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REVIEW

Scanning electrochemical microscopy: an analytical perspective

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Abstract Scanning electrochemical microscopy (SECM) has evolved from an electrochemical specialist tool to a broadly used electroanalytical surface technique, which has experienced exciting developments for nanoscale electrochemical studies in recent years. Several companies now offer commercial instruments, and SECM has been used in a broad range of applications. SECM research is frequently interdisciplinary, bridging areas ranging from electrochemistry, nanotechnology, and materials science to biomedical research. Although SECM is considered a modern electroanalytical technique, it appears that less attention is paid to so-called analytical figures of merit, which are essential also in electroanalytical chemistry. Besides instrumental developments, this review focuses on aspects such as reliability, repeatability, and reproducibility of SECM data. The review is intended to spark discussion within the community on this topic, but also to raise awareness of the challenges faced during the evaluation of quantitative SECM data.

Keywords Scanning electrochemical microscopy · Nanoelectrodes · Analytical figures of merit · Method validation

Introduction

Electroanalytical chemistry is a vital and relevant area of analytical chemistry, and has experienced a multitude of novel developments leading to new applications and advancements in electroanalysis based on advanced electrode materials, coupling of analytical techniques with electrochemical methods, and use of microelectrodes and nanoelectrodes. Thus, measurements in extremely small volumes and confined spaces with high temporal and spatial resolution are made possible along with the opportunity to perform high-resolution electrochemical mapping (i.e., electrochemical imaging) experiments. In particular, electrochemical imaging has significantly advanced in recent years, and is now applied in multidisciplinary research areas ranging from biomedical research to materials science, corrosion, catalysis, and energy-related topics such as fuel cells and battery research. Scanning electrochemical microscopy (SECM) [1, 2] and derived "hyphenated" electrochemical scanning probe techniques [3–8] now allow electrochemical/(electro)analytical measurements with nanoscopic and microscopic electrodes or nanopipettes. Positioning such probes in close proximity to the investigated sample surface allows high-resolution information on electrochemical processes or ion fluxes occurring at the solid–liquid and liquid–liquid interface to be obtained. Besides the combination with other scanning probe microscopy (SPM) techniques, SECM has also been coupled with spectroscopic techniques (i.e., surface plasmon resonance, Raman, and IR) [9–11], mass spectrometry [12], or an electrochemical quartz microbalance [13] to list just a few examples. The research activities in SECM are well documented by the breadth of original contributions and a steadily increasing number of review articles ranging from comprehensive overviews of the current state of the art to focused articles on specific application areas such as bioapplications, corrosion research, and energy-related applications [14–25]. SECM is considered an electroanalytical technique, as most classical electroanalytical techniques, including stripping voltammetry at mercury-coated microelectrodes [26–28], potentiometric

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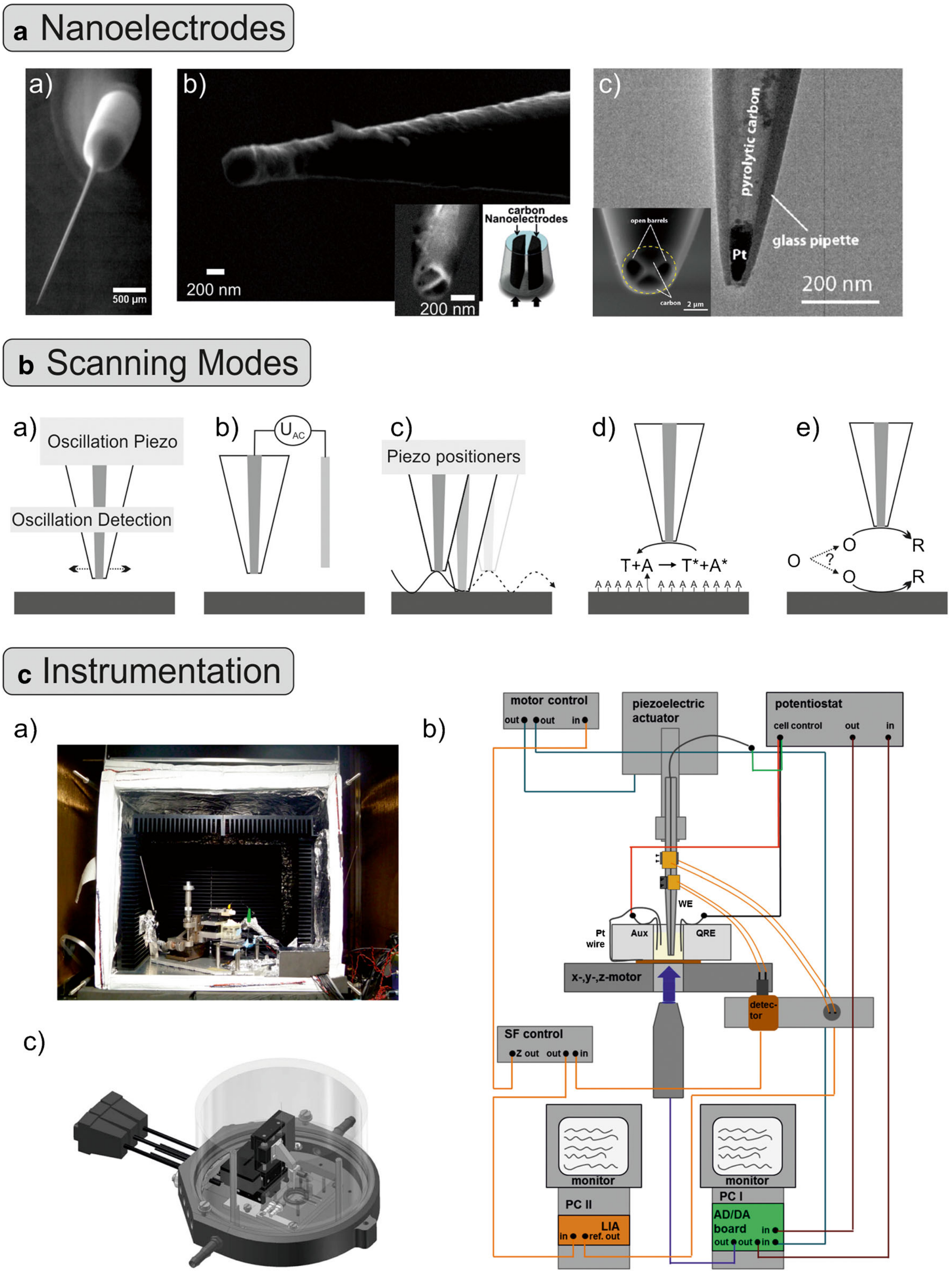
measurements [29–33], square wave voltammetry [34, 35], and electrochemical impedance measurements [13, 36, 37], have been demonstrated in SECM studies. Consequently, this technique is extremely useful in modern electroanalysis for obtaining quantitative data on specific analytes or processes.

Given the number of reviews already published on SECM, the present review is intended to give a critical perspective on SECM in terms of analytical figures of merit in the field of (electro)analytical chemistry highlighting in particular research from recent years. The International Union of Pure and Applied Chemistry (IUPAC), Analytical Chemistry Division (V) has given the following definition: “Analytical chemistry (which includes electroanalytical chemistry) is a scientific discipline that develops and applies methods, instruments, and strategies to obtain information on the composition and nature of matter in space and time, as well as on the value of these measurements, i.e., their uncertainty, validation, and/or traceability to fundamental standards.”

