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Marker ink based dye as a tool for intravital high-resolution visualization of cells on a silicon

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Abstract. When creating new bioelectronic devices, including bioimpedance cytosensors, there is a need for optical monitoring of cell interactions with bioelectrodes. Typically, fluorescent dyes are used for this purpose, which are usually expensive and require strict storage conditions. In this paper, we propose a technology for visualizing living cells on opaque surfaces such as silicon using do-it-yourself low-cost dye called ABDS (A Beauty Dye for Staining), which is based on permanent marker red ink. We have shown that the use of ABDS allows one to obtain more detailed images of cells in respect to cost-free reflected light modes without using any dye at all. Moreover, the ABDS utilization makes it possible to study cells at the edge of the opaque bioelectronic device cell culturing chamber, which is difficult when using simple reflected light modes due to strong optical distorsions caused by the formation of a cells medium meniscus.

Keywords: Cell visualization, HeLa, ABDS, silicon, PETG

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Краситель на основе чернил перманентного маркера как инструмент для высокоразрешающей визуализации живых клеток на кремнии

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Аннотация. При создании новых биоэлектронных устройств, в том числе биоимпедансных цитосенсоров, возникает необходимость в оптическом контроле взаимодействия клеток с биоэлектродами. Обычно для этих целей используются флуоресцентные красители, которые, как правило, обладают высокой стоимостью и требуют специальных условий хранения. В данной работе мы предлагаем технологию визуализации живых клеток на непрозрачных поверхностях, таких как кремний, которая основана на применении красителя под названием ABDS (A Beauty Dye for Staining) – самодельного дешевого реактива на основе чернил красного перманентного маркера. В ходе ее выполнения мы показали, что изображения клеток, окрашенных с помощью ABDS, обладают значительно лучшей детализацией по сравнению с фотографиями полученными с помощью не требующих расходных материалов режимов отраженного света. Кроме того, использование ABDS также позволяет изучать клетки на краю непрозрачной культивационной камеры биоэлектронного устройства, в то время как из-за оптических искажений, вызванных образованием мениска у клеточной среды, применение методов на основе регистрации отраженного света в таких условиях становится неэффективным.

Ключевые слова: визуализация клеток, HeLa, ABDS, кремний, PETG

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Introduction

Nowadays, ones of the rapidly developing areas of bioelectronics are impedance cytosensors engineering and living neurons based devices development [1–4]. These devices are typically presented as modified Petri dishes with an array of microelectrodes on the bottom, which are also called multielectrode arrays (MEAs). Although commercial MEAs are widely used in biology and medicine today to monitor cell proliferation, drug testing, cancer research, etc., these devices are still under active development, one of the challenges for which is the search and improvement of MEA materials. For example, one of the most promising materials for MEA fabrication – the silicon, semiconductor electronics fundamental brick – has been intensively used over the past decades to create high-resolution MEA combined with CMOS technology [5–7]. Such a scientific trend is motivated by the silicon biocompatibility, existence of the developed silicon industry,

and the ability to use integrated circuity approaches for improving the MEA spatial resolution. However, silicon, like many other semiconductors, is an opaque material in the visible range of light, which complicates the diagnostics of living cells lying on it. In particular, opaque silicon is not compatible with classical transfer light and phase-contrast modes for intravital cell monitoring, and only reflected light and fluorescence non-inverted microscopy are available as relatively simple silicon MEA optical bio-diagnostic. At the same time, the reflected light mode usually produces a low-contrast picture of cells, since its video signal includes background image data [7, 8], whereas cell images obtained using fluorescence microscopy are clearer [8, 9], but require the use of expensive dyes [10]. To overcome the last problem, it is possible to use self-fluorescence cells, which e.g. enable the expression of green fluorescence protein (GFP) [6]. However, most cells do not produce fluorescence proteins, and their gene modification may be more expensive than the use of dyes. Moreover, GFP staining provides images of low detalized cells.

Recently, we have introduced an intravital cell dye called ABDS, which can be prepared from permanent red marker inks [10]. ABDS allows high-resolution imaging of cells, allowing for much more detailed examination of cell morphology than is possible with reflected light and GFP, which provides the solution of the mentioned above cells visualization problem for silicon based MEAs. Thus, in this study, we aim to show the advantages of the ABDS usage for cell visualization on the silicon substrates.

