Bruker BioAFM Scanning Electrochemical Microscopy (SECM) Option

Introduction

Until the release of Bruker's exclusive PeakForce SECM™[1] mode for the Dimension Icon XR, the world's first complete commercial solution for AFM-based scanning electrochemical microscopy (SECM) with a spatial resolution less than 100 nanometers, SECM had been somewhat of a niche technique [2,3]. Bruker has now made SECM available for the JPK BioAFM NanoWizard® family (for the Vortis™ controller generation onwards). The innovative probe design enables AFM-based SECM with nanoscale resolution, ideal for a wide range of applications ranging from chemistry kinetics to biochemical signaling and environmental chemistry. The NanoWizard AFM family can be seamlessly integrated into high resolution optical microscopy and is safe and easy to operate in liquid. It is, therefore, perfectly suited for the simultaneous acquisition of topographic, electrochemical, electrical, and mechanical information with nanometer-scale lateral resolution.

Figure 2: *SECM option on NanoWizard 4 XP on a Zeiss Axio-Observer inverted optical microscope*

High quality, highly consistent nanoelectrode

The key to performing a reliable and reproducible SECM experiment is the cantilever. It needs to provide an isolated electric contact to an exposed nanoelectrode at the tip apex. Bruker, therefore, developed a nanoelectrode probe with proven performance, which led to a steadily growing number of peer reviewed publications [4,5,6,7]. SECM nanoelectrodes are fabricated using a wafer-based MEMS approach to guarantee a highly consistent probe quality.

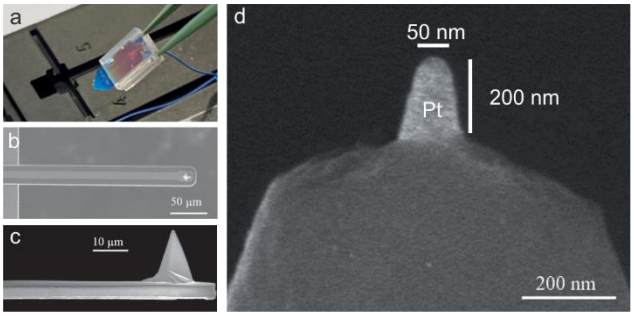

Figure 1: *(a) Pre-mounted PeakForce SECM probe; (b) SEM top view image of the cantilever showing the 15 µm wide Pt conductive path; (c) SEM side view image of cantilever; (d) SEM image revealing exposed Pt-coated tip apex with ≈50 nm end-tip diameter and ≈200 nm tip height (adaped from Nellist et al.[8])*

The pre-mounted PeakForce SECM probe which has already been contacted is shown in Figure(1a). The components of the assembled probes are rigorously tested for chemical compatibility. The mounted nanoelectrode probe is larger in size than a regular AFM probe, and the glass packaging has handling grooves for increased safety and ease of use. The rectangular cantilever is shown in Figure (1b, c).

A Pt conductive path runs between the tip and the base and provides the electrical conductivity. The apex of the tip, as shown in Figure (1d), has a Pt coated area of ~50 nm in diameter and a ~200 nm tip height. Apart from this small, electrochemically active region, the probe is fully insulated with a layer of SiO_2, essential for performing nano-electrochemical and nano-electrical measurements in liquid.

Bruker BioAFM SECM components

The SECM option for the NanoWizard AFM family has been developed to enable maximum ease of use and perfect optical integration for observation and control. The comprehensive package contains the following major components:

Probe holder: A specifically developed probe holder holds the PeakForce-SECM probe. For safe and easy handling, the probe is inserted into the holder using the handling grooves and locked with a spring-loaded pin (see Figure 3b).

Current preamplifier: The electrical contact from the SECM-probe is fed directly into a current/voltage converter attached to the AFM-head and located close to the probe (see Figure 3a). The preamplifier coverts the low tip-current into a robust, low impedance voltage inside the Faradaic cage formed by the AFM-head and stage, instead of sending the sensitive current signal via long cables to the potentiostat which is typically prone to pick-up noise. This is essential for obtaining a current noise floor of <1nA in the electromagnetically critical environment of a standard inverted optical microscope and means that an additional electromagnetic shielding of the whole setup is not necessary. The preamplifier works as a zero-resistance ammeter with a current range of ±10nA, whereas the tip-potential can be independently controlled with respect to the counter- and first working-electrode (sample). For safety reasons, the circuitry limits the tip-current to the measurement range, even though the probe can handle much higher currents. The voltage, proportional to the current, is fed to the Vortis AFM controller as a raw tip current signal and to the custom-designed bi-potentiostat.

