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Designing electrodes for electrical impedance spectroscopy in a four terminal setup

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Abstract: Electrical impedance spectroscopy has a high potential for monitoring and characterizing samples, e.g., biological cells. The technique is highly sensitive but artefacts often disturb it. This study discusses problems for miniaturization of the measurement volume needed to observe single cells in a 3D printed 4T setup. There is a capacitive artefact between 1 Hz – 1 kHz introduced by the distortion of the electric field due to fringing effects. Proposed solution for reducing distortion: use of thinner measurement electrodes.

I. Introduction

Electrical Impedance Spectroscopy (EIS) is a technique that monitors the change of electrical properties over a frequency range to investigate matter. Especially biological cells are often in the focus of these investigations [1-8]. This technique has an enormous potential as it can be used non-invasively without harming biological cells. Cell measurements are typically performed in suspensions [1-5] or in direct contact to the cells [6-8]. Cell suspensions can investigate only one specific cell line surrounded by a medium with already known electrical properties, which cannot be realized for direct measurements in practical applications so far. Thus, to determine the electrical property of a small number of cells or even a single cell [2, 3], measuring it in suspension is the most suitable solution. The main advantage of small-sized measurement volume is, that tiny variations have a higher impact on the output. Furthermore, to determine the electrical properties of cells as exact as possible only a single cell should be measured. Additionally, the smaller sample size reduces costs and time for growing cells. Thus, the size between measurement electrodes has to be reduced to a minimum. To extend the amount of information, the frequency range should be as large as possible. This paper explores limitations of the miniaturisation process for a four terminal experimental setup which enhances the output in low frequency range and gives design hints.

II. Theoretical Background

A device performing an EIS experiment applies a defined alternating voltage or current to the sample, and measures the resulting current or voltage while varying the input over a wide frequency range. There are several ways to represent the output, one often used is to plot impedance magnitude and phase shift over the frequency (Bode Plot). The main parameters of an EIS experiment are frequency range, amplitude of the voltage or current applied to the system, sample type, geometry and size as well as the experimental setup. One aim of the experimental setup is to reduce noise. It is possible to reduce noise such as the fluctuations of surrounding environment by increasing the amplitude of the input signal. It is important to limit the amplitude to a linear region (material dependent, typically around 10 mV) and to the cell preserving (below 100 mV) range [10]. The electrode material can improve the signal quality by injecting more current into the system without increasing the voltage. This is due to the exchange current density which depends on the electrode material and medium [9]. Further, the electrodes have to be good conductors, biocompatible and inert [11]. A rectangular chamber shape is a reasonable choice for simple determination of the cell constant [11, 12]. A further source of noise is the so-called double layer effect described in detail in [9]. It occurs in low frequency ranges and can be avoided by a four terminal setup (4T) [1, 5, 10] which is illustrated as a simplified circuit model in fig. 1. There exist three typical dispersions (α-, β-, γ-dispersion) which are measured by cell experi-ments [13]. The α-dispersion occurs in the low frequency range [13]. It could be shown that cell lines in a range below 1 kHz can be easily distinguished [1]. Thus, a four terminal design is advantageous for EIS experiments with cells [5].

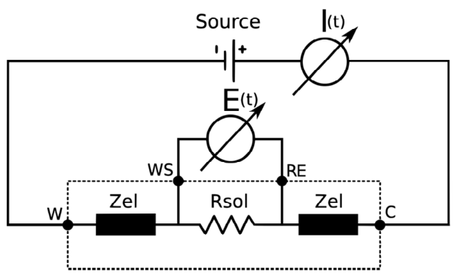

Figure 1: Four terminal setup as a simplified circuit model [5]. The current is injected by the working (W) and counter (C) electrodes. At the reference (RE) and working sense (WS) electrodes the suspension potential is measured. Zel represents the electrode impedance. Rsol represents the sample.

II. Material and Methods

A Gamry Interface 1000E device in potentiostatic mode was used to perform the experiments (applying a voltage and measuring the current). As sample, the Standard Con-ductivity of 1413 µS/cm (VWR Chemicals), a conductivity solution consisting solely out of potassium chloride was measured. Shielded coaxial cables and the four terminal design reduced noise and artefacts. The

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electrode material was stainless steel (V4A), the applied voltage was 10 mV rms. For all experiments, measurement chambers (fig. 2) produced by a stereolithography apparatus called Form 2 of the company FormLabs Inc. were used.

