BioLogic
Science Instruments

SECM for bio-sensors

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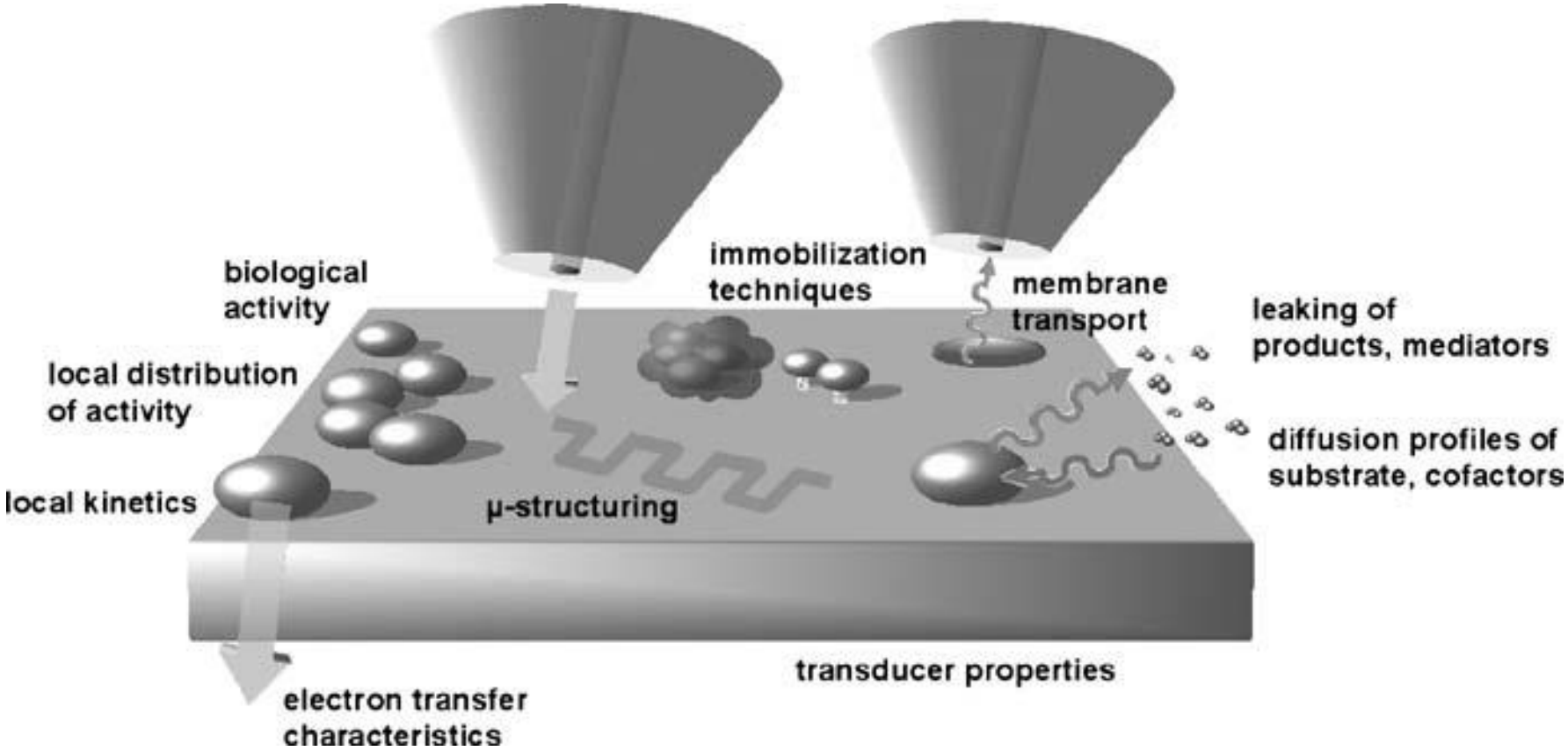
Product Manager

Prerequisite

- Understand the principles of dc-SECM
- Be aware of all the operating modes of SECM
  - Feedback (FB)
    - Positive (+FB)
    - Negative (-FB)
  - Generation/Collection (G/C)
    - Sample Generation/Tip Collection (SG/TC)
    - Tip Generation/Sample Collection (TG/SC)
  - Direct Mode (DM)
- Understand the principles of bio-sensors

Fields of interest



Fields of interest in biosensor research that can be addresssed by SECM.
Measurements are performed in electrolyte solution.