The first part of the definition is clearly covered within the field of SECM. Over the years a significant number of original contributions have been published on instrumental developments and improvements. Such developments can be categorized into (1) developments regarding improved hardware, positioning of the SECM probe, and imaging modalities, (2) controlling physical parameters such as temperature and surrounding atmosphere, which was initially shown for biological investigations, where fixed CO_2 content or oxygen-reduced atmospheres [38] play an important role, and (3) developments targeting reproducible fabrication schemes and characterization routines for SECM nanoelectrode probes and pipette-based electrodes, which certainly have a huge impact on the analytical figures of merit. Figure 1 highlights several examples of such recent improvements. Figure 1a illustrates several examples of nano-sized probes. Laser-assisted methods have been exploited for the fabrication of nano disk electrodes (Fig. 1a, panel a). Alternatively, instead of the use of solid metal or carbon, microelecrodes or nanoelectrodes can be based on micropipettes or nanopipettes known from scanning ion conductance microscopy (SICM) [46]. Novel concepts for performing electrochemical scanning probe experiments have been introduced; for example, small electrodes were also implemented into atomic force microscopy (AFM) probes [5, 6, 47, 48]. Exemplary nanopipette-based probes are illustrated in Fig. 1a, panels b and c. Among other groups [3, 49–51] and collaborative efforts between the groups of Matsue, Korchev [4], and Unwin [52], Unwin and coworkers made a significant contribution called “pipette-based” electrochemical SPM [42, 53–55], introducing imaging methods such as the scanning micropipette contact method [56], whereby a localized electrochemical cell is formed because of attractive capillary forces between the meniscus and the sample surface. Subsequently, they introduced a technique termed “scanning electrochemical cell microscopy” (SECCM) [57, 58] using double-barrel pipettes (i.e., theta capillaries). Both barrels are filled with electrolyte solution, and each barrel contains a reference/counter electrode (i.e., quasi-reference counter electrode). Again the meniscus forms a localized droplet cell when the pipette is near the sample surface. Ions migrate across the meniscus between the two barrels if a potential is applied between the electrodes. By addition of an oscillation of the pipette similar to the operation mode in SICM, an alternating current component of the ionic current is periodically altered in strong dependence on the meniscus height, which is used for distance control. Because of sophisticated hardware (i.e., electronics) and alternative scanning approaches such as spiral scanning, SECCM has been developed into a multifunctional electrochemical scanning probe technique, which even allows video-rate electrochemical imaging with high lateral resolution [41].

Fig. 1 Recent developments in scanning electrochemical microscopy ▶ (SECM). **a** Nanoelectrodes: pulled quartz pipette nanoelectrodes (a), dual carbon nanoelectrodes (b; the inset shows a magnification of the electrode tip and a schematic drawing), and a platinum-filled nanopipette probe (c; the inset shows a quad-barrel probe with two carbon-filled barrels). **b** Imaging modes. Modes for probe positioning: shear-force SECM (a), alternating current SECM (b), and intermittent contact SECM (c). Imaging modalities: surface interrogation SECM (d) and redox-competition SECM (e). **c** Instrumentation: isothermal chamber using vacuum insulated panels (white parts) and extruded aluminum heat sinks (black) for effective drift compensation (a), photoelectrochemical shear-force-based SECM setup with illumination from the bottom (b), and SECM head (positioning system, tip holder, and electrochemical cell) placed under a custom-made Plexiglas bell, which is in an argon-filled glove box along with the controller (temperature is measured with a thermometer inside the chamber) (c). (**a** Reprinted with permission from [39] (a), [40] copyright 2016 American Chemical Society (b), and [41] copyright 2016 American Chemical Society and [42] copyright 2015 American Chemical Society (c). **c** Reprinted with permission from [43] copyright 2012 American Chemical Society (a), [44] (b), and [45] (c))

Beyond efforts toward nanoscale probes, imaging modes allowing improved positioning of the SECM probe such as shear-force mode, alternating current mode [59], and intermittent contact mode [60] have been introduced. Panels a–c in Fig. 1b illustrate these modes. Briefly, they involve the lateral oscillation of the SECM probe at an amplitude dependent on the proximity of the surface (Fig. 1b, panel a), the application of alternate potential perturbation to the probe at varying frequency so that the acquired alternating current provides information on different surface features (Fig. 1b, panel b), and the vertical oscillation of the probe, sensing the presence of the surface, which allows a constant probe–sample distance to be maintained by use of the signal as input for a feedback loop (Fig. 1b, panel c). Likewise, imaging modalities providing enhanced information, including surface interrogation mode [surface interrogation SECM (SI-SECM)] [61] (seen in Fig. 1b, panel d), and redox-competition mode (redox-competition SECM (RC-SECM)] [62] (depicted in Fig. 1b, panel e), have been developed, further improved, and applied to various



emerging analytical problems. RC-SECM evaluates the extent to which the probe and substrate compete for the electrochemical conversion of the same redox molecule. SI-SECM involves the surface modification (e.g., by UV illumination) inducing adsorbed species, which are next electrochemically interrogated with appropriate reactants to evaluate the resulting changes in surface chemical composition. Capillary-based reference electrodes have been demonstrated for detection of local current densities based on an ohmic measurement principle termed "scanning ohmic microscopy"

[63, 64] providing information on adsorption and intercalation reactions with micrometer resolution; these are difficult to map via conventional SECM.

Whereas during the first decades of SECM, with few exceptions [65], SECM investigations focused on studying processes involving macroscopic and microscopic samples using solid microelectrodes, a remarkable trend is currently being experienced toward nanoscale studies. In recent years, studies on the electroactive properties of nano-objects such as nanoparticles down to the single nanoparticle level have emerged, which is clearly correlated with instrumental improvements and advancements in probe fabrication at nanoscopic dimensions. Given the fact that in SECM experiments the dimensions of the probe are strongly related to the achievable spatial resolution, such interest in nano-objects must be accompanied by appropriate fabrication procedures. Hence, reliable fabrication schemes allowing a more reproducible production of nanoelectrodes with well-defined shape and size, along with establishment of characterization routines for nano-sized electrodes [66–68], have been important steps toward improving the achievable spatial resolution down to the single particle level. Besides etching procedures and laser-pipette-puller-based fabrication of nanoelectrodes, pulling glass capillaries with orifices down to a diameter of 50 nm followed by postmodification steps for implementation of an electrode either as a ring (i.e., by sputtering a metal layer) or as a second barrel filled with carbonized material has been introduced. In terms of characterization, high-resolution scanning electron microscopy and in particular transmission electron microscopy [66] have improved the determination of the shape and size of the nanoelectrodes obtained. Surface modification of such nanoelectrodes by electrochemical deposition processes (e.g., platinum deposition) or modification with a mercury film [69] is required to increase the electron transfer kinetics if species beyond ferrocenemethanol should be detected. In addition, only few measurements with nano-sized probes have been performed in ”constant height mode” [70–73]; that is, where the probe is scanned at a fixed distance across the sample surface.

Hence, a distance control providing independent electrochemical signals was a major step toward deconvoluting electrochemical and topographical information, as the sample morphology can be recorded independently but simultaneously with the electrochemical information. Shear-force-based SECM was introduced fairly early as a current independent mode, and it has developed into a routine distance-controlled imaging mode applied by many researchers in the field. “Hyphenated” scanning probe techniques such as SICM–SECM and AFM–SECM have gained popularity, as SICM–SECM probes can be fabricated fairly easily in any laboratory environment. In respect to AFM–SECM, SECM is now available as a commercial add-on to AFM instrumentation [74].