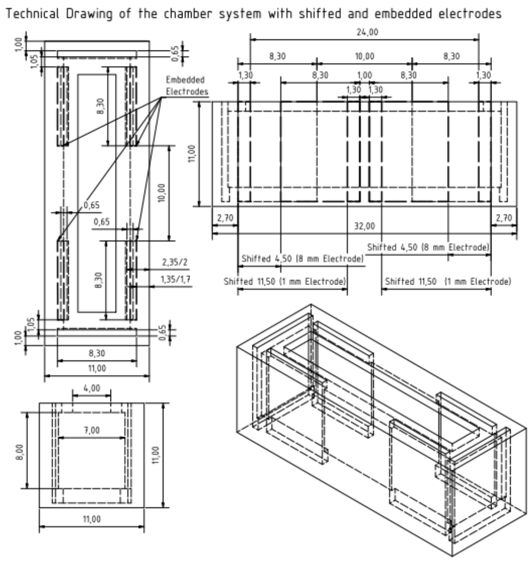

Figure 2: Technical drawing of the chamber system with different positions of reference and working sense electrode as well as positioning them embedded inside and at the wall. All dimension are in millimeter.

The size and position of the reference (RE) and working sense (WS) electrode were changed to investigate their influence while the remaining experimental setup stayed the same. The WS and RE electrode size varied between 1 mm and 8 mm width. The minimum distance between these electrodes is 1 mm. Further, electrodes embedded completely inside the wall of the chamber and in front of the chamber`s wall are investigated.

III. Results and Discussion

The influence of the RE and WS electrode depending on their position is evaluated. The embedded electrodes showed no remarkable differences compared to the electro-des positioned at the wall. Since a conductivity solution is the sample, no capacitive signal should be measured. Thus, all the phase shifts should be zero. Therefore, the phases observed in fig. 3 are artefacts due to fringing effects.

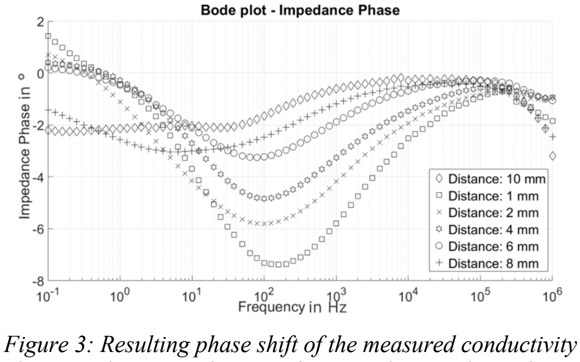

Figure 3: Resulting phase shift of the measured conductivity solution with varying distances between the two electrodes WS and RE. The used electrodes are not embedded in the wall of the chamber and have a width of 8 mm. Note that the introduced phase shift (artefact) increases with decreasing distance between WS and RE electrodes.

The same artefact as in fig. 3 can be observed for measurements with 1 mm-sized RE and WS electrodes but the phase shift is much smaller. This leads to the hypothesis that the artefact occurs due to distortion of the electric field. This is verified by a FEM simulation (fig. 4) which shows that between the RE and WS electrodes no uniformly distributed electric field exists.

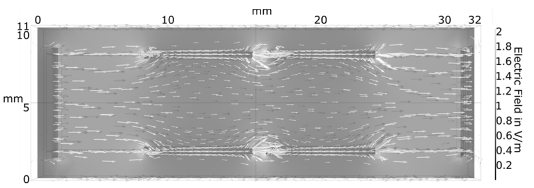

Figure 4: Comsol Multiphysics FEM simulation of electric field lines in the chamber design with a voltage of 10 mV rms applied to the working and counter electrode. RE and WS are 8 mm wide and have a distance of 1 mm.

IV. Conclusions

This work shows some of the challenges which have to be overcome for minimizing the sample volume while keeping the frequency range as large as possible. The size of the RE and WS electrodes should be kept as small as possible to avoid distortion of the electric field, especially if the distance between electrodes is reduced. Further, the position of these electrodes inside or at the wall shows no remarkable effect on the output.

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AUTHOR'S STATEMENT

Authors state no conflict of interest. Informed consent has been obtained from all individuals included in this study.

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