

REVIEW

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Scanning electrochemical microscopy: a new way of making electrochemical experiments

Received: 28 October 1999 / Revised: 10 January 2000 / Accepted: 21 January 2000

Abstract A short review is given on scanning electrochemical microscopy (SECM). The historic background of the technique is briefly summarized and the basic principles outlined. The three different directions of its use: chemical microscopic imaging, the measuring of physicochemical constants and coefficients, and use as a micro-machining tool are briefly discussed. The general built-up of the SECM apparatus is described. Preparation and use of several different measuring tips are introduced. A few examples are given of the application of SECM measurement in different studies.

Introduction

Different laboratories aiming at different goals have been working on the miniaturization of electroanalytical electrodes. For example, life scientists already in possession of detailed microscopic images of functional units of living objects have been interested in measuring the instantaneous, local concentration of different chemical species. They have very often been the pioneers of microelectrode preparation and have been using microelectrode positioning devices for decades. Some of these devices provide fraction of micrometer resolution and are mechanized. Laboratories equipped with high tech tools and fabrication experience have been interested in developing miniaturized analytical tools that can be mass produced. Electrochemists have been preparing electrodes with ultramicro dimensions to investigate their properties and to use them in studying electrode processes.

As is well known a new surface analytical technique, scanning tunneling microscopy (STM) was developed by

Binnig and Rohrer in the early eighties. This resulted in the award of the 1986 Nobel prize in Physics and it also initiated an ever growing family of measuring methods today called probe microscopy. The probe microscopic methods are based on a miniature measuring probe which is scanned over the target surface in three dimensions by a high resolution positioning device. The investigated target is often called substrate in SECM literature. The data reflecting the location of the probe and the local signal collected by the probe are stored in a computer. Special programs serve for image formation. Two very important advantages have made the probe microscopic methods very popular in a relatively short time. They can achieve very high resolution, on the one hand, and, on the other hand, the target does not have to be in a vacuum. The probe microscopes can study the objects in ambient conditions. Probe microscopy soon became established as an electrochemical method, for example to study the effect of electrode reactions, deposited layers or extent of corrosion. For example, electrochemists were the first to realize [1], that STM measurements can be made at the electrolyte solution/solid interface.

As probe microscopy became established and ultramicro size electrode electrochemistry became popular, it was a straightforward step to combine these two and develop the electrochemical version of probe microscopy, that we now call scanning electrochemical microscopy (SECM). The pioneering work in developing this method was mostly performed in the laboratories of A.J. Bard and W. Engstrom in the second part of the eighties. While Engstrom and coworkers showed that concentration profiles can be traced with a microelectrode attached to a positioning device [2, 3], the Bard laboratory concentrated its early SECM work on measuring tip-target surface interaction, investigating the so-called feedback effect of the amperometric tip [4, 5]. The first user-friendly electrochemical microscope was built and the basic feedback current equations were also developed in Bard's laboratory [6].

Several groups have now reported the successful construction of SECM apparatus and a commercial electrochemical microscope is also available. The first SECM

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symposium was held in Freiburg, Germany in 1997 and several excellent reviews have appeared about the method [4, 6–10]. SECM is still in the early stage of its development. A considerable part of its proposed prospective application field has yet to be tested and proved in practice. Three major fields of utilization of the technique can be distinguished today. One of them is the preparation of high resolution microscopic images of particular chemical significance from different targets. The second is the use of the technique to measure important physicochemical parameters such as reaction rate, diffusion coefficient or adsorption-desorption rate. Preliminary investigations predict that the technique may also have a future in the practice of high precision micromachining.

Basic principles

As mentioned above, the probe microscopy methods rely on a minute measuring tip, on a precision positioning device and on appropriate computerized data collection for image formation. This is valid for SECM with the additional feature that it uses electrochemical matter (e.g. signal formation, electrode reaction) in its operation. Depending on the role of the scanned tip the SECM methods are usually divided into two groups. Sometimes the tip is just a passive observer. It measures a certain electrochemically accessible property at a defined location and reports it to the computer. This is SECM with a passive tip. Very often, however, SECM experiments are made with an active tip. It interacts with the target and the signal is the result of this interaction. Active tip SECM can be used in all three above-mentioned areas of practice while obviously a passive tip cannot be a help in micromachining. With a passive tip, concentration profiles originally present can be imaged, while active tips can give information about different properties of the target without an originally present, electrochemically detectable characteristic concentration profile.

Three different modes of SECM imaging can be distinguished: the feedback mode, the generating collecting mode and the one-dimensional or penetration mode. In the following sections we shall shortly discuss these.

The feedback mode

The general principle of the feedback mode of SECM imaging is easy to understand if we consider the following. It is well known that with an ultramicro size amperometric working electrode polarized at a sufficiently high, constant potential a steady current ($i_{T,\infty}$) can be observed in quiescent solution containing appropriate electroactive species. This is so if the ultramicro electrode surface is in the bulk of the solution. The current is controlled by the mass transport of the electroactive species. For a disk shaped electrode of radius a , the following equation gives the steady state current.

$$i_{T,\infty} = 4nFDca \quad (1)$$

Here n is the number of electrons taking part in the tip electrode process, F is the Faraday constant, D is the diffusion coefficient, and c is the bulk concentration of the reacting species.

In pure electrochemical experiments the working electrodes are usually positioned in the bulk. In SECM the tip is brought into close vicinity of the target surface. This can affect the current in two different ways. The target surface can decrease the mass flow of the reacting species by blocking the diffusion path. This is called negative feedback because it decreases the amperometric current, i_T . Most often the active measuring area of the ultramicro electrode used as measuring tip is inlaid in an insulating shield. The size and geometry of the shield naturally also contributes to the negative feedback. Numerically obtained equations have been reported for the description of this effect. For diffusion controlled heterogeneous reactions and in case of a disc shaped tip with 1:10 disc to shield diameter ratio, the amperometric dimensionless current, $I_t = i_{T,L}/i_{T,\infty}$, can be expressed as:

$$i_{T,L}/i_{T,\infty} = 1/(0.15+1.5385/L + 0.58 \exp(-1.14/L) + 0.0908 \exp [(L-6.3)/(1.017L)]) \quad (2)$$

L is the dimensionless distance between the tip and the target surface, where $L = d/a$; d is the distance, a is the measuring disc radius.