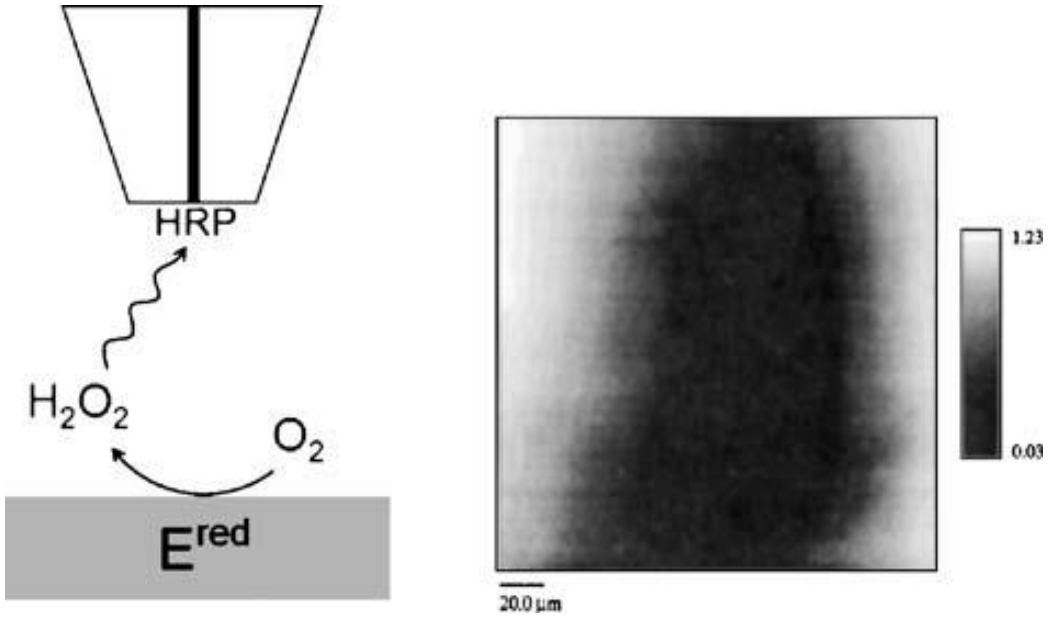
L. Stoica et al. Adv Biochem Engin/Biotechnol (2008) 109: 455–492

General advantages

- Provide spatially-resolved information on the local electrochemical activity at the surface
- Measurement performed in electrolyte solution = objects are not denatured
- No need to label the biomolecules with fluorescent dyes to allow detection, the detection comes for electron flow.
- Specific molecules can be produced at the tip, and the reactivity of the receptors towards these molecules can be specifically tested.

G/C mode for O_2 reduction activity

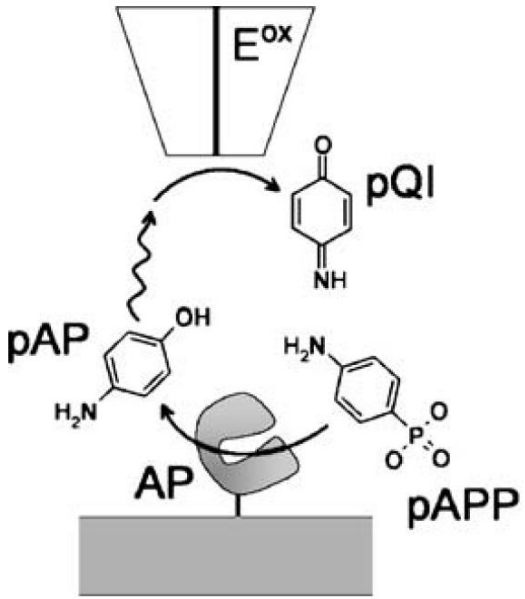
H_2O_2 detection using a micro biosensor: horseradish peroxidase (HRP) « wired » to an 8 µm diameter C electrode using an osmium redox polymer

