$ is a random vector, which allows to vary $\vec{p}$ -values in the range of 100%. For MC processing (items ii and iii), for each spectrum we have provided 11 randomized simulations and one simulation which used previous approximation results as a starting point. Further, the $\vec{p}$ -value which gives the lowest CNLS-error was selected as equivalent scheme estimation.

Results and Discussion

The results obtained from applying different strategies of choosing starting point are presented in Fig. 1. It can be seen that if approximation was performed without MC, all spectra did not

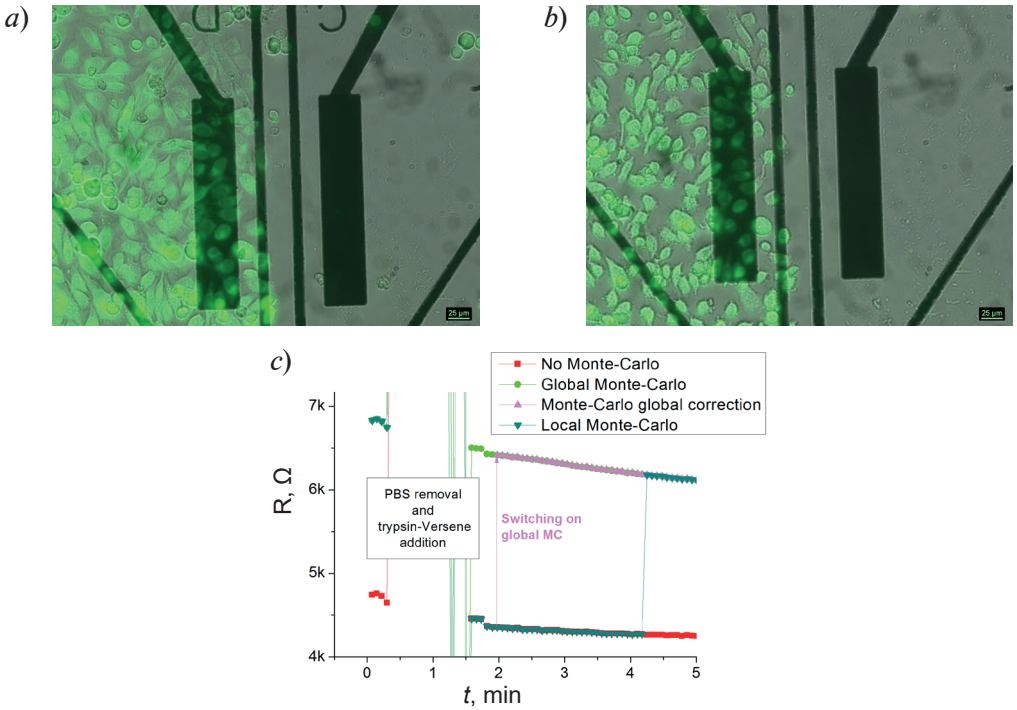

Fig. 1 Comparison of different CNLS processing strategies. (*a*) and (*b*) Photographs of the cells before and after trypsin-Versene addition respectively; (*c*) evolution of CNLS obtained resistance for different strategies of choosing starting point. Here and in Fig. 2, red color corresponds to no usage of MC (strategy i), green – usage of global MC (strategy iii), dark cyan – usage of local MC (strategy ii), magenta – global MC correction. It can be seen that addition of trypsin-Versene leads to cells de-adhesion (*a*) and (*b*). However, resistance, obtained with different strategies (*c*), varies greatly, and only the global MC approximation spectra completely match with the experimental one, this claim being justified by Fig. 2. Moreover, this approximation is one that behaves in accordance with the Giæver-Keese model [1]