Materials and Methods

To test the proposed method for the visualization of cells in opaque silicon, we have used HeLa cells and a MEA-like device fabricated from a silicon wafer [Si (111), surface coated with natural silicon dioxide], PDMS Sylgard 184 (DowCorning, Germany, 10/1 PDMS/hardening agent ratio), and a 3D-printed rings of polyethylene terephthalate glycol (PETG, models avaliable at https://cults3d.com/ru/3d-model/instrument/small-mea-ring-with-guide-frame). The rings were fabricated on a Flying Bear S1 3D printer (Zhejiang FlyingBear 3D Technology Co., Ltd, China) using PETG plastic from Guangzhou Zhiwen Technology (China). The devices were fabricated by gluing 3D printed rings to silicon wafers using PDMS according to the protocol [11]. The resulting MEA prototypes were then washed with soap and water, rinsed with deionized water, and sterilized with ethanol. The HeLa cells were obtained from the cell culture bank of the Institute of Cytology of the Russian Academy of Sciences. The day before the experiment, the cells were seeded on MEA prototypes and cultured at 37 °C and 5% CO_2 in DMEM medium (Biolot, Russia) with 5% FBS (Sigma Aldrich, USA) and 0.15% gentamicin (Biolot, Russia). Before the experiment, cells were stained with ABDS to visualize the membrane according to the protocol described in [10]. Finally, the cells' medium was then replaced by PBS with DAPI dye (ThermoFisher Scientific, USA) for cell viability check. The microphotographs of HeLa cells were taken using a Leica DM 4000 B non-inverted microscope equipped with a light emitted diode (LED) and mercury metal halide EL6000 fluorescent lamp (Leica, Germany) incident light-sources. Accordingly, the cell images were acquired in three modes: BF-BF mode, when the cells were illuminated with white incident light from the EL6000 without color filters (BF-BF is the name of the microscope software settings that provide such image registration); LED mode, when the cells were illuminated with incident white light from an LED source without color filters; and fluorescence mode labeled as N2.1 in the photographs by the name of the used cube filter [12], when the cells were illuminated with green light (516–560 nm) from the EL6000, with the fluorescence signal detected at 580 nm.

Results and Discussion

The obtained results are shown in Fig. 1 and Fig. 2. As can be seen in Fig. 1, at 10X magnification, clear images can be obtained by using reflected light modes without usage of any dyes as well as using cells visualizing with ABDS. But at higher magnification 20X both BF-BF and LED modes do not allow to visualize cells with sufficient clarity. At the same time, the visualization method which uses ABDS is free from such drawbacks and allows one to obtain a sufficiently clear image of cells even at 20X magnification. This effect can be connected with the wide-band spectrum of the EL6000 and LED light sources, which leads to chromatic aberrations smoothing the image [13]. Moreover, the usage of the white-band spectrum lighting can influence the semiconductor device functioning much stronger than narrow band fluorescence radiation. In addition, in the reflected