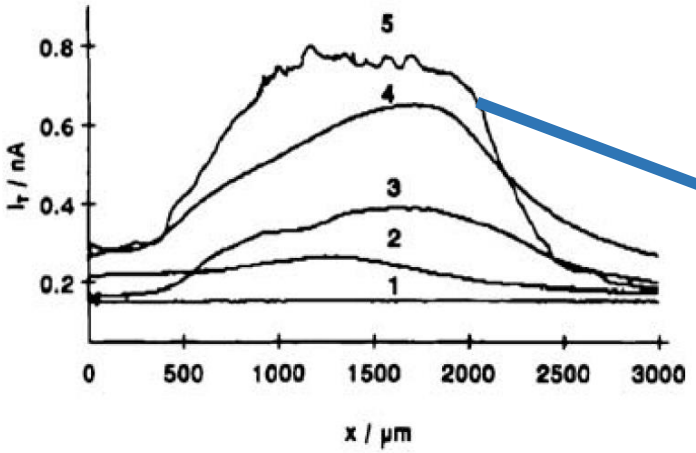


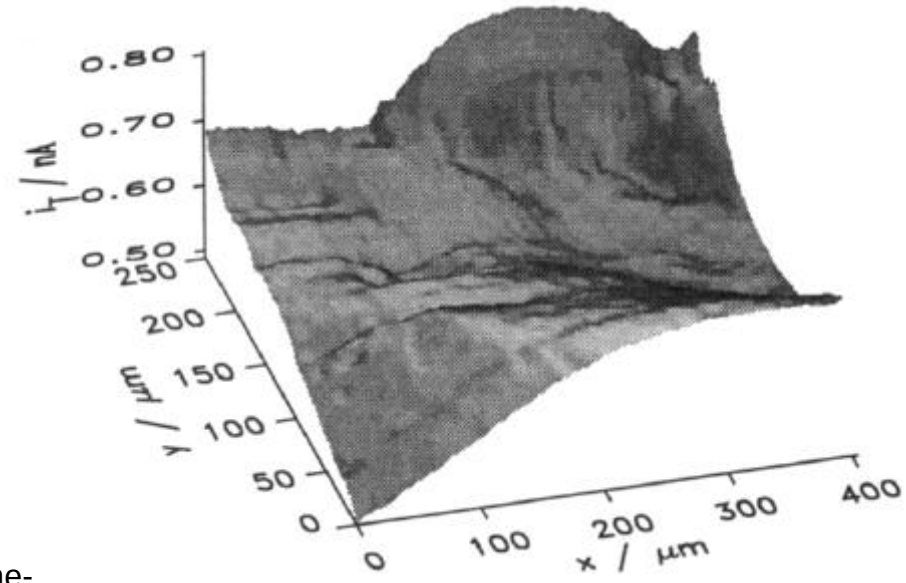
Application of a HRP-modified microbiosensor as SECM tip in the generation/collection mode.
Right: SECM image of the H_2O_2 concentration profile at an unbiased 25 µm Pt disk electrode acquired with a HRP-modified carbon fiber electrode.