In terms of instrumental developments and improvements, recent efforts were directed to ensure stable environmental conditions, which may have a significant impact on the establishment of SECM as an analytically validated method. For instance, surrounding temperature control is achieved by placing the SECM system in an isothermal chamber, avoiding thermal drifts of the positioning system, which is crucial for distance control for nanometer probes [43]. The presence of vacuum insulated panels and extruded aluminum heat sinks (white and black as seen in Fig. 1c, panel a) has been implemented into the SECM setup to ensure high thermal stability in the system. In addition, controlling or varying the sample temperature (e.g., by a Peltier-heated sample stage [75]) may enhance the electrochemical response. A well-controlled surrounding atmosphere is not only crucial in biomedical research, but is a prerequisite, for example, in battery research, where SECM experiments typically have to be performed in a glove box [76, 77]. Also, adding controlled illumination of the sample within the SECM setup has gained attention, in particular for the study of photocatalytic materials and their performance *in operando* [44, 78, 79]. One achieves this either by illuminating the sample, supported in transparent materials, from below (as seen in Fig. 1c, panel b) [44, 78] or by guiding the excitation light through the glass material of the ultramicroelectrode (UME) itself [79]. Recent efforts toward atmospheric stability in battery research by Wittstock’s group [45] have resulted in the custom-designed Ar-filled setup shown in Fig. 1c, panel c, including ports for the necessary electrical connections at the rear of the setup, and Ar inlet and outlet at the sides (see Fig. 1c, panel c). Recently, Bard and coworkers [73] published improvements in SECM instrumentation suitable specifically for nanoscale studies.

In 1992, Bard [80] described the emerging challenges in electroanalysis and electrochemistry that will overcome some of the existing limits such as time, space, potential range, temperature, and pressure. Twenty-five years later, some of these limitations have been clearly pushed beyond what was feasible at that time by the introduction of nanoelectrodes, sophisticated imaging modalities, and improved instrumentation. Despite these advances, the remaining challenges are predominantly related to the reliability and achievable analytical figures of merit (Fig. 2) of SECM-based electroanalytical measurements. In the following, we focus on challenges related to uncertainty, validation, and/or traceability in SECM studies, and discuss the challenges along with a few examples highlighting some of these aspects, selected for their illustrative ability.

Method validation

Electroanalytical methods require addressing and reporting of the analytical figures of merit schematically shown in Fig. 2. In general, maximum comprehensiveness of validation includes parameters such as trueness, precision, reproducibility,

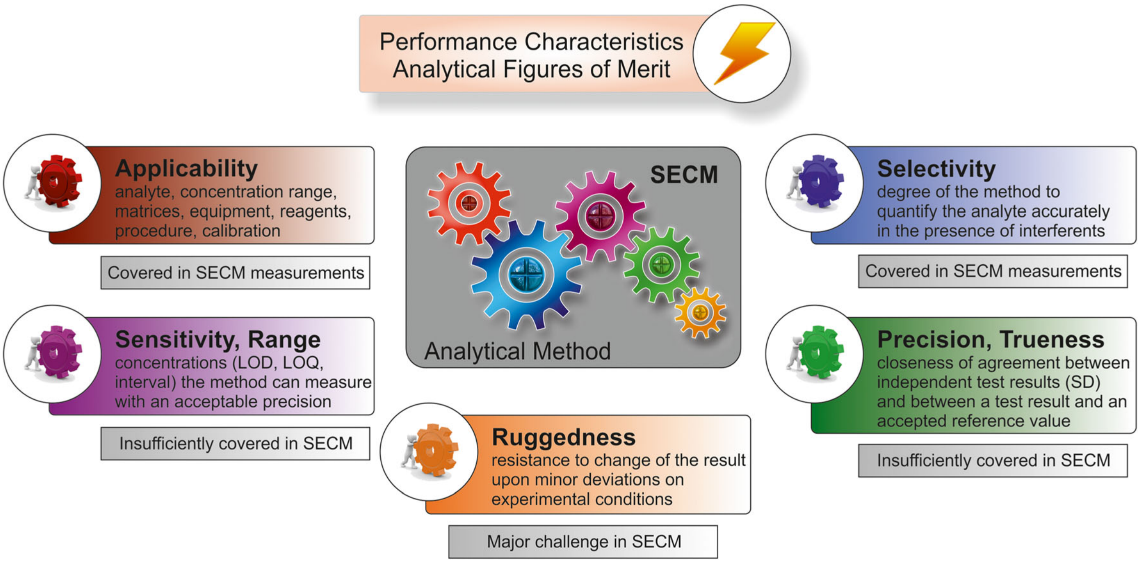


Fig. 2 Representation of the analytical figures of merit according to IUPAC [81]. Information, whether these figures of merits are addressed or pending for scanning electrochemical microscopy (SECM) is included. LOD limit of detection, LOQ limit of quantification, SD standard deviation

linearity, recovery rate, selectivity, robustness, limit of detection, and limit of quantification. However, what is actually needed for qualifying a measurement is strongly dependent on the purpose of the analysis. As those are general requirements for an analytical method to be validated, they eventually have to be standardized if SECM is to be considered in routine analysis. In the current section, the present state of and future prospects for the implementation of such figures of merits in SECM studies will be discussed according to the scope and limitations of the technique and its applications.

Challenges in method validation for SECM measurements

Validation of a given analytical method is built around the figures of merits displayed in Fig. 2. Moreover, in routine analysis, measurements and procedures are defined by national and international regulations, quality control procedures, proficiency tests, and standardization by defined international standards such as ISO/IEC 17025 [81]. In SPM such as near-field scanning optical microscopy, scanning tunneling microscopy, or AFM, ISO norms have been established for calibrating the probe and the "standardized" use of the technique [82]; interestingly, no standards have been conceived for SECM to date. Protocols for the analysis of surface geometric quantities [83] or experimental uncertainties such as drift [84] have been standardized for the SPM techniques mentioned above, but they have not yet been extended to SECM. This is of particular relevance, as SECM is likewise capable of determining geometric features, which are eventually affected by drift. ISO norms in SPM regulate the calibration and use of SPM in nanotechnology, whereas SECM was probably not included at that time, achieving mostly spatial resolutions in the micrometer range. Nowadays, SECM achieves resolution comparable to, for example, that achieved by near-field scanning optical microscopy using nano-sized electrodes and nanopipettes. Even SPM techniques mapping local electric currents have been validated and standardized in terms of usage and lateral resolution [82, 85]; however, again this procedure has not been extended to local electrochemical measurements yet. Given the broad range of applications and the different modes of operation using individually fabricated SECM probes, it is evident that these requirements are essential for a more widespread routine use of SECM.

Concomitantly, the number of companies now offering commercial SECM equipment, given in Table 1, has increased in the last few decades along with the widespread development of custom-built systems, including hybrid microscopes. It seems clear that the establishment of standardized equipment would be an important step toward the development of validated protocols.

The establishment of validated analytical methods for the calibration of the SECM response and its operation in quantitative analysis would be a prerequisite for the technique to be standardized. Analytical validation includes the entire analytical procedure from sampling to publication of the results, as well as reporting the associated uncertainties. For conventional (electro)analytical methods, such as those aiming to quantify a given analyte in a liquid sample or a certain solid material, validation is well established, and the certainty of the results must be clearly reported for the results to be accepted. However, for a wide range of feasible surface and interface analyses using SECM, no unified procedure has been conceived for data evaluation within uncertainty intervals. In many cases, simulations have been invoked to effectively

Table 1 Companies that offer commercial scanning electrochemical microscopy (SECM) systems

Company	Available systems/modes
Ametek Inc. (Berwyn, PA, USA)	SECM, AC-SECM, constant distance SECM (they also offer soft stylus probes)
BioLogic Science Instruments (Seyssinet-Pariset, France)	SECM, AC-SECM, intermittent contact SECM
Bruker (Billerica, MA, USA)	PeakForce Tapping AFM with SECM module
CH Instruments Inc. (Houston, TX, USA)	SECM
Keysight Technologies (Santa Rosa, CA, USA)	AFM system with SECM module
Heka Electronik GmbH (Lambrecht/Pfalz, Germany)	SECM, SICM, SECCM, SPECM, shear-force SECM
Sensolytics GmbH (Bochum, Germany)	SECM, AC-SECM, shear-force SECM

AC alternating current, *AFM* atomic force microscope, *SECCM* scanning electrochemical cell microscopy, *SICM* scanning ion conductance microscopy, *SPECM* scanning photoelectrochemical microscopy

support conclusions obtained by SECM experiments, although this does not entail clarity for the experimental uncertainties.