Based on Eq. (2), a working curve can be made and the position of the electrode can be estimated. More precisely, by fitting the experimentally obtained distance/current curve to this equation, the distance can be measured. The negative feedback effect can be used for high resolution topographic imaging of different surfaces. Scanning over the target, the smaller current will indicate convex, extending areas, extending micro projections, while higher current shows concave areas, pits, cracks, channels. The negative feedback effect appears in case of target surfaces of electric insulator character or in case of any surface if the electrode process at the tip is chemically irreversible.

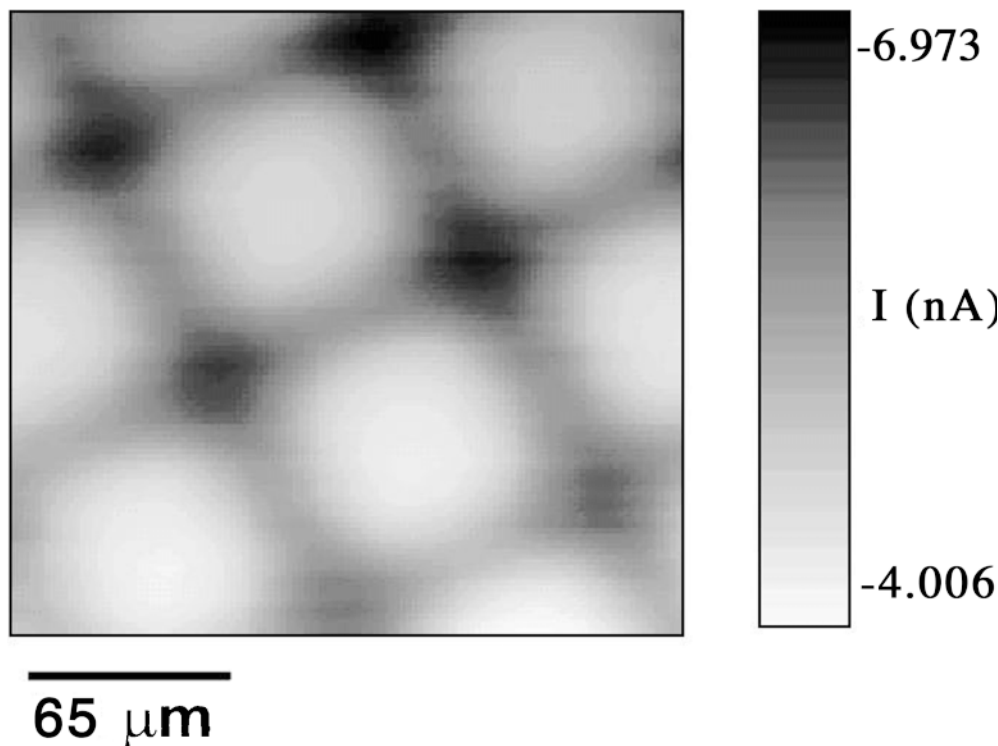
On the other hand, the proximity of a surface can increase the tip current. This is called positive feedback. It can happen at a conductive target, when the electrode process at the tip is chemically reversible and the spontaneous or adjusted electric potential of the surface supports a fast reverse reaction. In this case the adjacent surface regenerates the species reacted at the tip, increasing its local concentration in the gap between the two surfaces. Numerically obtained and experimentally proved equations are also available for positive feedback. Considering equal diffusion coefficient for the redox couple involved in the electrode process, large target surface and diffusion controlled electrode reactions, Mirkin et al. [11] obtained the following equation for the current ratio.

$$i_{T,L}/i_{T,\infty} = 0.78377/L + 0.3315 \exp(-1.0672/L) + 0.68 \quad (3)$$

The equation is valid for disc shaped electrodes with 1:10 electrode: shield ratio.

For experimental conditions coinciding with those considered above, Eq. (3) can be used for tip-target distance

Fig. 1 SECM image of copper minigrad made with an amperometric Pt tip ($a = 5 \mu\text{m}$) in ruthenium hexamine solution at about $10 \mu\text{m}$ distance. High current (darker image) is the result of positive feedback



estimation. It is obvious that positive feedback always indicates proximity of conductive surface area. In this case the current increase means protruding, convex spots.

As an example, Fig. 1 shows a SECM image of a copper minigrad prepared with platinum tip using ruthenium hexamine as mediator. The positive feedback over the copper results in a darker image.

Obviously the current measured over the investigated surface area reflects, on the one hand, the local topography, the distance and, on the other hand, the conductive or insulating nature of the sampled surface. Because of the opposite distance-current functions of the two feedback effects, questions can arise about the distinction between approaching a conductor or moving away from an insulating part of the surface. Tip position modulation (TPM) as developed by Wipf et al. [12] can help to determine the nature of the particular microarea of the studied surface. They use a piezo motor to modulate tip-target distance with a small amplitude sinus wave. The phase angle of the given frequency AC tip current shows the conductive or insulating character of the studied area. Using this TPM technique high resolution phase sensitive imaging or constant current imaging with tip-target distance control can be performed.

At the interface between two immiscible electrolyte solutions (ITIES) the tip current can increase or decrease similarly to the feedback phenomenon, if the interface is sharp and steady [13]. As the tip approaches a sharp ITIES, the current decreases because of hindered diffusion at the interface. This is true if the electroactive species reacting at the electrode surface is insoluble in the other solvent. The current can increase, however, if the product of the electrode reaction at the tip is recirculated by reacting

with an appropriate mediator dissolved in the other electrolyte phase. The speed of regeneration, that is the extent of the positive feedback, depends on the rate of the bimolecular redox reaction between the two liquid phases. In this case the current increase at the vicinity of the interface is the result of electron transfer between the phases.