HORROCKS ET AL. ANALYTICAL CHEMISTRY, VOL. 65, NO. 24, DECEMBER 15, 1993

G/C mode for immobilization studies





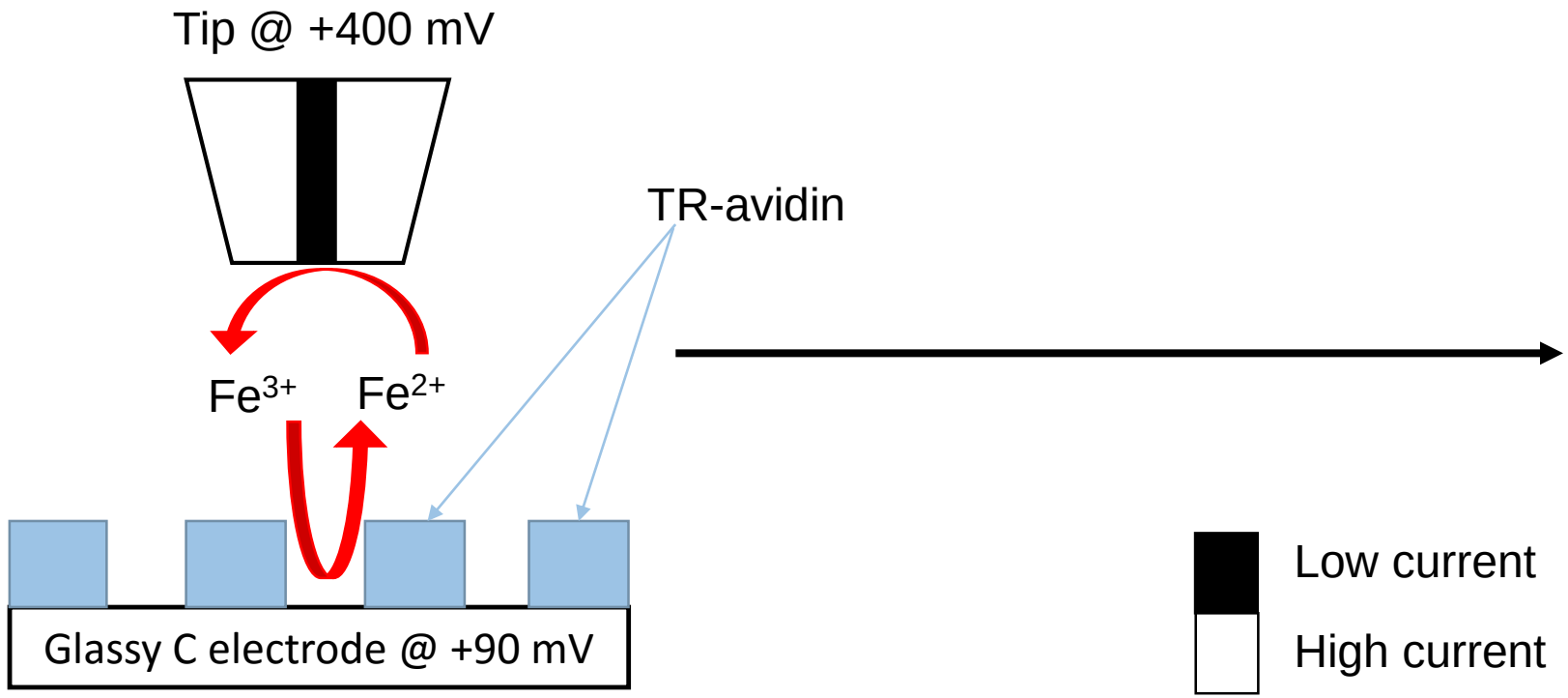


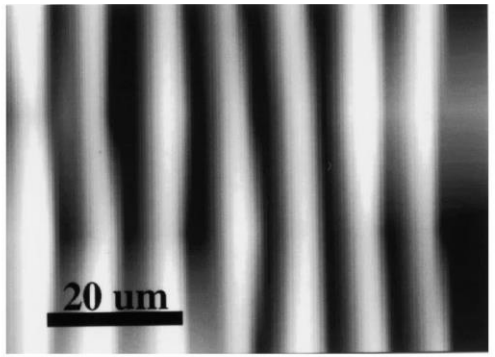
Horizontal line scans across spots with covalently attached monoclonal anti-digoxin Ab (antibody) after reaction with enzyme-labeled digoxin.
Dilutions of the incubated Ab solutions: (1) no Ab solution applied, (2) 1:3, (3) 1:4, (4) 1:2, and (5) undiluted antibody stock solution, 0.141 mg.mL^{-1} protein.

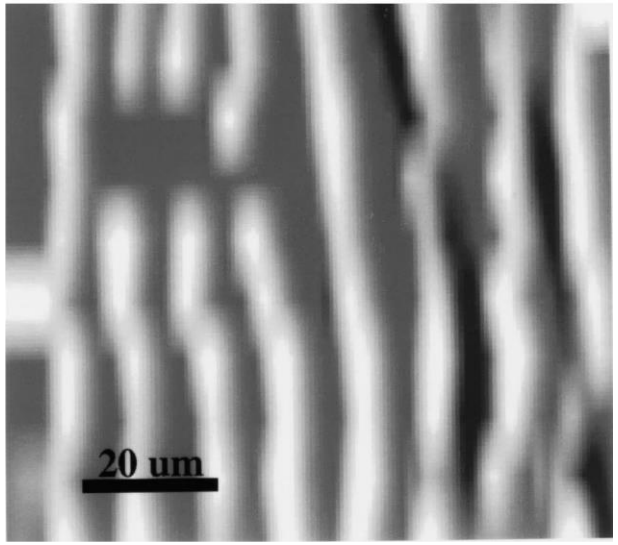
Increasing the antibody concentration increases the immobilization rate.
Spatial heterogeneity of the immobilization of the enzyme can be studied.