Bi-potentiostat: The potentials inside the electrochemical cell, relative to the reference electrode, are controlled by a custom-built potentiostat. The potentiostat connects directly to the counter electrode, reference and first working electrodes (sample). Nine different current ranges, from ±1n to ±100nA, can be applied to read the sample current, whereas the already converted tip-current (second working-electrode) is fed directly into the potentiostat from the preamplifier. The unit is powered by the AFM controller to avoid ground-loops which might interfere with the current sensing. Consequently, the USB connection to the host PC is galvanically isolated. The applied potentials and current from tip/sample-electrodes are fed to the auxiliary inputs of the AFM controller and recorded during AFM operation.

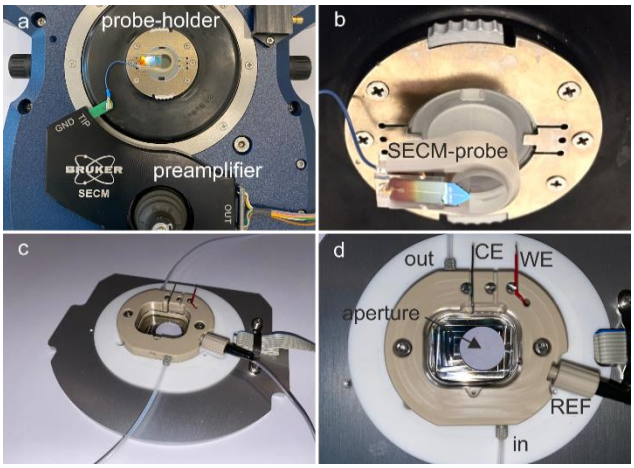

Figure 3: *(a) Bottom view of NanoWizard 4 XP head with attached current preamplifier; (b) detailed view of probe-holder with mounted SECM-probe; (c) complete EC-cell assembly with mounted reference electrode and perfusion tubings attached and (b) detailed top view of cell showing the positions of the three electrodes (WE, CE, REF)*

Electrochemical cell: The new electrochemical cell depicted in Figure 3 (c) and (d) was developed to host the SECM-probe. Its base enables optical transmission microscopy and samples of 35 x 30 mm in size can be mounted. A platinum wire counter electrode and a miniature Ag/AgCl-reference-electrode are used in conjunction with the tip and sample in a four-electrode configuration. Contact with the sample is made via a spring-loaded pin inside the cell body made of PEEK. If required, an additional port for a wire-type electrode can be used for a platinum or custom Ag/AgCl reference electrode. The cell body has three ports for liquid perfusion and gassing, and temperature can be controlled in the range of RT to 60°C.