Interestingly, at ITIES facilitated ion transport can also result in a tip current increase. Wei et al. [13] provided the following clear example. Let us consider a conventional micropipette filled with potassium chloride electrolyte solution and containing an internal reference electrode. The tip of this micropipette is inside an organic electrolyte phase. This contains an ionophore molecule which complexes the potassium ions. The internal reference of the pipette is polarized against a reference electrode and a steady current is achieved. The potassium ion outflux keeps the charge balance, that is the transference number of potassium ions equals 1. In this case the current is influenced by the diffusion rate of the ionophore to the pipette tip in the organic phase. If the tip approaches the water-organic solvent interface, then the ionophore potassium ion complex formed at the tip can easily reach the interface. At the interface the complex decomposes and the potassium ions enter the aqueous phase. This results in an elevated free ionophore concentration in the tip-surface gap, and the increased free ionophore concentration can increase the tip current.

Generation/collection mode

The straightforward application of microelectrodes is used to gather information about instantaneous local con-

concentrations of different species. So if the target, or as it is often named, the substrate, generates a concentration profile the measuring tip of an SECM apparatus can collect information about it. This mode of the SECM is called substrate generating/tip collecting mode (SG/TC) [2, 8]. The tip can be an amperometric ultramicro electrode, ion selective potentiometric microsensor or a conductometric probe. The image prepared with this technique reflects the spatial distribution of different species, the concentration profiles. Many experiments carried out with positioning device-linked microelectrodes in the life sciences can be considered as one-dimensional SG/TC microscopy. Diffusion-, corrosion-, heterogeneous catalytic reactions-, and metabolic processes of microorganisms generated concentration profiles have all been successfully imaged, as will be mentioned below.

In other cases the scanning tip generates concentration disturbances at different areas of the studied surface and the targeted substrate collects the information. This is the tip generating, substrate collection mode (TG/SC). The tip can do the generation by controlled potential electrolysis or simply by diffusion. In the first case the tip can be an amperometric ultramicro electrode while a small size open micropipette can be used to deliver a constant diffusion flux of an appropriate species. A good example of the TG/SC operation is the imaging of active spots on a conductive target surface. For this the potential of the substrate is kept constant to support a certain electrode process of the tip generated species. Then the amperometric current intensity-tip location image reflects the local activity of the substrate area with some topographic information.

Penetration mode

The tip of an ultramicro electrode can penetrate axially in the Z direction into soft films, gels or biological tissues; however, in these structures it can not move laterally. X-Y imaging is not possible without damaging the target or breaking the tip. A one-dimensional penetration experiment can serve to collect information about concentration profiles or to investigate the properties of the film, layer or structure. One-dimensional concentration data collection experiments in biological media are a good example for experiments in this penetration mode [e.g. 15]. Similarly, concentration profiles in enzyme-containing gels during enzyme catalyzed reactions have been imaged with an axially moving penetrating measuring tip [16, 17]. The properties of mediator-loaded thin films formed on metal surfaces can be studied with a penetrating amperometric tip. Positive feedback could be observed in mediator-containing films cast on a conductive metal surface as the tip moving inside the film approached the metal surface [18].

Measurement of reaction rates

In amperometric SECM the current measured depends on both the mass transfer and on the reaction rates. Since the

mass transfer can be changed by changing the distance between the tip and the substrate, values can be obtained for finite heterogeneous rate constants from results of SECM tip current measurements. For the tip current $I_T(E,L)$ at electrode potential E and distance from the substrate $L = d/a$ in case of finite electron exchange rate at the tip and diffusion controlled mediator regeneration Mirkin and Bard [19] derived the following equation.

$$I_T(E,L) = [0.68 + 0.78377/L + 0.3315 \cdot \exp(-1.0672/L)] / (\theta + 1/k) \quad (4)$$

$$\text{Here } \theta = 1 + e^{\frac{nF(E-E^0)}{RT}} \cdot \frac{D_O}{D_R} \text{ and } k = k^0 e^{\frac{-anF(E-E^0)}{RT}} / m_0, k^0$$

is the rate constant, E is the tip potential, E^0 is the formal potential.

m_0 is the mass-transfer coefficient and can be given as

$$m_0 = 4D_0[0.68 + 0.78377/L + 0.3315 \exp(-1.0672/L)] / (\pi a) = i_T(L) / (\pi a^2 n F c) \quad (5)$$

It can be seen that by decreasing the distance $L=d/a$ the mass transfer coefficient increases and the current will be controlled by the electron transfer kinetics. Therefore, from the dependence between tip current and L the rate constant of the electron transfer reaction proceeding at the tip can be estimated.

A more accurate, general equation was also derived for $(I_T(E,L))$ [20]. Information can also be obtained about the heterogeneous rate constant of a slow electrode reaction at the target from the experimentally recorded current-distance curve, fitting it to the following dependence derived by Wei et al. [13]:

$$I_T(L) = I_S(1 - I_T^{ins} / I_T^{con}) + I_T^{ins} \quad (6)$$

$$I_S = 0.78377/L(1/1+L) + [0.68 + 0.3315 \exp(-1.0672/L)] / [1 + F(L,\Lambda)] \quad (7)$$

I_T^{con} and I_T^{ins} are the dimensionless ($I_T(L)=i_{T,L}/i_{T,\infty}$) tip current intensities at the given distance in case of conductive and insulating substrate, respectively, I_S is the current at the substrate resulting from the slow electrode reaction at the target surface, $\Lambda = k_f d/D$, k_f is the rate constant of the reaction at the substrate and $F(L,\Lambda) = (11/\Lambda + 7.3)/110 - 40L$. Obviously using the same approach, the tip current-distance dependence allows one to estimate the rate constant of the reverse chemical reaction which results in the regeneration of the species reacting at the tip.

An electrode reaction at the tip can be used to deplete the solution film between the substrate and the tip and the surface dissolution rate can thus be determined. If the depleted species is on the surface of the substrate, the tip current intensity reflects the dissolution rate. The theory, however, for this case is more complicated. Macpherson and Unwin [21] made model calculations for different experimental conditions of such dissolution studies. The dissolution rates of potassium ferrocyanide [21] and copper sulfate [22] ionic single crystals were investigated with this technique.