G. Wittstock et al., Analytical Chemistry, Vol. 67, No. 79, October 7, 7995

G/C mode for immobilization studies



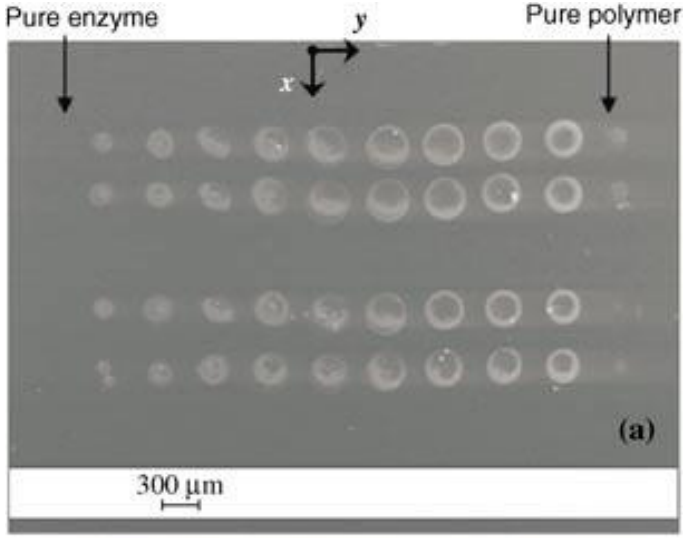




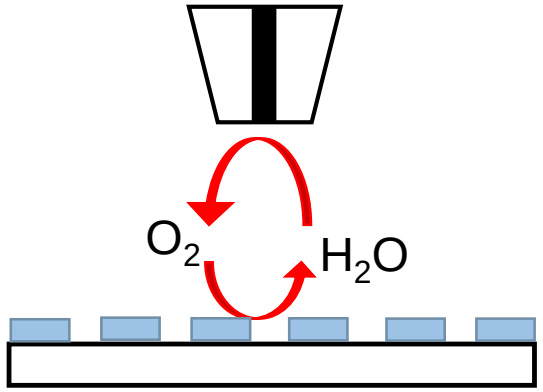
Microchannels were created to allow a diffusion of the mediator between active site of immobilized enzyme (on TR-avidin) and the surface of the carbon electrode.
SECM is used to probe electron transfer kinetics on the modified surface.

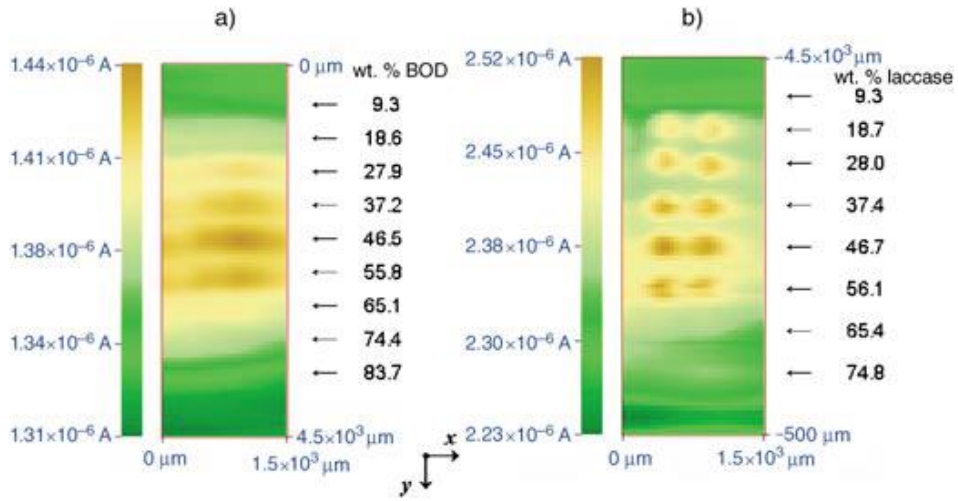
W.B. Nowall et al. / Biosensors & Bioelectronics 13 (1998) 1237–1244

G/C mode for composition optimization



SEM photograph of two arrays of redox-polymer–laccase spots made with 6.5 wt.% cross-linker. Each two-dot row corresponds to a particular composition.