Seamless software integration

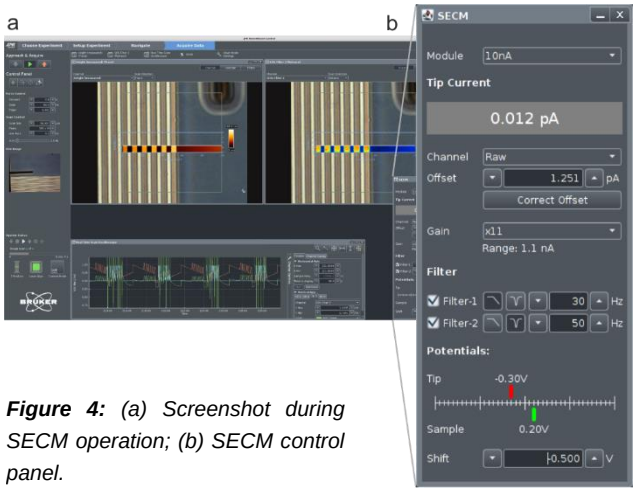

Figure 4: *(a) Screenshot during SECM operation; (b) SECM control panel.*

SECM has been seamlessly integrated into JPK BioAFM's new SPM Software V7 version which provides workflow-based user guidance. To ensure maximum flexibility, the SECM capability is not restricted to any particular AFM operating mode. The SECM panel displays all of the necessary controls, as shown in Figure 4 (b). In particular, two freely configurable, chained digital filters are used for conditioning the SECM current signal. This approach is significantly more flexible than the fixed frequency analog filters typically found in potentiostats for signal conditioning. Both the filtered and raw signals can be captured during AFM measurements. Recording and saving the raw signal without any filtering being applied enables extensive offline processing of the acquired SECM data.

As well as performing classical AFM-SECM imaging in an interleaved acquisition pattern (lift / hover), the software supports the recording of electrochemical volume data "cubes" using QI™ or force mapping for multi-dimensional imaging on soft / viscous samples. These rich datasets can be processed by a set of powerful operations, either during acquisition or offline by the data processing software. Thus, multi-dimensional views of various sample properties such as height, adhesion, stiffness and SECM current at various heights can be generated.

A variety of applications

In its over 30 years of existence, SECM has established itself as the tool of choice for studying the spatially resolved electrochemical reactivity of surfaces, including the quantitative study of the kinetics of both electrochemical and chemical reactions [9]. However, in the past, the relatively large, micron-sized electrodes (ultramicroelectrodes) limited the spatial resolution, and crosstalk between the electrochemical signal and topography limited the number of applications.

The commercial availability of high quality nanoelectrode probes for AFM-based SECM has overcome these limitations by reducing the size of electrodes to the nanometer scale and decoupling the reading of the electrochemical current from the acquisition of the highly resolved topography. Furthermore, advanced operation modes such as PeakForce Tapping, PeakForce QNM [10, 11] and QI™ (quantitative imaging) [12] enable the simultaneous, high resolution capture of mechanical sample properties in conjunction with SECM, even on soft and fragile biological samples, e.g. live cells [13, 14, 15] , bacteria [16, 17], viruses [18, 19], protein layers and many more. In addition to collecting electrochemical information, the highly insulated SECM probe, with only the tip being exposed, is ideal for performing nano-electrical measurements in liquid.

The perfect optical integration of Bruker's BioAFM systems allows users to optically identify areas of interest and/or conduct SECM experiments under optical control. The unparalleled optical design of NanoWizard AFM heads, which have a free optical path, enables transmission-based contrast methods such as DIC and optical phase contrast using standard optical microscope condensers. Furthermore, SECM can also be combined with fluorescence and advanced fluorescence techniques (Confocal, FLIM, TIRF, FRET, FCS) for colocalized image acquisition or direct stimulation of electrochemical activity.

A growing number of applications arises from the diverse SECM operational configurations, such as:

- localized corrosion
- electrocatalytic behavior of nanoparticles
- enzyme catalyzed reactions
- charge-transfer mechanisms
- adsorption and desorption phenomena
- diffusion processes across membranes
- hydrogen peroxide release from bacterial films
- physiological activity of single live cells

Electrode charge transfer

Optical microscopy can be used to directly target specific locations on transparent electrode structures. Micrometer- or nanometer-sized electrodes are not only crucial elements in most optoelectronic devices such as displays and solar cells, but are also used in cell biology to derive values like impedance/potential as a measure of live cell activity or for

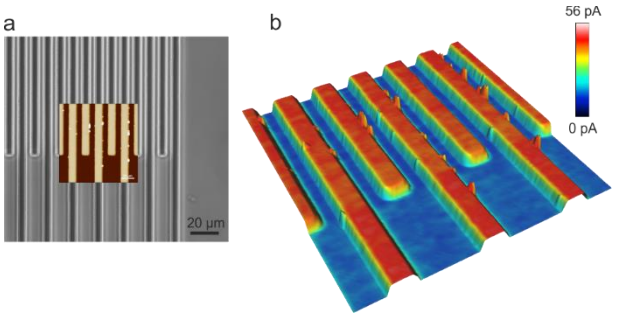

Figure 5: *(a) Overlay of optical image (40x, Ph2) and AFM topography of platinum microelectrodes (width 5 µm, height 200 nm) on glass captured in tapping mode; (b) 3D topography with SECM current skin recorded in lift scan 50 nm above sample. The Faradaic current ($[Ru(NH_3)_6]^{3+}$ → $[Ru(NH_3)_6]^{2+}$) is enhanced in the presence of the electrodes by redox-cycling. (tip potential set to -0.3V, electrodes potential 0.0V, 5 mM $[Ru(NH_3)_6]^{3+}$ in 0.1 M KCl).*