In terms of SECM, an additional inherent problem is that a method validated for a specific analyte that is present in a certain matrix or at a surface in a given concentration range still may not be considered valid if the system or the matrix changes. This may give rise to substantial variations in experimental conditions and parameters, introducing additional error sources [81]. If such a prerequisite were strictly applied for SECM electroanalysis, critical limitations may arise because of the broad range of applications and the diversity of samples investigated, further complicating the endeavor of validation and standardization of SECM-based analytical methods. To the best of our knowledge, no SECM-based electroanalytical procedure has been completely validated to date establishing generic analytical figures of merit. This may be related to the fact that SECM has to date found little application in routine analysis compared with other analytical techniques; however, a significant number of publications suggest that future applications of SECM may include routine analysis.

Analytical figures of merit for SECM can be summarized as follows according to [81]:

- Applicability range for the measurable magnitude related to the probed species (i.e., concentration, generation rate, amount of immobilized molecule or entity, etc.) or the probed property at the surface or interface (i.e., electron transfer kinetics, permeability, catalytic activity, etc.)
- Applicability of the experimental conditions, in terms of the matrix (i.e., kind of surface or interface, nature of the immobilizing scaffold, etc.), electrolyte, redox mediators, etc.
- Suitable calibration routines for SECM probes, and the response toward the detected species or property within the applicability range, which should be validated with the analysis of residuals errors rather than the linear regression coefficient
- Establishing the limits of detection and quantification from calibration procedures for a specific target analyte
- Sufficient selectivity of the response ensuring that the information obtained and conclusions are unambiguous, and reflecting a clear distinction of different parameters affecting the SECM probe response
- Trueness and precision of the result obtained within uncertainty ranges of the quantified values, and traceability during the acquisition of randomly acquired replicates ensuring that no influence of systematic experimental conditions occurs
- Robustness of the results if deviations from the experimental conditions occur

Evidently, some of the requirements listed may not be immediately applicable or feasible for SECM measurements, in particular when complex systems such as biological/biomedical samples are being studied, but others directly or indirectly related to the validation procedure are achievable or they have already been established. For example, some limitations and uncertainties of the SECM response, and in particular the influence of the surface morphology on the electrochemical activity, have been addressed via distance-controlled imaging approaches, thereby minimizing or eliminating topographical features affecting the electrochemical signal. Importantly, since the main interest in quantitative determination is ensuring the accuracy and reliability of the results obtained, estimating uncertainties is frequently regarded as the crucial step during the validation of (electro)analytical procedures [81]. As a result, most efforts toward reporting reliable analytical information extracted from SECM measurements have attempted to satisfy this demand, as highlighted by examples summarized in "Advances in validating SECM methods."

To date, other aspects of the analytical validation procedure have been only sparsely reported in the SECM literature. For instance, the definition of the applicability ranges and conditions appears a rather challenging task considering the multitude of application scenarios where SECM measurements have been performed. In addition, analytical reports rigorously providing calibration parameters, including uncertainties and analysis of residual errors, are, to the best of our knowledge, not commonly found in the SECM literature. Besides, very few reports provide accurate information on the limits of detection and quantification achieved. Ultimately, given the ability of SECM to characterize physicochemical surface phenomena, it may be accepted that calibration routines for the system response are not always required. Instead, physicochemical models have been typically applied to evaluate the probe response (e.g., the determination of electron transfer kinetic constants using data from amperometric probe approach curves). These parameters may in fact be considered analytically acceptable providing that the extracted information can be traced back to the uncertainty of the response.

The assessment of the robustness is probably the most challenging task during the validation of SECM methods. In general, to address this issue for a given (electro)analytical method, a minimum number of laboratories are required to perform the same measurements under equivalent conditions and compare their results (i.e., comparable to international collaborative ring trials or round robin tests). Such test measurements would require that the measurements be executed using comparable equipment (e.g., the achievable accuracy and step size of positioners may differ significantly), by different staff members, and in different laboratory environments. Most commercialized or standardized analytical techniques fulfill this requirement, whereas the robustness of SECM-based methods may be significantly underestimated. In SECM, it is common practice to use custom-built SECM systems and SECM probes/electrodes, which may differ because of different fabrication and polishing procedures [86]. Recently, several SECM reports on the use of carbon-based materials such as graphene [87, 88] or nanoparticles [89, 90] appeared that spiked significant interest, and may serve as suitable examples for validating the robustness of SECM if several groups would participate in such interlaboratory comparison studies.

A significant challenge during interlaboratory validation of SECM methods is the diversity of systems responding to similar physicochemical principles. This is illustrated by the determination of apparent electron transfer kinetic constants using feedback-mode SECM. To extract this information from so-called approach curves (i.e., current–distance curves), the resulting SECM current response is modeled with respect to the kinetic constant of the electron transfer between the sample and the redox mediator with respect to the probe/electrode geometry [91]. These generic models have been exploited in several fields, arising as powerful analytical procedures to determine electrochemical surface properties within their uncertainty ranges; for example, for self-assembled monolayers (SAMs) [92–94], liquid–liquid interfaces [95, 96], conductive materials [97], and photoinduced electron transfer phenomena [96, 98–100].

Similarly, electroanalytical methods to determine the permeability of membranes and films via their influence on the feedback response during SECM measurements have been conceived for surfaces of different origin ranging from deposited films on conductive materials [101, 102] to lipid layers and cell membranes [103, 104]. Elegant studies have recently been reported that generically model and simulate the tip-current response for such complex porous electroactive systems [104, 105], thereby facilitating the future establishment of validated methods.

An alternative strategy versus interlaboratory validation may be the establishment of certified reference materials (CRMs), which is well established for the general validation of analytical methods. To date, there are no CRMs existing or reported for SECM. Thus, only materials or electroactive species (i.e., typically, outer sphere redox species) for which the SECM response is well "accepted" yet with an associated uncertainty have been used as a quasi-reference for estimating the reliability in SECM. For example, the characterization of SECM probes/electrodes using cyclic voltammetry in solution containing a known concentration of an outer sphere redox mediator of known diffusion coefficient is performed in a given electrolyte, which is a well-established procedure to determine the quality and dimension of an SECM probe [106]. Thereby, ideal and clean electrode surfaces are usually assumed, which may sometimes be erroneous [107]. The electrochemical response of SECM probes on an electroactive material behaving in a well-characterized fashion (e.g., platinum electrodes when cycled in sulfuric acid solution) may be complementarily considered for probe characterization in the absence of appropriate redox species [108]. Also, the positive and negative feedback behavior at well characterized solid–liquid interfaces may be used to derive the probe geometry and determine the effective RG value (i.e., the ratio between the radius of a conductive micro disk electrode and the radius of the surrounding insulation material) [109].

Similarly, reference materials that concomitantly consume the analyte probed at the UME would facilitate the validation of SECM methods based on RC-SECM as long as variations in the environment and matrix do not significantly alter the probe response. This is particularly challenging during the investigation of, for example, living specimens, whose respiratory activity has been analytically and statistically investigated by quantification of the depletion of the oxygen reduction reaction current at a UME tip, as oxygen is consumed in the respiration cycle. Despite the demonstrated potential and interest in studying the life cycle of cells [110–112] and embryos [113–115], no suitable CRM emulating such systems has been reported to date. Indeed, it is difficult for most biological samples to be mimicked by or be studied with use of CRMs, for example, for the analysis of species released from stimulated cells. Such analyses can be performed only by comparing the tip-current response toward the redox species, which are expected to be released, first in bulk solution, and then near the biological target [116, 117].