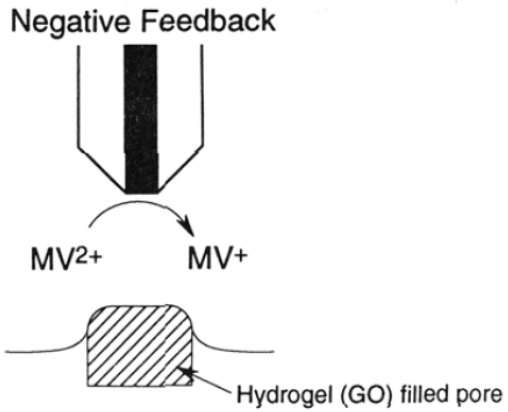


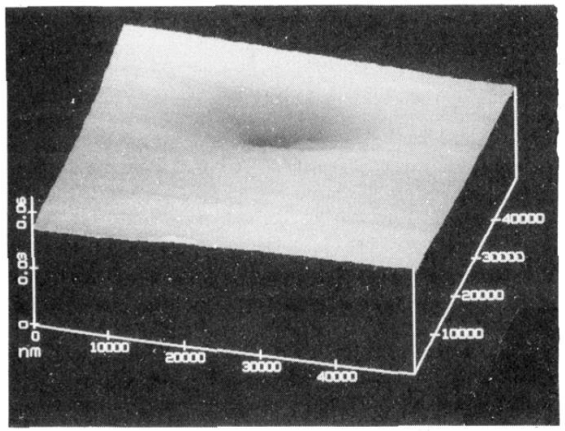
Sample O_2 electroreduction current for BOD and laccase enzyme with respective percentage.

O_2 produced at the tip is collected at the sample.

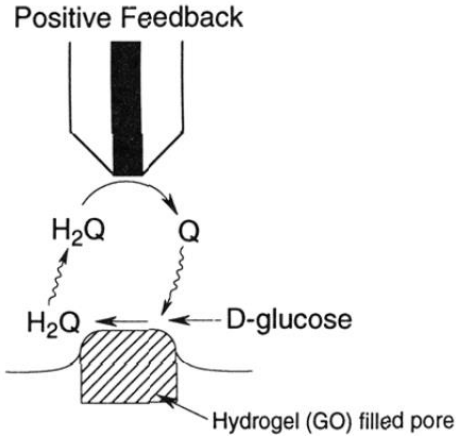
SECM is used to optimize the composition ratio of polymer/enzyme.
In this case the application is Bio-fuelcell.

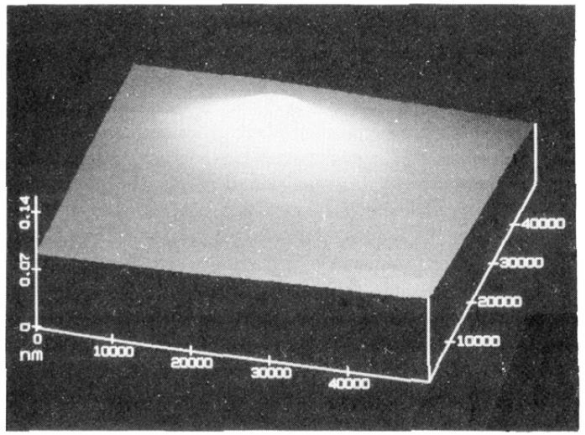
Feedback mode to image enzyme reactivity



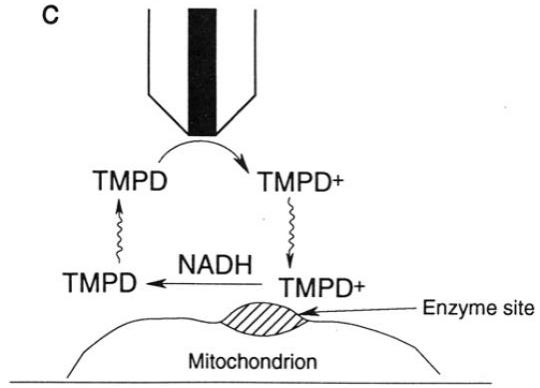


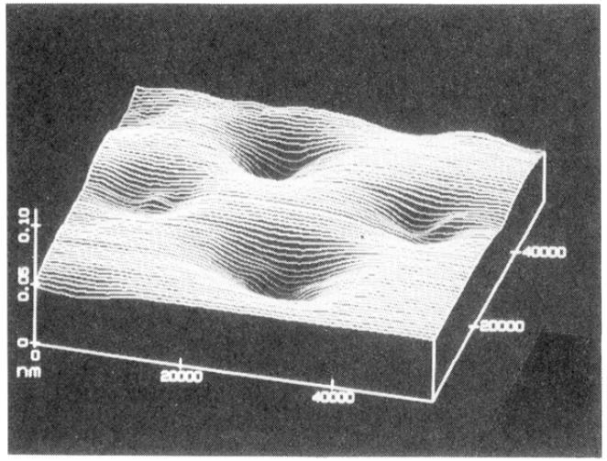
Negative feedback image of a glucose oxidase enzyme trapped in a pore. MV^{2+} = methylviologen





Positive feedback image of a glucose oxidase enzyme trapped in a pore. Q: quinone; H_2Q: hydroquinone

