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POSITIONING AND CONTROL OF SCANNING ELECTROCHEMICAL MICROSCOPY

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Abstract. Positioning problems of scanning electrochemical microscopy (SECM) are very important by means of living cells damaging by moving ultramicroelectrode (UME). The working principle and operating modes of SECM are introduced. Investigation of redox activity of living cells are outlined. Problems, which arise in investigations of living cells by constant distance and constant height modes are discussed. Technical challenges and advances in application of SECM in living cell investigations are provided.

Keywords: scanning electrochemical microscopy, living cells, ultramicroelectrode, human cells, approaching curves, shear-force SECM.

Introduction

SECM was introduced as a technique, in which the local electrochemical activity of surfaces could be visualized (Bard *et al.* 1989). The advantage of SECM is that the technique can be applied for in-situ studies without any damage to the system of interest, and this feature is very important for surface-activity analysis of enzymatic biosensors (Morkvenaite-Vilkonciene *et al.* 2014, 2015). SECM can be applied for high-resolution imaging of chemical reactivity, electrocatalytic activity, and topography of enzyme-based interfaces formed in enzyme immunoassays (Yasukawa *et al.* 2007), biosensors and biochips (Zhao, Wittstock 2005; Lau *et al.* 2014). Until now, SECM has been widely used for investigating the viability of living cells (Morkvenaite-Vilkonciene *et al.* 2016). SECM is capable to provide local information from electrochemically active surface in several different ways: i) amperometric; ii) potentiometric; iii) electrochemical impedance. Measurements of local electrochemical impedance gives a lot of information about surface's reactivity, by mean of applied equivalent circuits models (Morkvenaite-Vilkonciene *et al.* 2017). One of the most informative SECM modes is based on the vertical movement of an UME *vs* sample (approaching curves) because it allows to register a concentration profile. Feedback modes are described mathematically, and from such measurements it is possible not only to determine the kinetics of a surface, but also to calculate the geometry and quality of the electrode (Cornut *et al.* 2011). To achieve reaction kinetics from approach curves, the redox competition model was created (Ivanauskas *et al.* 2016). It was found that the absolute current signal depends not only on electrochemical reactions close to the electrode, but also on the diffusion rate, when some chemicals are added to the solution.

Approach curves, from which information about diffusion of electroactive species could be determined, are obtained by moving the UME vertically until zero position is reached. However, there are some problems: if UME is of non-ideal geometry, the current signal never reaches zero at zero distance. Therefore, some techniques for distance determination should be used, and several of them are discussed in this paper.

Scanning electrochemical microscopy

The main part of the SECM is the ultramicroelectrode (UME), with a diameter in the micrometer range, it is usually used as a moving working electrode in an electrochemical cell (Bard, Mirkin 2001). UME can be moved in three

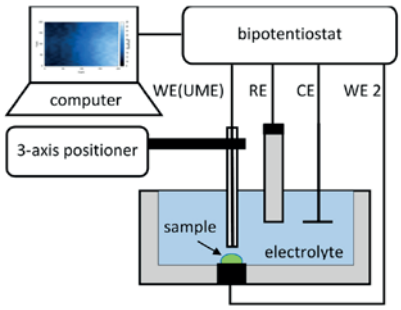


Fig. 1. Scheme of typical SECM experiments. WE(UME) – working ultramicroelectrode, RE – reference electrode, CE – counter electrode, WE 2 – second working electrode, connected to a substrate

directions and the current is registered as a function of the coordinates. The experimental SECM scheme is shown in Figure 1. Here a four-electrode electrochemical cell is shown with an UME as a moving working electrode, reference and counter electrodes, and the substrate is connected as a second working electrode. All electrodes are connected to the bipotentiostat. The control and movement of the UME and also current registration at the same time are controlled and recorded by a computer program.