It is accepted that the robustness of an analytical method may be alternatively addressed by "spiking–recovery" strategies based on the addition of a known amount of well-defined tracer analyte to the matrix at an early stage of the analytical process [118]. This strategy is particularly suitable for the determination of analytes in complex bulk samples; however, a similar strategy for surface analysis does not appear straightforward. Nonetheless, it may be conceived practical if, for example, known amounts of an analyte of interest are immobilized on a surface—with or without controlled release function—when the surface electrochemical response is being mapped. Indeed, this immobilization analysis procedure has been demonstrated to be suitable for the determination of enzyme activities; for example, β-galactosidase present in biofilms [119] or cytochrome *c* immobilized on SAMs [120]. Even biomolecules without native redox activity may be investigated via labeling with redox-active moieties such as *p*-aminophenol [121–123] or benzoquinone [124, 125]. The resulting redox-active molecules are electrochemically addressed at the probe, and the current determined is translated into a final concentration of the analyte with the corresponding uncertainties.

Hence, although it may be stated that SECM allows the acquisition of qualitative and eventually quantitative information, the latter may not be considered analytically validated. Therefore, mainly general trends on the behavior of the sample are extracted when the experimental conditions are varied. This appears to sufficiently satisfy the demands of most analytical questions addressed via SECM. To the best of our knowledge, in most contributions where quantitative data have been reported, including the corresponding uncertainties, the SECM measurements were conducted with the UME placed stationary close to the sample surface or by recording approach curves. This strategy significantly reduces the number of parameters one may statistically evaluate (e.g., maximum current, applied potential, and electron transfer kinetic constant.) in comparison with imaging experiments, where morphology and distribution of the electroactive sites at the sample surface play a major role during the localized response. Indeed, analytical evaluation of data obtained via 2-D SECM measurements would generally require tests of significance. Such statistical tests are scarcely reported in the SECM literature; for example, during the investigation of the morphology of living cells [126], the correlation of morphology and surface activity of CoCrMo alloys [127], the correlation of intracellular and respiratory activity of cells [112], or the distinction between cancerous and healthy cells [128]. Yet, a universally accepted method is still missing for the investigation of local activity, in particular investigations involving dynamic phenomena, where evolution is monitored by SECM. Conversely, it is more frequent that several maximum values acquired during 2-D scans are averaged and evaluated (i.e., discarding the spatial resolution of the information obtained); for example, during the analysis of the maximum performance of photocatalysts [129–131]. This is particularly remarkable since the ability to collect information from a representative cross-section or area via 2-D electrochemical analysis is regarded a major and nearly exclusive advantage of SECM. In comparison, AFM data derived from 2-D scans can be evaluated in terms of roughness parameters, and histograms can be obtained from height distributions or feature distributions (e.g., particle sizes) as valid information after appropriate statistical analysis. It appears only logical that a future focus in SECM should include efforts toward statistically validated and sound electrochemical information, which in turn may further facilitate the application of SECM-based methods as a routine electroanalytical technique.

Again, we would like to point out that the difficulty in applying the analytical figures of merits for SECM is also because they may not be universally applicable as for other electroanalytical techniques. SECM studies dealing, for example, with approach curves or SI-SECM share the problems with interfacial analysis but not with imaging techniques. Hence, it is particularly difficult to come up with universally accepted strategies for handling and quantifying uncertainties in SECM measurements. This problem may be further concealed by the fact that SECM measurements can be related to continuum simulation of reactivity-transport problems. Comparison with such simulation is important to establish imaging modes (trueness) but has little value in dealing with uncertainties as is common in concentration determination.

Advances in validating SECM methods

Although to date no fully validated SECM methods according to the criteria defined by IUPAC have been reported [81], this section reviews SECM research where at least certain aspects, such as reliability and repeatability, were investigated, providing, for example, appropriate uncertainty intervals. The acquisition of repetitive measurements and the clarification of the number of replicate measurements performed is a prerequisite when precision and reliability data are being reported. There are indeed a significant number of SECM articles with reported uncertainty intervals but without information on how many replicates were made. Hence, the traceability of the results is compromised by incomplete statistical information provided. In addition, one may quantitatively estimate analytical parameters from 2-D scans by averaging all the data points acquired across a defined region of interest provided that the surface heterogeneity is of minor influence and the system investigated does not significantly change with time during the scan acquisition. The traceability of the results is still limited, since this ultimately reflects a single measurement.

A different situation may be conceived when one is analyzing SECM data obtained from stochastically behaving systems. Since the random nature of such phenomena demands statistical analysis of the data obtained even within a single experiment be performed, the resulting conclusions may be accepted as analytically valid in spite of the single measurement. Exemplarily, the characterization of the frequency and mean parameters of the arising events has permitted the analysis of membrane porosity data when membranes are blocked by individual particles, thereby reducing the current at the SECM probe sensing species diffusing through the pores [132]. Similarly, investigation of the diffusion-controlled events resulting from collisions of metal oxide nanoparticles in the small gap between the SECM probe and a conductive or insulating surface [133] and investigation of the behavior and collisions of metal nanoparticles in small cell volumes by SECCM [134] have been demonstrated.

For analytical methods providing quantitative information, calibration routines or the application of theoretical models is required. In the simplest case, the distinction of the registered SECM current response obtained at two well-defined states of a system may readily report the desired answer as a "yes/no" decision. An example is the distinction between healthy and tumorous cells, which is usually accompanied by a statistical analysis of significance (e.g., Student's *t* test) [128]. Herein, we focus on examples where the SECM response has been converted into quantitative data, or at least discussed in quantitative terms. Notwithstanding, it should be noted that there are a breadth of SECM studies and measurements that provide valuable information without using statistics, which is outside the scope of the present review.

Given the direct correlation of the measured probe current to the redox-active species at a surface or in solution and its mass transfer in SECM experiments, the data collected can be used for the electroanalytical determination and quantification of concentration or diffusion coefficients. This can be readily applied in RC-SECM or generation–collection (GC) SECM, provided that no additional feedback response occurs. Indeed, diffusion coefficients in several media have been determined with UMEs with the probe placed a few micrometers from the substrate behaving as the source of the redox species [135, 136].

RC-SECM or GC-SECM experiments have also been used for the determination of concentrations to derive a rate or flux of redox-active molecules of (bio)analytical interest. For example, the catalase activity of *Vibrio fischeri (Gammaprotoebacteria*, *Vibrionaceae*) was estimated via hydrogen peroxide concentration profiles [119]. For biomolecules that do not exhibit reaction paths involving redox-active species, SECM characterization is commonly conducted with an immobilization and labeling strategy. Typically, the sample investigated is labeled with enzymes catalyzing a reaction forming a redox-active molecule, which can then be detected at the SECM probe. Commonly applied labeling enzymes providing analytically reliable data include ß-galactosidase catalyzing the conversion of *p*-aminophenyl-ß-D-galactopyranoside to *p*-aminophenol [121–123], and horseradish peroxidase catalyzing the oxidation of hydroquinone to benzoquinone [124, 125]. The quantitative estimation of the fluxes of the generated species allows the qualitative evaluation of cells in terms of senescence (i.e., aging), which was statistically evaluated by a Student's *t* test in terms of the level of significance [123]. Yet, the major interest in such measurements is related to the actual quantification of the amount of immobilized biomolecules [122, 124, 125]. From an analytical perspective, it is of interest to take advantage of the linear relation between the maximum probe current and the amount of immobilized biomolecules, thus allowing calibration procedures including the linear range of the method, the limit of detection, and the limit of quantification [124, 125].