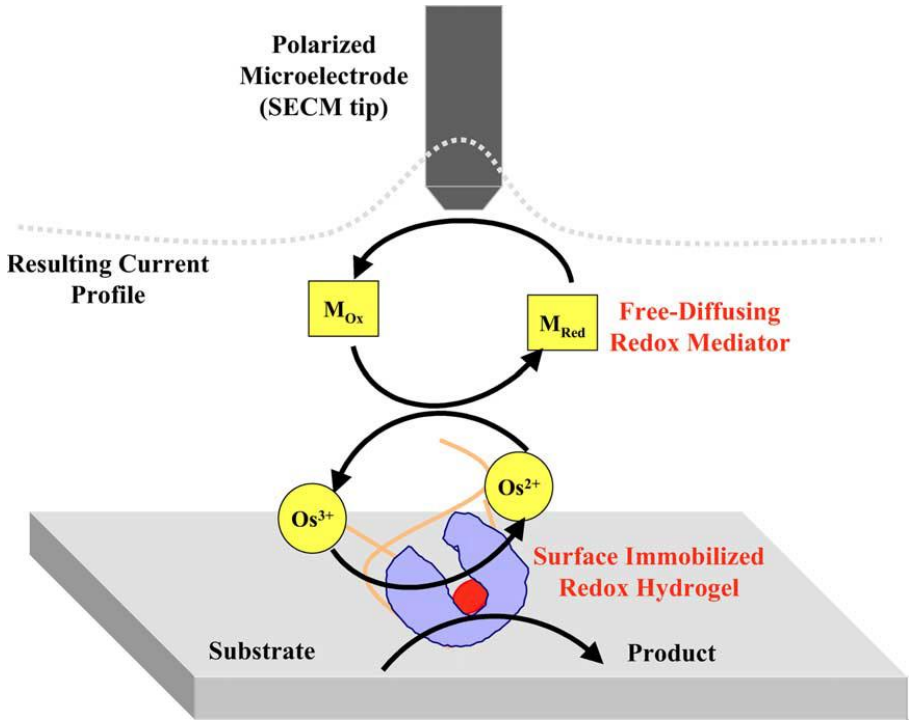




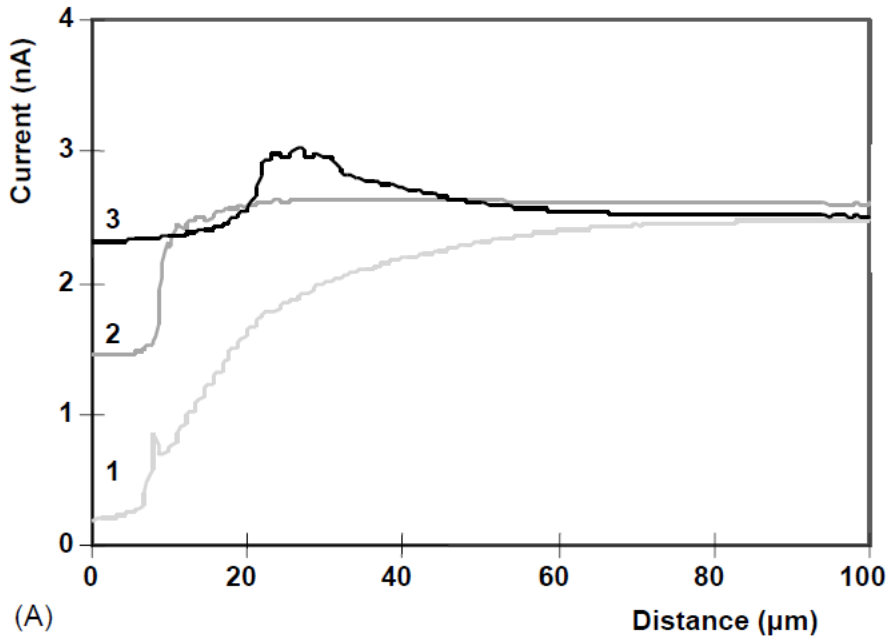
Positive feedback image of a glucose oxidase enzymes immobilized on a mitochondrion

D. T. Pierce, A. J. Bard, Anal. Chem. 1903, 65, 3598-3604

Feedback mode to optimize chemistry



Strategy used: the target surface is covered with a thin film of polymer containing a redox relay (Os^{3+}) and the enzyme.
Enzyme substrate is in solution as well as a mediator to regenerate the relay.