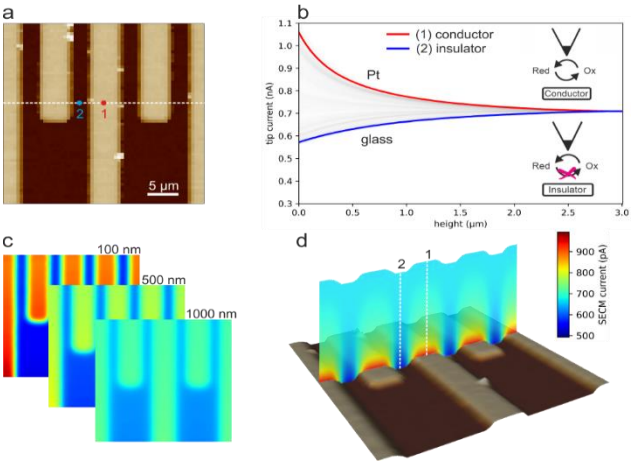

Figure 6: *(a) AFM topography of platinum microelectrodes (same as figure 5) captured in force mapping mode (60 x 60 points); (b) density plot of all collected SECM current approach curves, individual curves on electrode (1) and glass (2) are highlighted showing the expected behaviour of redox-cycling and blocking; (c) reconstructed electrochemical current images at increasing distance and (d) vertical slice through the recorded SECM current volume (tip potential -0.3V, electrodes potential 0.0V, 10 mM* $[Ru(NH_3)_6]^{3+}$ *in 0.1 M KCl).*

Watch animation on Youtube

the direct stimulation of individual cells. Figure 5(a) shows an overlay of an optical image and the AFM topography of an electrode structure made of platinum on glass. The Faradaic current of the reduction of ruthenium ($[Ru(NH_3)_6]^{3+} + e^- \rightarrow [Ru(NH_3)_6]^{2+}$) at a tip potentia l of -0.3V is recorded at a distance of 50 nm between the tip and sample in an interleaved pattern shown in Figure 5(b). The redox-cycling (tip: $[Ru(NH_3)_6]^{3+} \rightarrow [Ru(NH_3)_6]^{2+}$, sample: $[Ru(NH_3)_6]^{2+} \rightarrow [Ru(NH_3)_6]^{3+}$) enhances the electrochemical current in the presence of the conducting sample electrodes, whereas the current drops in the presence of the insulating glass as a result of Ru-complex diffusion to the tip electrode being blocked.

SECM data cube recording

In addition to capturing the charge transfer between the tip and sample electrodes at constant distance, approach curves can be used to study the local interfacial charge transfer dynamics in the volume above the sample. Arrays of force-distance curves, recorded in the force mapping or QI™ mode, can be used to simultaneously capture the electrochemical current versus distance, as shown in Figure 6. Here, the same electrode structure shown in Figure 5 is used to capture a 60 x 60 points force map, while biasing the tip at -0.3V to trigger the reduction of $[Ru(NH_3)_6]^{3+}$, but fixing the sample potential at 0.0V. This allows a re-oxidation of tip-generated $[Ru(NH_3)_6]^{2+}$ should the diffusion layer of the tip come into close proximity to the conducting sample electrode. Individual approach curves (see Figure 6, b) for position (1) on the platinum and (2) on glass show the pronounced enhancement (redox-cycling) and blocking of the electrochemical current while approaching the surface. The strength of this acquisition setup is that the SECM current is captured across the whole volume in a single measurement. Thus, the diagram of the electrochemical activity can be computed as arbitrary sections across the volume as shown in Figure 6, (c) for slices parallel and (d) perpendicular to the sample surface.