For example, Fig. 3a shows the linear correlation between the amount of an immobilized cancer-related antigen labeled with horseradish peroxidase and the maximum SECM current response recorded via linear scans [124]. Fig. 3b provides the associated calibration function for similarly labeled immobilized DNA obtained by correlation of the maximum current recorded during probe approach [125].

RC-SECM has been applied to determine the oxygen content and concentration gradients in oxygen-consuming samples in materials science. For instance, SECM was used for the rapid optimization of O_2-electroreduction catalysts assembled as surface spots of different composition [137], and for monitoring the spontaneous formation of metal oxide films by quantitative analysis of the oxygen depletion with respect to a fully aerated electrolyte [138]. Estimation of the oxygen consumption of a living specimen is another relevant application area of SECM. These studies have allowed the characterization of the respiratory activity of living cells [110–112, 139–141] and embryos [113–115]. Possibly because of the variability that such living entities commonly exhibit, particular attention has been paid to the reliability of the results via statistical analysis, including the reporting of uncertainty intervals. The most common noninvasive strategy involves the estimation of the vertical profile of the oxygen reduction reaction current proportional to the oxygen concentration between bulk solution and a sample surface. From the data, the concentration gradient and the respiratory activity can be estimated according to spherical diffusion theories. For such studies, usually repetitive measurements for the estimation of the uncertainty intervals are reported and statistical analysis, including, for example, *t* tests quantifying the significance of the data obtained, is performed.

As an example, Sugimura et al. [115] investigated the respiratory activity of in vitro fertilized porcine and somatic cell nuclear transfer embryos via the annotated analysis of variance, and tests of significance ($p < 0.05$) during data evaluation. This resulted in the box-and-whisker diagram reproduced in Fig. 4 for the oxygen consumption at different growth stages of the embryos after implantation.

The current response during approach can be evaluated by appropriate models to obtain apparent heterogeneous electron transfer constants for a given redox mediator. Such information was used to distinguish cell states from biopsy specimens, but was also applied for other biomolecules. For example, the

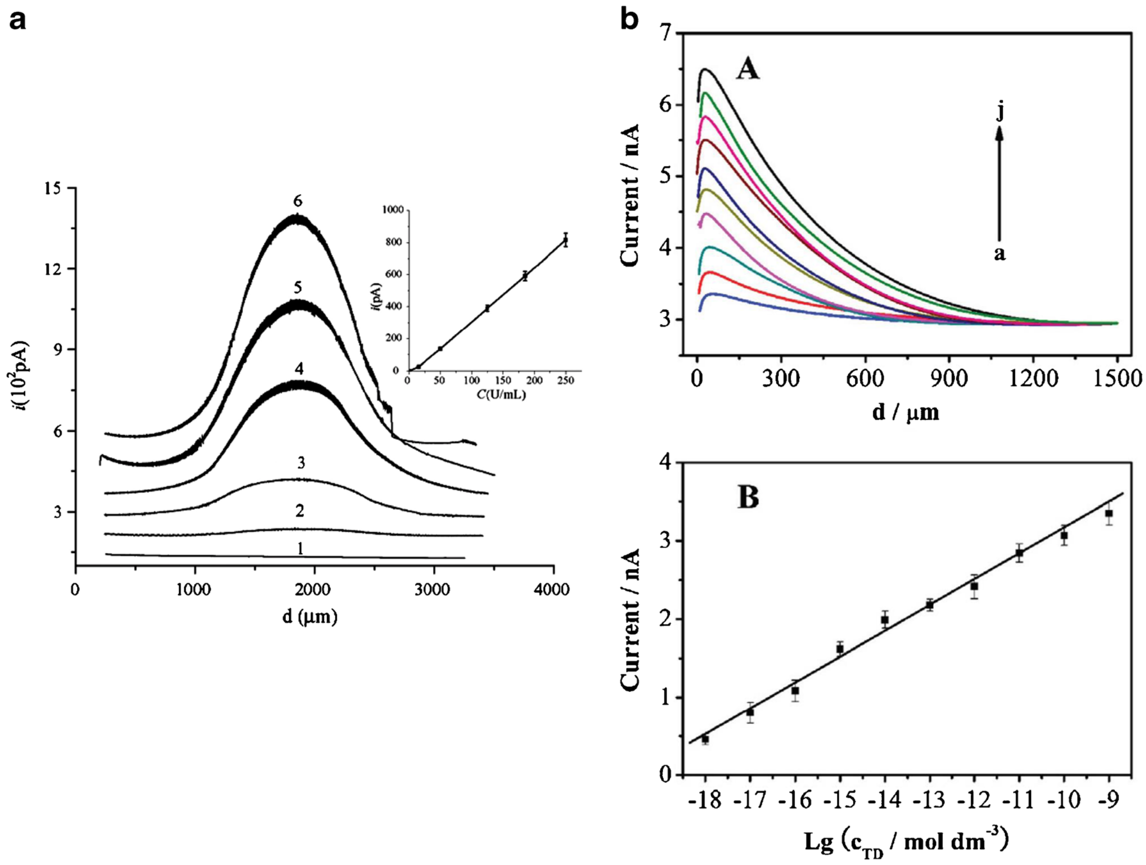


Fig. 3 **a** Scanning electrochemical microscopy (SECM) scans across the labeled spot fabricated with different cancer-related antigen CA15-3 concentrations (U mL^{-1}): 0 (1), 15 (2), 50 (3), 125 (4), 185 (5), and 250 (6). Spot size 1.0 mm in diameter. The inset: shows the relationship between peak current and CA15-3 concentration. **b** *Z*-approach curves for a fabricated DNA biosensing platform detecting the target DNA at different concentrations: 1 aM (a), 10 aM (b), 100 aM (c), 1 f. (d), 10 f. (e), 100 f. (f), 1 pM (g), 10 pM (h), 100 pM (i), and 1 nM (j) (A) and linear plot of signal current versus the logarithm of target DNA concentration (B). In both cases, phosphate-buffered saline containing hydroquinone at 1.0 x 10^{-3} mol L^{-1} and H_2O_2 at 1.0 x 10^{-3} mol L^{-1} was used as the electrolyte, with the SECM tip biased at -0.4 V versus Ag/AgCl. (**a** Reprinted with permission from [124]. **b** Reprinted with permission from [125])

metastatic and nonmetastatic state of immobilized cells could be discriminated by adequate selection of the redox mediator on the basis of its ability to permeate the lipid membrane and interactions with the intracellular media [142, 143]. Similarly, the feedback response was shown to vary after the interaction of cells with a significant amount of metal nanoparticles [144], yet no trend was evaluated for the exposure to progressively increasing amounts of particles. Qualitative information accessible via the electron transfer kinetic constant was evaluated in respect to the presence and location of mismatched nucleotides in double-stranded DNA immobilized on a gold surface [145]. The variation in feedback response of ferrocyanide as a redox mediator is highlighted after interaction of DNA with Zn^{2+} ions following a cyclic response associated with the location of the mismatch along the chain, which is confirmed by similar trends observed during electrochemical impedance spectroscopy studies.