The rotating ring-disc electrode configurations have often been used to determine the rate constants of homo-

geneous chemical reactions following an electron exchange step. SECM is also applicable for such measurements. Let us suppose that the apparatus is used in TG/SC mode and that both the tip and the substrate currents are measured under potential control. The species generated at the tip reacts in a reverse electrode reaction at the substrate, however, while approaching it a homogeneous reaction takes place resulting in electroinactive species. In this case the rate of this homogeneous reaction affects both currents. The higher the reaction rate in the gap the lower the flux of the unreacted species at the substrate surface, that is the smaller the current at the substrate. The reaction time can be changed by changing the gap. The tip current reflects the rate of the homogeneous reaction following the electron exchange step through the feedback effect. The higher the reaction rate, the smaller the observed contribution to the tip current from positive feedback. A molecule needs a time period of d^2/D to diffuse distance d between the tip and the substrate. About 10 μs are required for a quite small 0.1 μm gap (supposing $D=10^{-5}$ cm^2/s). The half life of the intermediate should be close to this for reliable measurement. The highest reaction rate constant measurable with this technique calculated in this way is 10^5 s^{-1} for a first order reaction and 10^8 $\text{M}^{-1}\text{s}^{-1}$ for a second order reaction with a reactant concentration of 1 mM. The dimerization rates of dimethyl fumarate and fumaronitrile radical anions [23] and those of acrylonitrile [24] and 4-nitrophenolate [25] were measured successfully with this technique.

The concentration profile generated by a heterogeneous surface reaction can be imaged with a passive SECM tip, e.g. an ion-selective micropipette [26]. The flux of the reacting or produced species can be extracted from the profile recorded under steady state conditions, and the rate constant can be determined from this flux.

As reported previously [27], quantitative kinetic data can be obtained about adsorption/desorption processes with the SECM technique. In such experiments the measuring tip is positioned close to the surface investigated. The adsorption/desorption equilibrium is perturbed by a potential step inducing an electrode process at the tip surface. The extent of this can be controlled by the tip-target distance or by the parameters of the potential step. From the comparison of the steady state tip current and the transient chronoamperometric curves, information can be gathered about the adsorption/desorption kinetics. The electrode process depletes the investigated species from the solution gap between the tip and the target. The desorption counterbalances this. Surface diffusion from areas in adsorption equilibrium also competes with the concentration depleting effect of the electrolysis. Taking advantage of the different time scales, however, the two effects can be separated. The adsorption/desorption process of H^+ ions was investigated on metal oxide crystals by Unwin and Bard [27] and on a platinum surface by Yang and De-nault [28].

Micromachining with SECM tip

An ultramicro size metal or graphite electrode connected to a fine positioning device can be used to deliver electrolytically generated reagents to different locations in a controlled time. Therefore, it can be used as a micromachining tool. Microstructures can be created on different surfaces or inside surface films in the electrolysis cell by wet etching or deposition. For example, copper and GaAs surfaces have been successfully etched with platinum microelectrodes [29–31].

To make a surface pattern by etching, the tip is brought into close vicinity of the surface and reagents such as bromine or $\text{Os}^{3+}(\text{bipyridyls})_3$ are generated. The etching resolution is controlled by the generating tip size, the tip-target distance and the rate of the etching reaction. With slow reactions, as in the case of Si(111) etched by bromine, the locally produced reagent can diffuse a relatively long distance making the etched spot shallow and broad, while with fast heterogeneous reactions sharp and deep engraving pits can be achieved. Tian et al. [32] propose a so-called confined etchant layer technique (CELT) for improving the resolution. In this method a scavenging reagent is employed which reacts with the freshly generated active etchant so avoiding its lateral diffusion. This technique was successfully used by Zu et al. [33] for Si(111) wafer etching.

Bard and coworkers [34] prepared micrometer-wide silver stripe structures with SECM in Nafion film. They added silver ions into the electrolysis cell, positioned the tip over the Nafion layer and adjusted the reductive tip potential. The structure was produced by slow motion of the tip. Using ruthenium hexamine as reagent reduced at the tip palladium and gold structures could be prepared inside polyvinylpyridine film.

Wittstock and Schuhmann [35] used the SECM tip to form enzymatically active micro spots on a gold support. In the first step they formed a self-assembled monolayer of n-alkane thiolates. Then small spots of the gold surface were regenerated by reductive desorption of the n-alkane thiolates using the SECM as micromachining tool. Onto the regenerated spots glucose oxidase enzyme was covalently linked through a self assembled monolayer. The SECM was also used to image the patterned active enzyme spots prepared in this way on the surface.

Instrumentation

SECM measurements require a relatively simple apparatus made of four main parts: a high resolution tip positioning device, an electrochemical measuring instrument, an electrochemical measurement cell, and a computer. The measuring tip can be considered as part of the cell. The individual parts are commercially available or can be made with well established procedures. However, developing a home-made apparatus is quite a challenging job. Good machine shop skills with electronic circuitry build-

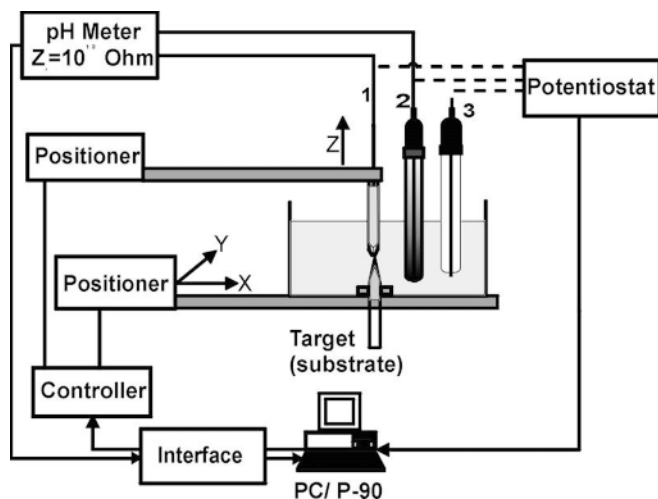


Fig. 2 General built-up of the SECM apparatus; 1 measuring tip, 2 reference, 3 counter-electrode

ing knowledge and good programming experience have to be combined. The product of these efforts, the functioning SECM is a small device. Together with its computer unit it easily fits on a normal laboratory bench. Figure 2 shows the schematic design of a SECM apparatus.