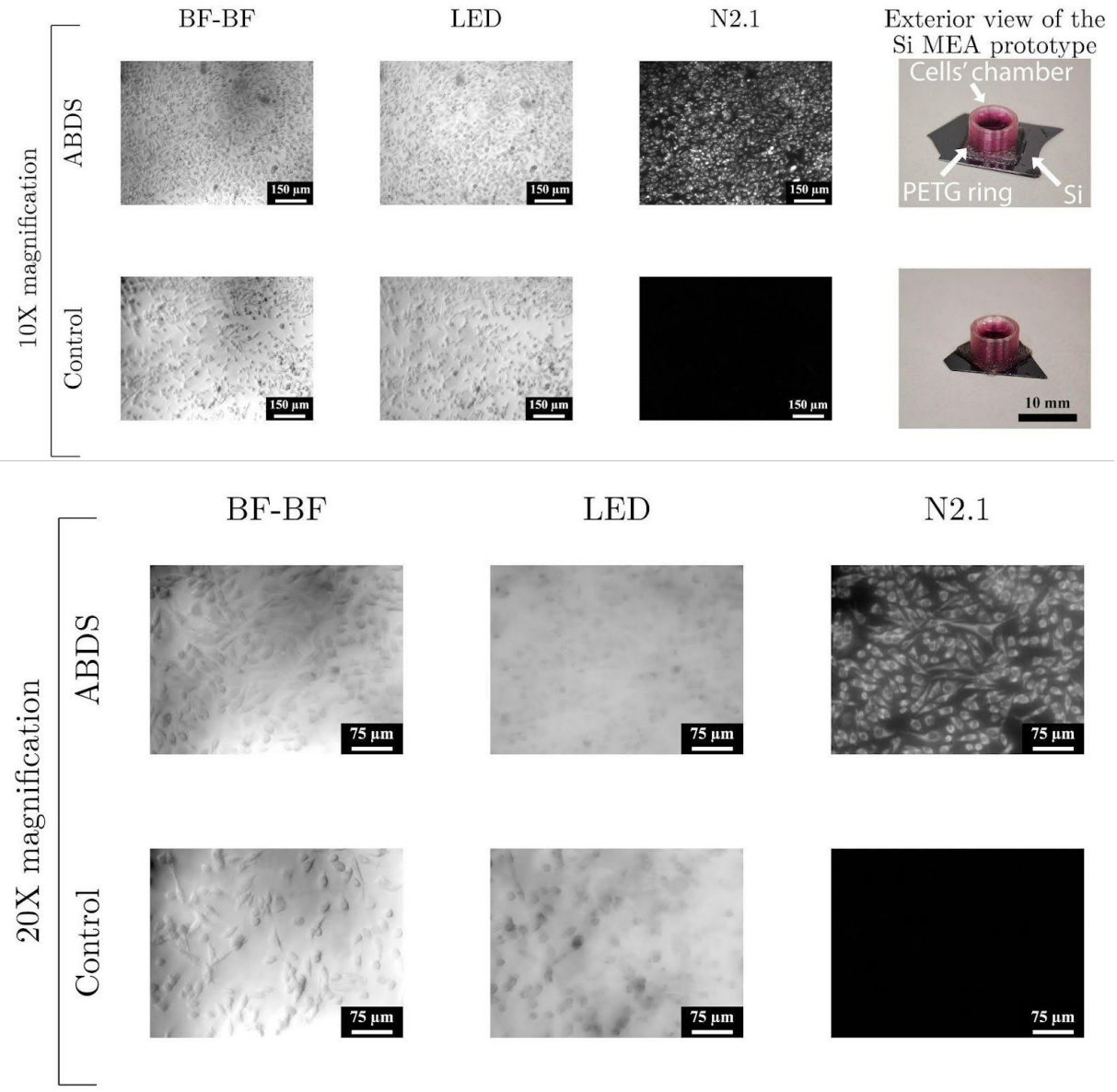

Fig. 1. Visualization of cells on the surface of a silicon MEA prototype. It is seen that at 10X magnification, cells are clearly visible both in reflected light (BF-BF and LED) and ABDS staining (N2.1 cube) modes. However, at 20X magnification, visualization of cells using ABDS achieves significantly higher image clarity compared to reflected light. ABDS staining allows to see in detail the membrane of the cells, nuclei shadow, and focal contacts. Control images, where cells were not treated with ABDS, show no background silicon fluorescence for the ABDS (N2.1) channel

light mode, no output optical filters are used, which leads to blinding the CCD-camera (Leica DFC 345 FX, Leica, Germany) and dropping the image quality. In contrast, the ABDS signal is pumped and detected in relatively narrow bands, which eliminate the blinding and aberration effects.

It is also worth noting that ABDS staining allows one to examine cells located near the edge of the silicon MEA prototype Petri dish (compare with [11]). In Fig. 2, it is clearly seen that in the BF-BF and LED modes images the cells cannot be distinguished at the edge due to the optical distortions caused by meniscus formation, while the ABDS fluorescence in the cells allows a clear image to be obtained since for the fluorescence signal there are less optical artifacts. During experiments we do not detect significant DAPI fluorescence, which confirms the biocompatibility of the proposed staining technique.

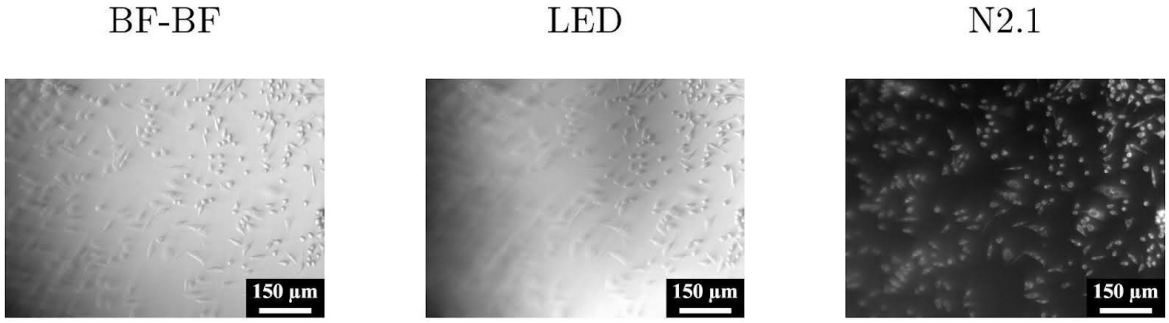

Fig. 2. Visualization of HeLa cells near the edge of a Petri dish on the surface of a silicon MEA prototype (20X magnification)

Conclusion

In this work, we proposed a method for visualizing cells on the opaque surface of silicon wafers using ABDS dye, created on the basis of a red permanent marker ink. The advantages of the resulting technology are its low cost, versatility, and significantly better clarity of the resulting images compared to simple reflected light regimes. We hope that our proposed method for visualizing cells on opaque substrates will significantly simplify the development of new cytosensors and the selection of optimal materials for their manufacture.

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