A major interest during the estimation of kinetic constants is the quantification of the charge transfer properties of electroactive materials, interfaces, or modified surfaces such as novel electrode materials with catalytic properties. This is clearly confirmed by the recently emerging interest in SECM studies of graphene [87, 97]. The determination of the heterogeneous electron transfer rate constant with respect to the applied overpotential provides a linear correlation within a 90% confidence interval [97]. Although additional labeling with redox-active molecules is required to characterize electrochemical properties at inherently insulating surfaces, the transfer of charge between a top layer and an underlying material after their modification with redox-active label molecules, for example, for SAMs is made possible [92–94, 120]. The behavior of SAMs in respect to tunneling effects, and their overall electrochemical response have been modeled considering both contributions, electron transfer and tunneling phenomena, and have been corrected for the presence of pinhole defects [92–94, 120]. In addition, predictions in terms of the chain length [92, 93], surface coverage, and substrate potential [94, 120] are accessible via SECM analysis of SAM-coated surfaces.

Estimates of the electron transfer kinetic constant have been reported for photochemically active materials [96, 98–100], and interfaces of two immiscible electrolyte solutions (ITIES)

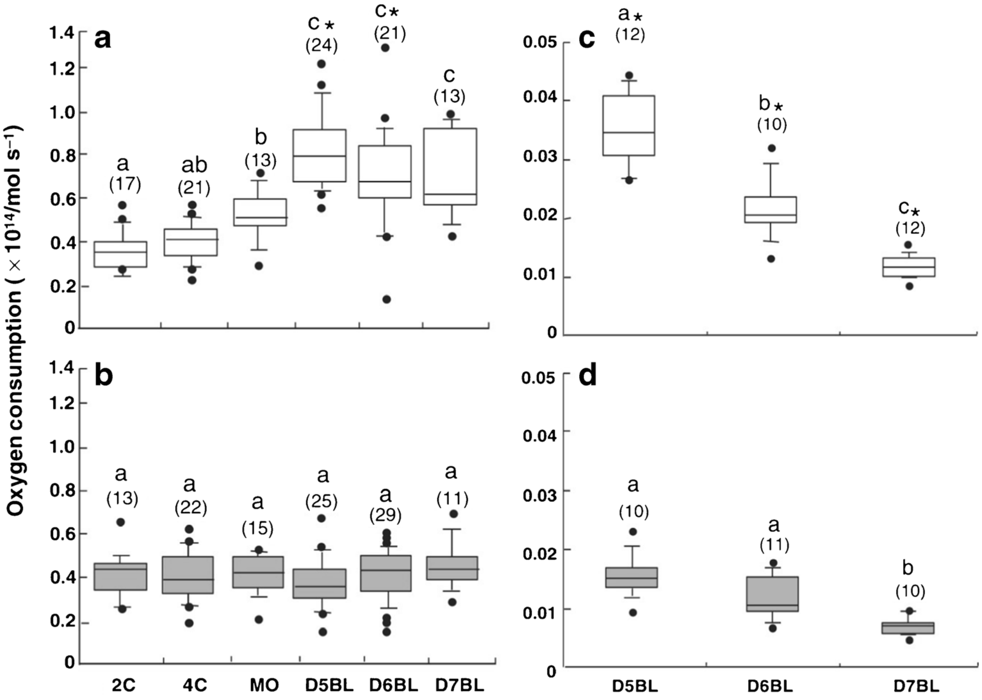


Fig. 4 Oxygen consumption in the preimplantation in vitro fertilized (IVF) and somatic cell nuclear transfer (SCNT) embryos. The box plot graphs represent oxygen consumption in the preimplantation IVF (A, C) and SCNT (B, C) embryos. The box indicates two quartiles, namely, the 25th and 75th percentiles, and the line indicates the median. The whiskers indicate the maximum and minimum values within the acceptable range that is defined by the two quartiles. The circles denote the outliers. Labels on the *x*-axis in B and D refer to the two-cell stage (2C), four-cell stage (4C); morula stage (MO), and day 5 (D5BL), day 6 (D6BL), and day 7 (D7BL) in the blastocyst stage after in vitro culture. The data on oxygen consumption per cell at D5BL, D6BL, and D7BL are presented in C and D. The number of evaluated embryos in the respective stages is indicated in parentheses. Different letters (a, b, c) indicate significant differences within each panel ($p < 0.05$). Asterisks indicate a significant difference with respect to oxygen consumption between the IVF and SCNT embryos ($p < 0.05$). (Reprinted with permission from [115])

[95, 96]. The activity of photocatalysts evaluated via electron transfer under UV–vis illumination is based on the excitation of electrons, and the subsequent formation of electron–hole pairs, which react with the reducible or oxidizable redox mediator probed at the SECM electrode [98–100]. From the extracted kinetic data, it is possible to establish the optimum composition and photochemical conversion conditions, including suitable wavelength or light intensity. In respect to ITIES, the kinetics of the electron exchange between two species—each of them dissolved in one of the immiscible liquid phases—permits the evaluation of the charge transfer across the interface, and the quantification of its eventual inhibition by the formation of blocking layers [95]. Moreover, combining both types of analysis for the electroanalytical determination of the electron transfer abilities at ITIES under illumination was demonstrated by Li and Unwin [96]. In this contribution, the photoinduced excitation of tris(bipyridine)ruthenium(II) in the aqueous phase could be determined at a platinum UME placed in the organic phase following the electron exchange of the ruthenium complex with 7,7,8,8-tetracyanoquinodimethane (TCNQ) serving as a redox mediator (Fig. 5a). SECM data fitted with simulations, allowing a quantitative determination and linear correlation of the fluxes of TCNQ with respect to the light intensity (Fig. 5b) and derivation of the concentration of redox species in the organic phase (Fig. 5d) along with the dependence of the rate constant on the driving force (Fig. 5c).

An enhanced current response when feedback mode is used may also result from an incremental change in charge or mass transfer processes different from the electron transfer process. Such systems do not follow the same physicochemical models established for electron transfer yet result in a similar feedback-type response. Hence, alternative models are needed that consider these phenomena instead of electron transfer processes, and that convert the current data into relevant information on the actual surface properties. For instance, the kinetic constants of the enhancing charge transfer phenomena, for example, facilitated ion transfer via ITIES, may be determined with SECM. The corresponding Tafel plot then allows the associated transfer coefficient to be estimated [146].

In addition, SI-SECM has emerged in the last decade, providing an enhancement of the faradaic probe current determined as a result of the electrochemical conversion of a redox mediator similar to feedback-mode SECM. However, this method is devoted to the interrogation of the extent of a surface modification resulting in generated species, which in fact remain adsorbed. Thus, SI-SECM allows the detection of surface modifications such as the