Most electrochemical microscopes use inchworm type piezoelectric linear motors for fine positioning. The inchworm working principle provides long travel lengths with very fine, subnanometer resolution of the piezo translator. Two motors assembled perpendicularly provide the scanning of the horizontal X-Z plane. This stage holds the measurement cells, while the measuring tip is held by a fine holder located on the third piezo motor, that is on the vertical scanner. In other cases the three motors mounted together to form a 3-axis stage move the tip in all three directions and the cell stays steady.

The resolution of the piezo motor is much higher than is required by most SECM measurements. Therefore, to be more cost efficient and to obtain a more robust device stepper motor driven mechanically positioner-based SECM have also been constructed [36]. The stepper motor driven positioners can achieve spatial resolutions as high as 75 nm [37], high enough for most SECM measurements. It is convenient if the microscope contains a three-dimensional manual positioner stage for rough but quick tip movements.

Small size measurement cells holding just a few mL electrolyte solution are usually used in SECM. The target is attached to the cell bottom, so it has to be flat. To avoid false or tilted images or tip crash the cell bottom has to be carefully set to horizontal. The cell has to be open to allow the tip to scan. Therefore, if the study requires oxygen-free or completely dry conditions the whole mechanical part of the microscope has to be placed into an airtight box. The Faraday cage around the cell is also essential, especially in the case of the potentiometric working mode. SECM is less affected by vibration than STM, however, the presence of a vibration-free support is beneficial.

A bipotentiostat capable of measurements in the sub nA range is needed as voltammetric measuring unit. Often one channel is used to control the electric potential of the substrate and the other to control and measure the tip current. The potentiostat is connected to the computer via an A/D and D/A converter. Preamplifiers are used for the tip current measurements. For potentiometric measurements a high impedance voltage follower as a separate unit or part of a mV meter is connected to the tip. It is advantageous if this is located in the close vicinity of the tip. In this way the noise level can be reduced. The reference electrode, which is most often stationary, can be inside the cell or can be connected through a current bridge.

Commercial interface cards are available to connect the microcomputer part of the SECM to the measuring instrument and the positioner. The computer controls tip movement and data acquisition and forms the image. Several software programs have been developed, and with the fast development of computer techniques they are becoming ever advanced. When microscopic imaging is done the tip follows a preestablished moving pattern scanning over a predetermined area. In SECM imaging practice tip travel rates as slow as 1–5 $\mu\text{m/s}$ are used. Therefore, it takes quite a long time to image a $100 \times 100 \mu\text{m}$ size target area, especially if the tip passes on the same course twice, as is usual. Finding areas of interest with the tip positioner on a micrometer scale can be a tedious job. To simplify this, an automatic target location algorithm was developed and incorporated in the operation program of a SECM [37].

The lack of commercial apparatus initially delayed the widescale application of the technique. However, an advanced apparatus produced by CH Instruments, Inc. recently appeared on the market.

Measuring tips used in SECM

The performance of SECM measurements and their attainable resolution are determined by the measuring tip size. As was mentioned earlier a large number of procedures have been developed for the preparation of ultramicro electrodes. Several of them have been used successfully in SECM experiments. The early work in electrochemical microscopy was performed mostly with noble metal or graphite disc electrodes. Their diameter was a few micrometers, and the disc was shielded. These electrodes were prepared by sealing platinum, gold wires or graphite fibers in glass capillaries. The electrical contact was made with mercury or with silver epoxy through a metal wire placed in the lumen of the capillary. The end plate was sanded off and the electrode disc formed was finely polished. The glass shield was beveled to a symmetrical cone with the electrode disc in the center. Platinum wires down to 1 μm can be purchased. Such ultrathin wires have a silver metal coating for easy handling. When the wire is in place before sealing in, the silver coating is digested with nitric acid. The shielding is usually performed with electric heating coil under light vac-

uum. The robust electrodes prepared in this way are still in use in different laboratories. Electrodes with submicrometer dimensions are prepared by chemical or electrochemical etching. The etched conical side of the microwire has to be insulated electrically. The sealing in glass and subsequent polishing cannot provide very small electrode sizes and therefore different waxes (e.g., Apiezon wax) [11], polymer coatings or paints are used for insulation. The insulating layer is broken off at the tip by heat treatment or electric shock to expose the electrode tip. Recently Unwin and coworkers [38] developed a procedure for making a nanometer-sized platinum electrode for SECM imaging with electrochemical etching in saturated sodium nitrite solution and electrophoretically prepared insulating layer.

Demaille et al. [39] reported an interesting approach for the preparation of ultramicro spherical gold electrodes by self-assembly of gold particles and 1,9-nonanedithiol molecules at the tip of glass micropipettes. The micropipettes were carbonized internally, immersed in the dithiol solution, then immersed in Au sol for 2 h. The contact was made with Ga/In alloy which was in contact with the carbonized wall, but not with the microsphere at the tip. The gold ultramicro electrode was proved to work as SECM tip.

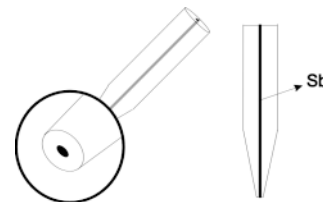
Chemically modified electrodes are becoming more and more popular. As was shown by Horrocks et al. [40] they can be used in SECM to solve special tasks. In their work a carbon fiber microelectrode was chemically modified by a horseradish peroxidase enzyme layer electrically 'wired' to the carbon surface with a redox polymer chain. The chemically modified amperometric tip was used to monitor local hydrogen peroxide concentration.

Ion selective microelectrodes have also been prepared and used in the practice of SECM. Denault and coworkers [41] prepared silver and silver/silver chloride microdisc electrodes of 10 and 50 μm diameter to probe silver and chloride ion fluxes during the electrochemical reduction and stripping of silver ions. These electrodes were made of silver wire soldered in glass capillaries. Using them the release and uptake of chloride ions by polyaniline films during controlled potential redox reactions could be monitored.