Transport across membranes

SECM can be operated in collection mode to capture mass transport across membranes. For example, the transport of a mediator such as $[Ru(NH_3)_6]^{3+}$ across individual pores is detected by the SECM tip as sketched in Figure 7 (a). Here, two polycarbonate membranes with well-defined 400 nm or 100 nm pores (Nucleopore™ track etched membrane) are used to separate different volumes, one containing and one free of $[Ru(NH_3)_6]^{3+}$. The tip is biased to -0.3 V to trigger the reduction of the ruthenium complex if present, so the detected Faradaic current is an expression of the local concentration of the mediator. The topography and electrochemical current are captured simultaneously in PeakForce Tapping mode, without the need for interleaved

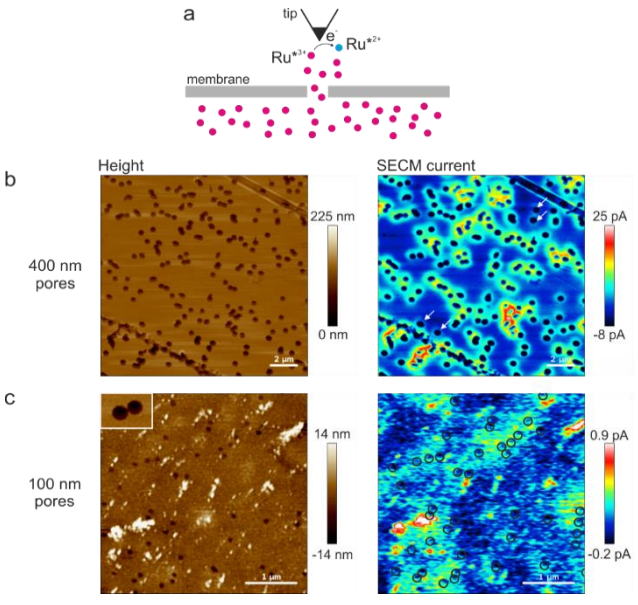

Figure 7: *(a) SECM detection scheme of mass transport across porous membranes; (b) topography and electrochemical current for 400 nm and (c) 100 nm pores (polycarbonate membrane, Nucleopore™) simultaneously capured in PeakForce Tapping while biasing the tip to -0.3 V for the reduction of* Ru^{3+} *(10 mM* $[Ru(NH_3)_6]^{3+}$*, 0.1 M KCl).*

scanning on non-conducting samples. In the case of the 400 nm pores shown in Figure 7(b), the current image clearly displays a higher concentration of $[Ru(NH_3)_6]^{3+}$ in the proximity of the pores, as expressed by the halo around most of the pores, while some of the pores appear less active (white arrows). The observed current variation is in the order of 10…20 pA. However, the electrochemical current drops significantly if the 50 nm tip enters the pore as a result of blocking of the active tip-electrode area. In the case of 100 nm pores as shown in Figure 7(c), the electrochemical current is significantly reduced by the lower mass transport of the ruthenium complex. Regions of higher spatial pore density with higher electrochemical activity can be clearly seen. Here, the observed current variation is less than 1 pA.

Conclusions

The new SECM package greatly extends the capabilities of Bruker's BioAFMs and provides users with numerous new possibilities, such as:

- High performance, fully commercial, complete SECM solution in combination with inverted optical microscope techniques, including phase contrast, fluorescence, and super-resolution microscopy
- Versatile SECM experiments in biologically relevant environments, with the highest number of AFM operating modes available, such as, PeakForce Tapping, QI™, force mapping/volume, Tapping Mode etc.
- Highest resolution SECM, multi-dimensional imaging with a spatial resolution of < 100 nm
- Simultaneous electrochemical, electrical, and mechanical imaging in liquid
- Proven SECM reliability and performance as a result of specifically designed, quality assured, and easy-to-use, commercially available SECM probes

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JPK BioAFM Business
Bruker Nano GmbH
Am Studio 2D, 12489 Berlin, Germany
productinfo@bruker.com
www.bruker.com/bioafm · www.jpk.com