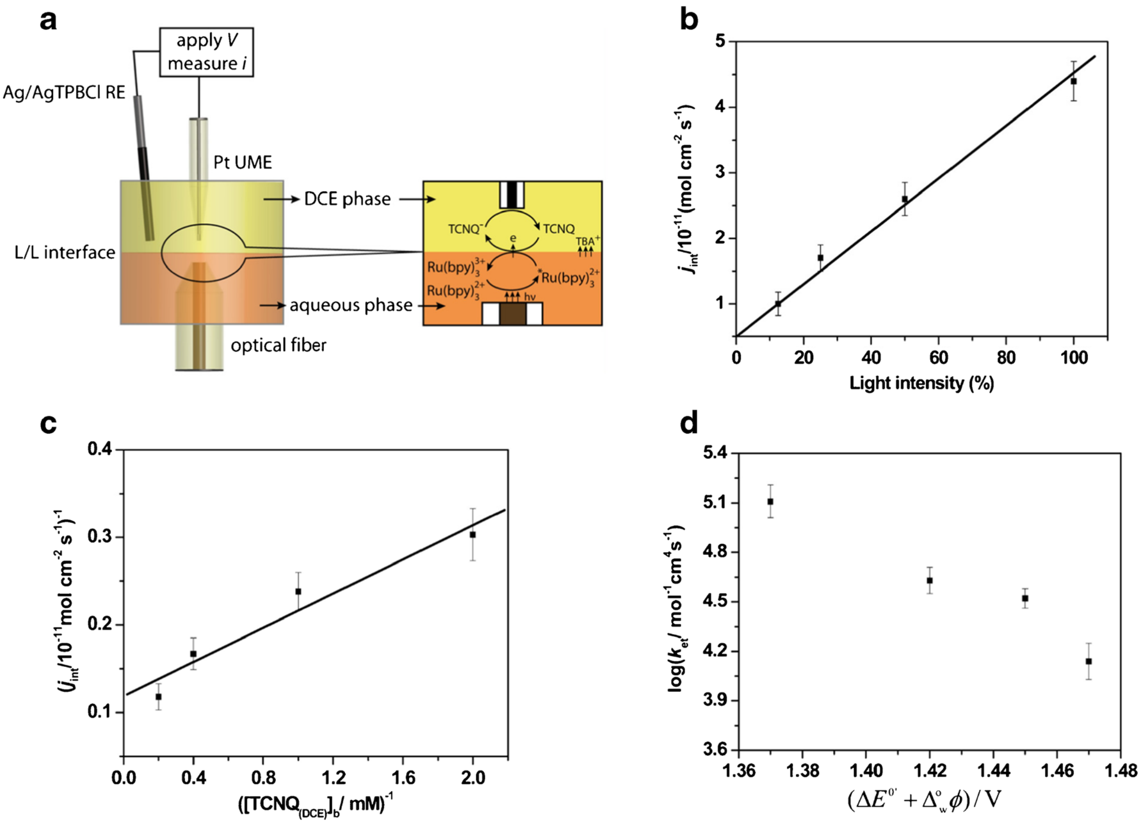


Fig. 5 **a** Scheme of the scanning electrochemical microscopy/photoelectrochemical setup used in the study of photoinduced electron transfer processes between tris(bipyridine)ruthenium(II) ($Ru(bipy)_3^{2+}$) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) at the interfaces of two immiscible electrolyte solutions (ITIES). **b**, **c** Linear correlation of the flux of TCNQ with light intensity (**b**) and the concentration of TCNQ (**c**).**d** Dependence of the photoinduced electron transfer kinetic constant with the driving force, given by the sum of the difference in the formal potentials of the $TCNQ^{0/\cdot}$ and $Ru(bipy)_3^{2+*/3+}$ couples (ΔE^{0}') and the Galvani potential difference across the ITIES ($\Delta_w^0\ \phi$). 1,2 Dichloromethane (DCE), L/L liquid–liquid, reference electrode (RE), tetrabutylammonium (TBA), tetrakis(4-chlorophenyl) borate (TPBCl), UME ultramicroelectrode. (Reprinted with permission from [96] copyright 2015 American Chemical Society)

photochemical generation of adsorbed OH• radicals, which exchange electrons with an appropriately selected redox mediator, thereby enhancing the current response [78]. The linear trend of the interrogated charge with the time delay between the generation

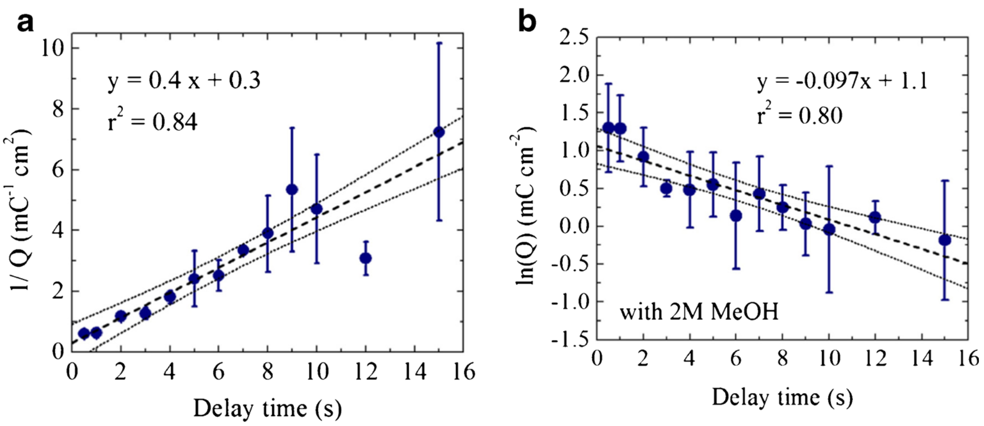


Fig. 6 **a** Reciprocal plot of the interrogation charges of OH• as a function of delay time. **b** Plot of the interrogation charges of OH• as a function of delay time in the presence of excess MeOH (2 M) in solution. The interrogation charge was calculated as the integral of the interrogation current versus time curves acquired after irradiation in 1 mM K_2IrCl_6 and 0.1 M Na_2SO_4 aqueous solution. The trend lines (dashed lines) and 95% confidence intervals (dotted lines) are drawn to show the slope and *y*-intercept deviations. (Reprinted with permission from [78] copyright 2013 American Chemical Society)

of the OH• radicals and the surface interrogation of a bismuth vanadate semiconductor photocatalyst is significantly different in the absence and presence of a radical scavenger (e.g., methanol) within a 95% confidence interval, as illustrated in Fig. 6. This SI-SECM study therefore allowed the determination of the rate constant of the reaction between a scavenger and a photochemically generated radical at a photocatalyst surface [78].

Finally, the enhanced mass transfer occurring at films and membranes because of their porosity may be analyzed to obtain quantitative data, for example, on permeability, which will significantly alter the feedback current response [101, 102, 104, 147, 148]. Several approaches conceived for SECM data treatment have been proposed for estimating permeability and porosity values. For instance, permeability values were determined by comparing the SECM current response obtained above a sample surface of interest with that obtained under the same conditions above an insulating substrate (i.e., inducing purely negative feedback), which is applicable, for example, to the study of thin film properties [101, 102]. In another example, porous membranes were investigated according to the diffusion across the porous material, and have been simulated by numerical approaches. Last but not least, stress-induced permeability of living cells when exposed to cytotoxic agents was monitored. It was confirmed that such agents result in an increase in the permeability of the cell membrane [104, 148]. The extracted vertical profiles of the tip current obtained in feedback mode with use of a variety of redox mediators reflect the trends of the cell membrane vulnerability to increasing exposure to toxic agents, as deduced from the permeability values obtained. Only very recently, a unified theoretical model has been proposed describing the influence of surface porosity on the feedback response, allowing the evaluation of all relevant parameters characterizing conductive porous layers [105].

Conclusions and perspective

Recent developments toward nanoscale electrochemical imaging in particular using nanopipettes and nanoelectrodes evidently render SECM-based analytical techniques competitive methods among the family of SPMs. Nowadays, SECM provides comparable temporal and spatial resolution along with the advantage of molecular selectivity because of the (electro)chemical information that can be obtained within a remarkably broad area of applications. Although "conventional" SECM using microelectrodes is a valuable technique in electrochemical surface science, it is evident that when quantitative data are reported, relevant analytical figures of merit are frequently only partially addressed. SECM and "hyphenated" SECM techniques have evolved toward studying complex problems and samples in at least close to real world scenarios, as demonstrated by the multitude of biological systems analyzed by SECM-based techniques. However, to obtain quantitative and traceable data, and for SECM to enter the domain of routine analysis, parameters such as the reliability and reproducibility underlined by appropriate analytical statistics have shifted into the focus of attention. With this contribution, the authors intended to critically review the lack of attention to analytical figures of merit in the field of SECM, and hope to spark not only interest and discussions, for example, at future SECM workshops, but also strategies toward improving the reliability of SECM data, potentially by the introduction of standardized reference samples and methods.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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