Since heterogeneous reactions often result in local pH changes, efforts have been made to develop and use pH sensitive SECM tips. A procedure has been worked out in Bard's laboratory for the preparation of an ultramicro size, pH sensitive antimony disc electrode [42]. It was observed that this electrode can also be used as an active amperometric tip. In this way the tip-sample surface distance can be measured and set in the feedback mode. Subsequently, potentiometric pH imaging can be performed. The antimony tip was successfully used in various electrochemical microscopic studies, e.g. [43]. The schematic design of a double function Sb SECM tip can be seen in Fig. 3.

Of all the electrochemical sensors ion-selective micropipettes can be prepared with the smallest measuring tip sizes. Life scientists developed well established universal procedures for preparation of micropipettes selec-

Fig. 3 Schematic design of a double function Sb SECM tip



tively sensing different ions long before the introduction of SECM. According to these procedures, micropipettes are made and at the tip the inner walls are siliconized. A small column of an ion-selective cocktail of lyophilic character is introduced into the tip, and the pipette is back filled with the internal filling solution. Finally, the internal reference electrode, most often a silver chloride coated wire, is introduced. Obviously such micropipettes were employed in ion microscopy. The first such experiments, however, revealed serious drawbacks. On the one hand, micropipettes are delicate tools, and, on the other hand, as passive tips they do not detect the target-tip distance. Therefore, 'fatal tip crash' onto the target surface is a common accident. Furthermore, without knowing the distance it is difficult to use the measured ion concentration values to obtain quantitative conclusions.

To overcome this difficulty a double-barrel configuration was proposed by Wei et al. [26, 44].

According to their suggestion one of the barrels contains the ion-selective micropipette, while the other serves for distance measurement. As has been shown the distance measuring barrel can be an amperometric gallium metal microelectrode operating in feedback mode [44] or

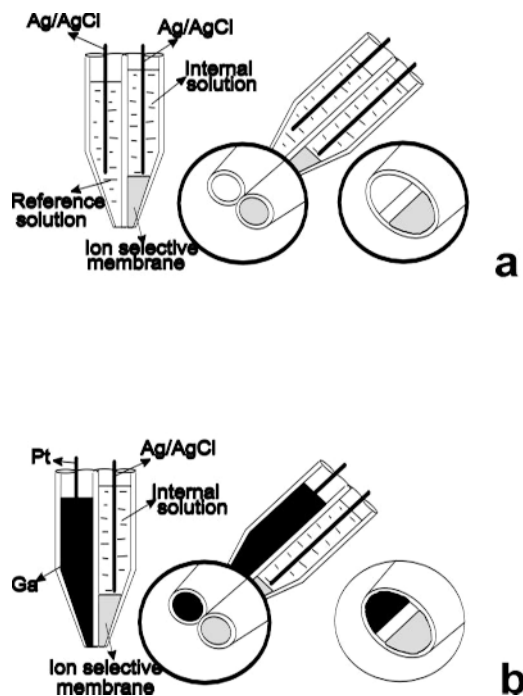


Fig. 4 Double barrel micropipette SECM tips. *a* – with open pipette distance calibrating auxiliary barrel; *b* – with amperometric Ga disc for distance estimation. For explanation see text

an open end silver/silver chloride reference barrel. The later operates in the following way: a small DC voltage is connected between the distance measuring barrel and a silver/silver chloride reference electrode placed in the cell. The current is measured. As the micropipette approaches the target surface the blockage of ion migration results in a decrease in current. For a quantitative treatment, see [26]. With a double-barrel configuration ion-selective K^+ , Zn^{2+} , NH_4^+ micropipette electrodes were prepared and used in SECM for ion concentration profile mapping. The two different type double-barrel electrodes are shown in Fig. 4.

More robust, all solid state ion-selective electrodes prepared on polypyrrole modified carbon fiber electrodes have also been used in SECM [45].

Conclusion and outlook

Some applications of SECM measurements were mentioned above. Here, without trying to be comprehensive, a few interesting tasks will be listed in which application of the technique was advantageous.

SECM can be used as a tool to obtain information on corrosion processes occurring on metal or on semiconductor surfaces. The precursor sites of corrosion can be identified, the passivating film break down can be monitored and the site of pit formation reaction can be detected [46–48]. Corrosion of biomaterials such as Hg-Ag dental amalgam crystals or Co-Cr-Mo alloy was investigated with the so-called ion release and deposition imaging mode using the SECM technique [49]. Some details about the working mechanism of corrosion type cyanide ion-selective electrodes could be obtained [43].

As was mentioned, dissolution rates of different crystals at different orientations can be measured. Spatial distribution of dissolution activity can be imaged [50, 51] and adsorption, desorption processes can be investigated.

Reactions catalyzed by immobilized enzymes have often been investigated by electrochemical microscopy. For example, Pierce et al. [52] detected immobilized glucose oxidase. Wittstock and Schuhmann [35] prepared and imaged micrometer size glucose oxidase spots with an SECM tip. The activity of immobilized enzymes could be increased locally by changing the pH on the micrometer scale [53] or the locally performed Br_2 or Cl_2 reagent production could deactivate the immobilized enzyme in close proximity [54]. An interesting observation was made by Horrocks and Mirkin [55] in the case of urease adsorbed on a gold surface with an ammonium ion-selective tip. They could reversibly deactivate/reactivate the enzyme by potential scanning of the gold substrate, that means they could turn the hydrolase enzyme on or off electrically [55]. SECM measurements were used in biosensor research to determine the optimal reaction layer thickness for enzyme or bacterial sensors [56].