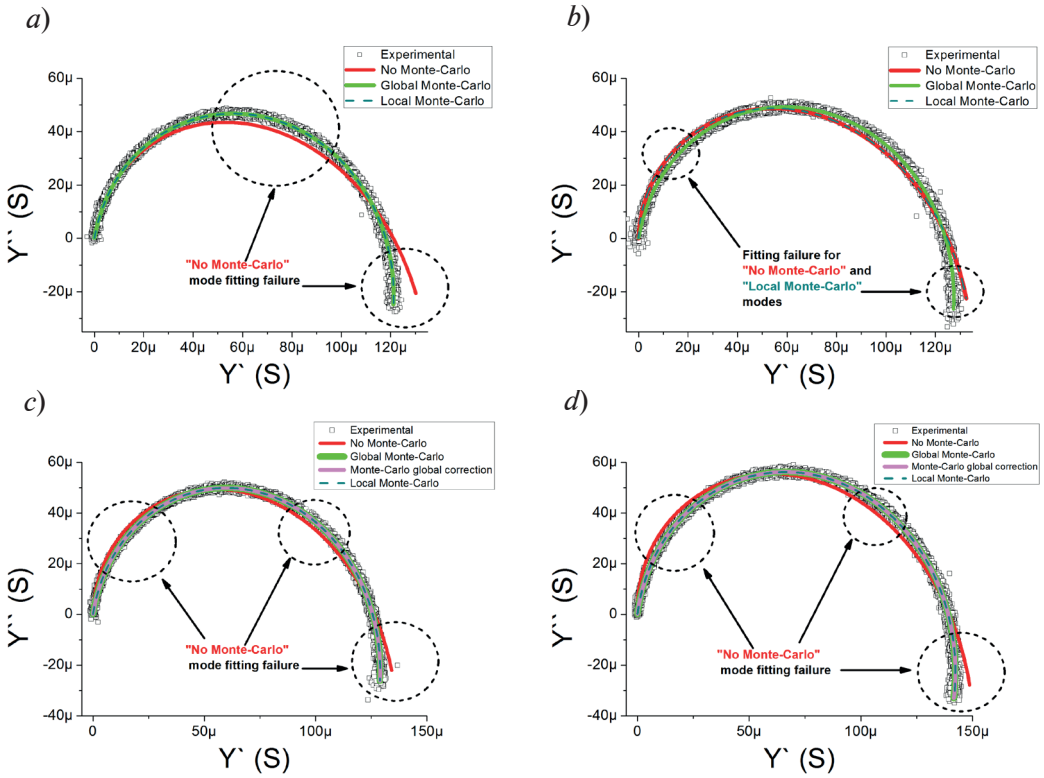

Fig. 2 Comparison of admittance loci of the cell/electrode interface for different CNLS processing strategies for the different time moments: (*a*) 0 min, (*b*) 1.5 min, (*c*) 2 min, and (*d*) 5 min. Squares scatter plot corresponds to experimental data, other line types are similar to Fig. 1. Dashed circles are highlighting spectrum parts with significant difference between CNLS approximation and experimental data. It can be seen that addition of trypsin-Versene leads to corresponding change of the spectra (compare loci with Fig. 1). Also, in the beginning local MC and global MC is indistinguishable, but, after PBS removal, global MC is the only one that match with experimental spectra. Moreover, after start of global MC correction, both global MC coincide with each other

match with the experimental one. In case of local MC strategy, starting spectra did match, but, after some manipulation with medium and as a result vastly different impedance signal, spectra stopped matching and approximation started behaving similarly to the strategy without MC. In the end, the local MC was able to find the minimum of the working function (Eq. 1) and provide an exact approximation. On the contrary, the global MC strategy did not only match experimental spectra all the way, but it also was able to succeed with a starting point, taken from failed local MC approximation, proving its supremacy.

Thus, the obvious strategy with using the result of the previous spectra approximation presents itself as not a stable one, due to the fact that change in the impedance was too large for the Nelder-Mead alone to be able to go back to the previous level. Even usage of local Monte-Carlo, which allowed us to run several simulations simultaneously in the search of the minimum, did not prove itself useful. Moreover, because in the end local MC was able to find exact approximation, usage of such a strategy led to the incoherent evolution of the resistance, which is showing sudden change in the state of the cells where in fact there is none. In a situation like that it is important to not only rely on the approximation of the previous spectra, but also have a universal starting point, located near the supposed minimum of the working function Eq. (1).

Conclusion

In this paper, we considered three different approaches to choosing a starting point for CNLS approximation and tested them on the bioimpendance experimental data. The strategy of not using Monte-Carlo showed itself as the most unreliable one; local Monte-Carlo was more stable, but still failed on some spectra; global Monte-Carlo was proved to be the most promising one out of them, being able to make accurate approximation for the whole duration of the experiment.

Thus, the combined approach, which consists of ruining several simulations using a universal starting point and the result of the previous approximation, was found out to be useful method for choosing a starting point. We believe that the results of this study will help in the development of new, more reliable devices for the needs of healthcare and modern biology.

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REFERENCES

1. **Stupin D.D., Kuzina E.A., Abelit A.A., Emelyanov A.K., Nikolaev D.M., Ryazantsev M.N., Koniakhin S.V., Dubina M.V.,** Bioimpedance spectroscopy: basics and applications, ACS Biomaterials Science & Engineering. 7 (2021) (6) 1962–1986.

2. **Stupin D.D., Koniakhin S.V., Verlov N.A., Dubina M.V.,** Adaptive filtering to enhance noise immunity of impedance and admittance spectroscopy: Comparison with Fourier transformation. Physical Review Applied. 7 (2017) (5) 054024.

3. **Ning K., Wang X., Xu X., Abassi Y.A.,** The xCELLigence system for real-time and label-free monitoring of cell viability. Mammalian Cell Viability: Methods and Protocols. (2011) 33–43.

4. **Ross M.J., Schoonman J., Lehnen A.P.,** Applicability and power of complex nonlinear least squares for the analysis of impedance and admittance data. Journal of Electroanalytical Chemistry and Interfacial Electrochemistry. 131 (1982) 77–95.

5. **Bondarenko A.S.,** Analysis of large experimental datasets in electrochemical impedance spectroscopy. Analytica chimica acta. 743 (2012) 41–50.

6. **Lagarias J.C., Reeds J.A., Wright M.H., Wright P.E.,** Convergence properties of the Nelder–Mead simplex method in low dimensions. SIAM Journal on optimization. 9 (1) (1998) 112–147.

THE AUTHORS

BOITSOVA Natalia A.
natab2002@yandex.ru
ORCID: 0009-0006-2524-7618

VERLOV Nikolai A.
verlov_na@pnpi.nrcki.ru
ORCID: 0000-0002-3756-0701

ABELIT Anna A.
anna.abelit@gmail.com
ORCID: 0000-0003-3859-6981

STUPIN Daniil D.
stu87@ya.ru
ORCID: 0000-0002-5470-9301

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