Positioning of the probe electrode

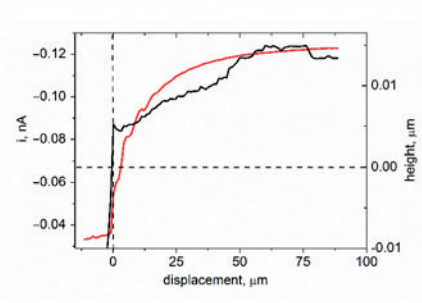
All SECM experiments can be carried out in constant height and constant distance modes. In constant height mode the UME is moved only laterally in the x and y directions, while in constant distance mode UME can be moved in x-y-z directions. The constant height mode is appropriate for the evaluating surface smoothness (if roughness is smaller than the UME radius) in samples (Li, Yu 2008). In this mode the UME current depends on the distance between UME and the surface of interest and on the reactivity of compounds immobilized on the surface. To determine the distance, which is the most suitable for appropriate resolution of SECM constant height mode measurement, the current *vs* distance dependence could be measured in feedback mode by approaching the UME to the surface of interest; and distance between UME and sample could be calculated from the SECM theory, where $i_T/i_{T,\infty}$ (ratio of UME current and steady-state current far from electrochemically active surface) can be related to d/a (the ratio of distance between sample and UME and UME radius). However, this approach is not accurate and can lead to the tip crashing or damaging the biological sample. Attempts to overcome these restrictions include: tip positioning to distances outside the feedback range (Yasukawa *et al.* 1998), into cavities (Shiku *et al.* 2001; Hirano *et al.* 2004; Shiku *et al.* 2004; Kaya *et al*. 2003; Torisawa *et al.* 2003; Torisawa *et al.* 2005), embedding the cells or efforts to subtract topographic contributions after cell death (Zhu *et al.* 2008). Moreover, as living cells are irregular in dimension, the tip-to-cell distance varies with the tip position. These limitations can be overcome by using a shear-force based constant-distance control (Ludwig *et al.* 1995). The microelectrode vibrates at its resonance frequency with typical amplitudes of only a few nanometers with the use of a piezo-pusher (Hengstenberg *et al.* 2000). Simultaneously, a laser beam is focused onto the very end of the vibrating electrode and the resulting Fresnel diffraction pattern is projected onto a split photodiode. The amplitude and phase information about the vibrating tip is obtained by the amplification of the differences in signals from the split photodiode with respect to the agitation signal used by a lock-in amplifier. With decreasing tip-to-sample distance, increasing shear forces between tip and sample surface lead to a damping of the vibration amplitude and to a phase shift, which can be used to continuously keep a predefined damping value related to a constant distance of about 50±100 nm by means of a software-controlled feedback loop (Hengstenberg *et al.* 2000). Another method of the shear-force detection is accomplished by mechanically attaching a set of two piezoelectric plates to the scanning probe (Ballesteros Katemann *et al.* 2003a). One of the plates is used to excite the SECM tip causing it to resonate, and the other acts as a piezoelectric detector of the amplitude of the tip oscillation. Increasing shear forces in close proximity to the sample surface lead to a damping of the vibration amplitude and a phase shift, effects that are registered by connecting the detecting piezoelectric plate to a dual-phase analogue lock-in amplifier (Ballesteros Katemann *et al.* 2003a). Also, a shear force-based method is able to work at various tip-to-sample separations. It can hence detect complete diffusion profiles in the surroundings of sources or sinks of redox-active species (Nebel *et al.* 2010). In particular, coupling SECM with scanning probe techniques, such as atomic force microscopy (AFM) (Macpherson *et al.* 1996) and scanning ion conductance microscopy (SICM) (Comstock *et al.* 2010), shear force (Ludwig *et al.* 1995; Ballesteros Katemann *et al.* 2003b; James *et al.* 1998; Ballesteros Katemann *et al.* 2003a) and impedance-based techniques, (Alpuche-Aviles, Wipf 2001) as well as led to efficient strategies to control the tip-to-sample separation. In the combined technique of AFM-SECM, AFM tip is used as a working electrode and as the force sensor at the same time (Eckhard *et al.* 2007; Kranz, Wiedemair 2008). This technique allows to achieve the best resolution and

to measure different properties of the surface. For living cells, it could be force curves measured at the same time with current-distance curves. To investigate the influence of toxic chemicals, or to distinguish cancer cells from the healthy ones. Both data are interesting: cells, depending on whether they're healthy, become softer/harder and at the same time more/less active.

Positioning experiment

The accurate determination of distance was performed by approaching UME to the surface (Fig. 2a). Usually, current *vs* distance curve minimal current value is zero, because diffusion of electroactive species to the UME is blocked. However, accurate distance could not be known, since diffusion could not be blocked close to surface due to microroughness of UME surface. Therefore, at zero-distance the small amount of current is still registered. It is clear from the results that height signal decreases sharply, and this means that UME is very close to the surface. This distance is evaluated as zero distance. Meanwhile, when current is decreased, zero distance is not seen, because electrode always show some current. It is seen from the picture, that decrease of current meets change in the height, but in zero distance current is still measured, and if no control occurs, the sample could be damaged.

a)



b)

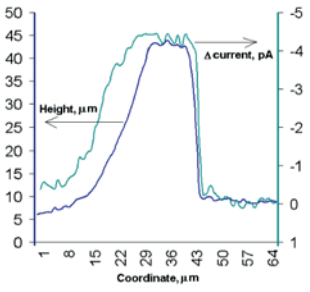


Fig. 2. a) UME positioning curve, registered by shear-force SECM; b) Horizontal scanning of cells by 20 µm distance from surface. Scanning was performed by measuring the current and topography at the same time.

Conclusions

Positioning of UME is crucial factor in SECM researches. Current, if concentration of electroactive species is constant, depends on distance from the surface of interest, and on UME geometry. Close to the surface, if UME geometry is ideal, current should be close to zero. However, UME with non-ideal geometry gives small current signal even if UME reaches zero distance. This leads to tip crashing or sample damage. Therefore, new techniques for UME positioning is needed, and this will be part of our next work.

Disclosure statement

Authors do not have any competing financial, professional, or personal interests from other parties.

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SKENUOJANČIOJO ELEKTROCHEMINIO MIKROSKOPO POZICIONAVIMAS IR VALDYMAS

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Santrauka

Tinkamas skenuojančiojo elektrocheminio mikroskopo (SECM) ultramikroelektrodo (UME) pozicionavimas yra aktualus eksperimentuojant su gyvomis ląstelėmis, nes gali pažeisti ląstelių paviršių. Šiame straipsnyje pateikiami SECM veikimo principas ir darbo režimai. Nagrinėjamas gyvų ląstelių oksidavimo ir redukavimo aktyvumas. Straipnyje pateikiama problemų, kurių kyla matuojant pastovaus aukščio ir pastovaus atstumo metodais, analizė. Pateikiami techniniai pozicionavimo sprendimai, iššūkiai ir progresas, taikant matuoti SECM gyvoms ląstelėms.

Reikšminiai žodžiai: skenuojantysis elektrocheminis mikroskopas, gyvos ląstelės, ultramikroelektrodas, žmogaus ląstelės, priartėjimo kreivės, šlyties jėgos SECM.