Figure 5 shows the images of oxygen (Fig. 5a) and H_2O_2 (Fig. 5b) concentration profiles in 5 mM glucose solution in the vicinity of an immobilized glucose oxidase

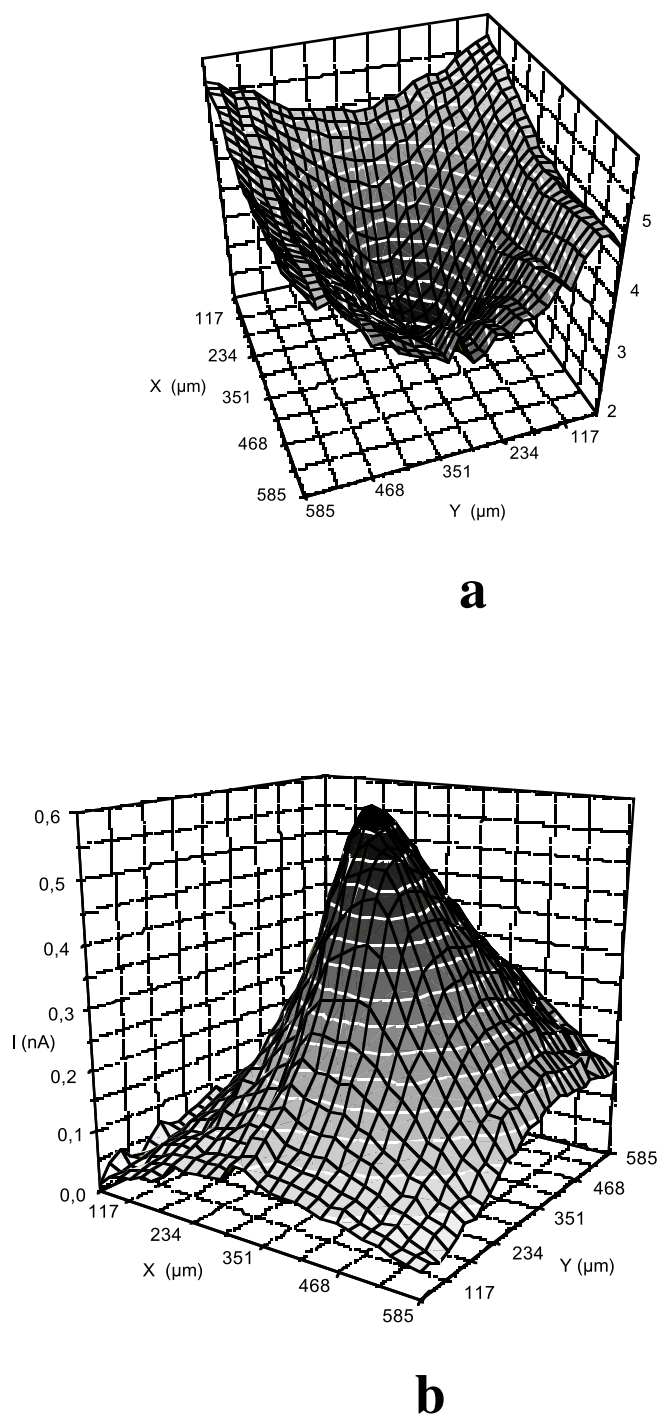


Fig. 5 SECM image of immobilized glucose oxidase containing micro disc. The measurement was made with amperometric Pt tip ($a = 5 \mu m$) at about $10 \mu m$ distance. Image *a*) shows the oxygen reduction current (tip potential $-0.6 V$), image *b*) the oxidation current of H_2O_2 (tip potential $0.6 V$). Glucose concentration 5 mM, phosphate buffer pH = 6

containing micro disc. Due to enzyme catalyzed glucose oxidation the oxygen concentration decreases and the H_2O_2 level increases locally over the active surface area. The same Pt tip polarized to $-0.6 V$ and $0.6 V$ was used for oxygen and H_2O_2 imaging, respectively.

In studying the electrochemical behavior of electrochemically prepared redox polymer films the charge balancing ion fluxes during controlled potential experiments could be monitored well with an SECM tip positioned close to the film [57–60].

SECM is an excellent tool for monitoring ionic or molecular transport through synthetic and biological porous membranes as was proved by several experiments, e.g. [61–64].

The individual contributions of diffusion and electroosmosis to the ionophoretic transport of a molecule through individual pores of a porous membrane could also be evaluated quantitatively [65].

The SECM technique was used to attempt the detection of a single molecule by electrode reaction. The idea was to make an ultra micro electrode of 10 nm diameter, and position it to the very close vicinity of a metal substrate to entrap a 10^{-18} mL solution film of 1 mM reversible electroactive species concentration. In this case statistically one single molecule is inside the film. At appropriate potential the molecule travels between the two electrodes and a current flows. If it makes the round trip fast enough, the current can be detected. Using an about 15 nm Apiezon wax-sealed Pt-Ir tip and indium-tin oxide substrate Fan and Bard [66] obtained a fluctuating current in the picoampere range. The electroactive species was in this case [trimethylammonium]-methyl ferrocene. After careful analysis of the results it could be verified that the noisy signal was the result of the electrode process of a single molecule. This illustrates the great power of the SECM technique.

The broad scale application of SECM as a new, more effective way of performing electrochemical measurements in investigating different processes and material properties is expected in the near future. It can be foreseen that the combination of the technique with other methods, such as electrochemoluminescence [67], laser spectroscopy, and quartz crystal microbalance measurements [68], will help to solve special problems.

Acknowledgement This work was supported by grant NO.OTKA T 030968.

References

- Liu HY, Fan FRF, Lin CW, Bard AJ (1986) *Chem Soc* 108: 3838–3845
- Engstrom RC, Weber M, Wunder DJ, Burgess R, Winquist S (1986) *Anal Chem* 58: 844–852
- Engstrom RC, Meaney T, Tople R, Wightman RM (1987) *Anal Chem* 59: 2005–2011
- Bard AJ, Fan FRF, Pierce DT, Unwin PR, Wipf DO, Zhou F (1991) *Science* 254: 68–74
- Bard AJ, Fan FRF, Kwak J, Lev O (1989) *Anal Chem* 61: 132–138
- Bard AJ, Fan FRF, Mirkin MV (1993) In: Bard AJ (ed) *Electroanalytical Chemistry*, Vol. 18, Marcel Dekker, New York pp 243–373
- Acra M, Bard AJ, Horrocks BR, Richards TC, Trieichel DA (1994) *Analyst* 119: 719–726
- Martin RD, Unwin PR (1998) *Anal Chem* 70: 276–284
- Mirkin MV (1999) *Mikrochim Acta* 130: 127–153
- Mirkin MV (1996) *Anal Chem* 68: 177A
- Mirkin MV, Fan FRF, Bard AJ (1992) *J Electroanal Chem* 328: 47–56
- Wipf DO, Bard AJ, Tallman DE (1996) *Anal Chem* 68: 1373–1400
- Wei C, Bard AJ, Mirkin MV (1995) *J Phys Chem* 99: 16033–16042
- Shao Y, Mirkin MV (1997) *J Electroanal Chem* 439: 137–143
- Nagy G, Moghaddam B, Oke A, Adams RN (1985) *Neurosci Lett* 55: 119–124
- Nagy G, Toth K (1993) *Bioforum* 16: 308–313
- Kolev SD, Nagy G, Pungor E (1991) *Anal Chim Acta* 254: 167–175
- Fan F-R R, Mirkin MV, Bard AJ (1994) *J Phys Chem* 98: 1475–1481
- Mirkin MV, Bard AJ (1992) *Anal Chem* 64: 2293
- Mirkin MV, Richards TC, Bard AJ (1993) *J Phys Chem* 97: 7672
- Macpherson JV, Unwin PR (1995) *J Phys Chem* 99: 3338–3351
- Macpherson JV, Unwin PR (1994) *J Phys Chem* 98: 1704–1713
- Zhou F, Unwin PF, Bard AJ (1992) *J Phys Chem* 96: 4917–4926
- Zhou F, Bard AJ (1994) *J Am Chem Soc* 116: 393–402
- Treichel DA, Mirkin MV, Bard AJ (1994) *J Phys Chem* 98: 5751–5757
- Wei C, Bard AJ, Nagy G, Tóth K (1995) *Anal Chem* 67: 1346–1352
- Unwin PR, Bard AJ (1992) *J Phys Chem* 96: 5035–5041
- Yang YF, Denault G (1996) *J Chem Soc Faraday Trans* 92: 3791–3803
- Mandler D, Bard AJ (1989) *J Electrochem Soc* 136: 3143–3155
- Mandler D, Bard AJ (1990) *J Electrochem Soc* 137: 2468–2475
- Mandler D, Bard AJ (1990) *Langmuir* 6: 1489–1497
- Tian ZW, Fen Z, Tian ZQ, Yhuo X, Mu J, Li C, Lin H, Ran B, Xie ZX, Hu W (1992) *Faraday Discuss* 94: 37–45
- Zu Y, Xie L, Mao B, Tian Z (1998) *Electrochimica Acta* 43: 1683–1690
- Caston DH, Lin CW, Bard AJ (1988) *J Electrochem Soc* 135: 785–793
- Wittstock G, Schuhmann W (1997) *Anal Chem* 69: 5059–5066
- Engstrom RC, Meane T, Tople R, Wightman RM (1987) *Anal Chem* 59: 2005–2011
- Kovács B, Csóka B, Nagy G, Kapui I, Gyurcsányi RE, Tóth K (1999) *Electroanalysis* 11: 349–355
- Slevin CJ, Gray NJ, Macpherson JV, Webb MA, Unwin PA (1999) *Electrochem Comm* 1: 282–288
- Demaille C, Brust M, Tsionsky M, Bard AJ (1997) *Anal Chem* 69: 2323–2328
- Horrocks BR, Schmidtke D, Heller A, Bard AJ (1993) *Anal Chem* 65: 3605–3617
- Denault G, Toise-Frank MH, Peter LM (1992) *Faraday Discuss Chem Soc* 94: 23–31
- Horrocks BR, Mirkin MV, Pierce DT, Bard AJ, Nagy G, Tóth K (1993) *Anal Chem* 65: 1213–1221
- Tóth K, Nagy G, Horrocks BR, Bard AJ (1993) *Anal Chim Acta* 282: 239–247
- Wei C, Bard AJ, Kapui I, Nagy G, Tóth K (1996) *Anal Chem* 68: 2651–2663
- Gyurcsányi RE, Nybäck AS, Tóth K, Nagy G, Ivaska A (1998) *Analyst* 123: 1339–1343
- Casillas N, Charlebois S, Symrl WH, White HS (1994) *J Electrochem Soc* 141: 636–641
- Casillas N, Charlebois S, Symrl WH, White HS (1993) *J Electrochem Soc* 140: 142–153
- Wipf DO (1994) *Colloids and Surfaces A* 93: 251–262
- Gilbert JI, Smith SM, Lautenschlager EP (1993) *J Biomed Mater Res* 27: 1357–1366

50. Macpherson JV, Unwin PR (1995) *Phys Chem* 98:1704–1713
51. Macpherson JV, Unwin PR (1995) *Phys Chem* 98:3338–3345
52. Pierce DT, Unwin PR, Bard AJ (1992) *Anal Chem* 64:1795–1801
53. O'Brien JC, Schumaker-Parry J, Engstrom RC (1998) *Anal Chem* 70:1307–1311
54. Shiku H, Takeda T, Yamada H, Matsue T, Uchida I (1995) *Anal Chem* 67:312–313
55. Horrocks BR, Mirkin MV (1994) *J Chem Soc Faraday Trans* 94:1115–1121
56. Hliva P, Kapui I, Czakó L, Nagy G (1998) *Magy Kém Foly* 104:223–231
57. Lee C, Anson FC (1992) *Anal Chem* 64:528–533
58. Kapui I, Gyurcsányi RE, Nagy G, Tóth K, Arca E, Arca M (1998) *J Phys Chem B* 102:9934–9939
59. Troise MHF, Denuault G (1994) *J Electroanal Chem* 379:405–411
60. Arca M, Mirkin MV, Bard AJ (1995) *J Phys Chem* 99:5040–5047
61. Scott ER, Laplaza AI, White HS, Phipps JB (1993) *Pharm Res* 10:1699–1709
62. Scott ER, Phipps JB, White HS (1995) *J Invest Dermatol* 104:142–145
63. Nuges N, Denuault DJ (1996) *J Electroanal Chem.* 408:125–140
64. Scott ER, White HS, Phipps JB (1993) *Anal Chem* 65:1537–1545
65. Bath BD, Lee RD, White HS, Scott ER (1998) *Anal Chem* 70:1047–1058
66. Fan F-R R, Bard AJ (1995) *Science* 267:871–874
67. Fan F-R R, Cliffel DE, Bard AJ (1998) *Anal Chem* 70:2941–2948
68. Cliffel DE, Bard AJ (1998) *Anal Chem* 70:1993–1998