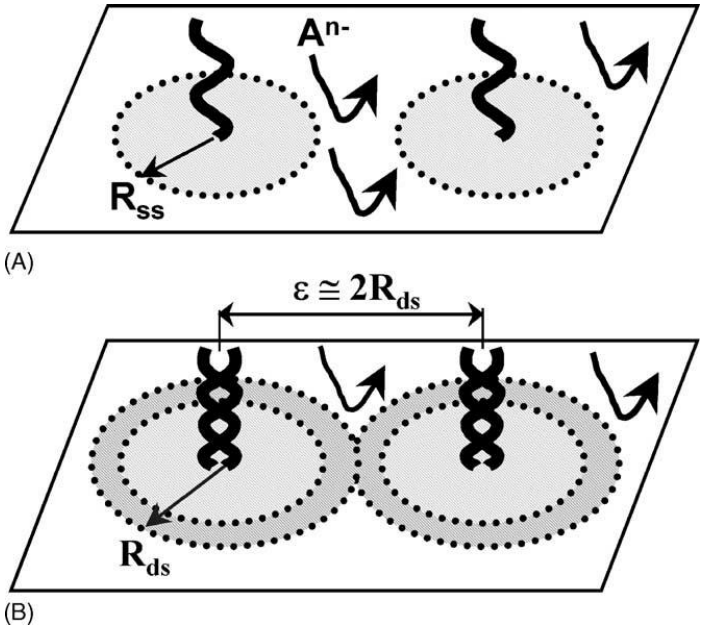


Approach curves performed on a glass surface
1) BSA, PVI without Os and PEGDGE (cross linker)
2) QH-ADH (enzyme), PVI without Os and PEGDGE
3) QH-ADH, PVI13dmeOs and PEGDGE

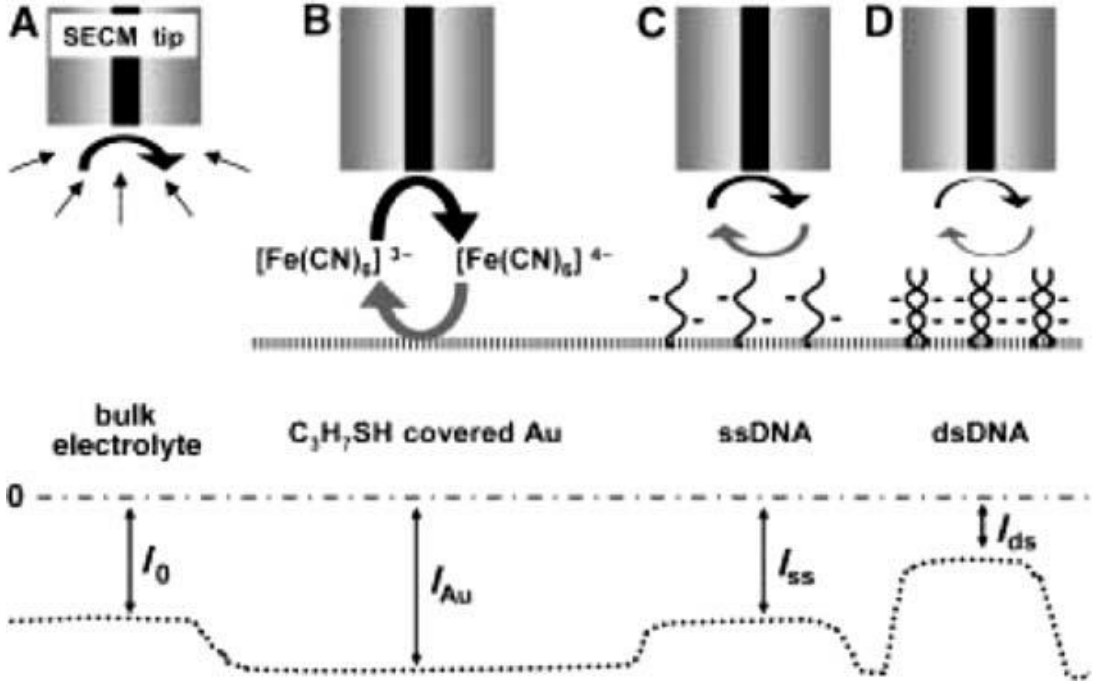
The surface moves from an insulator behavior towards an almost conductor behavior due to the effect of the enzyme, and the positive effect of the presence of the redox relay in the hydrogel.

M. Niculescu et al. / Biosensors and Bioelectronics 19 (2004) 1175–1184

Feedback mode for DNA characterization



DNA blocks the diffusion of anions to the conducting surface due to the phosphate groups on the DNA strand. Double stranded DNA will block the anions more than single stranded DNA.

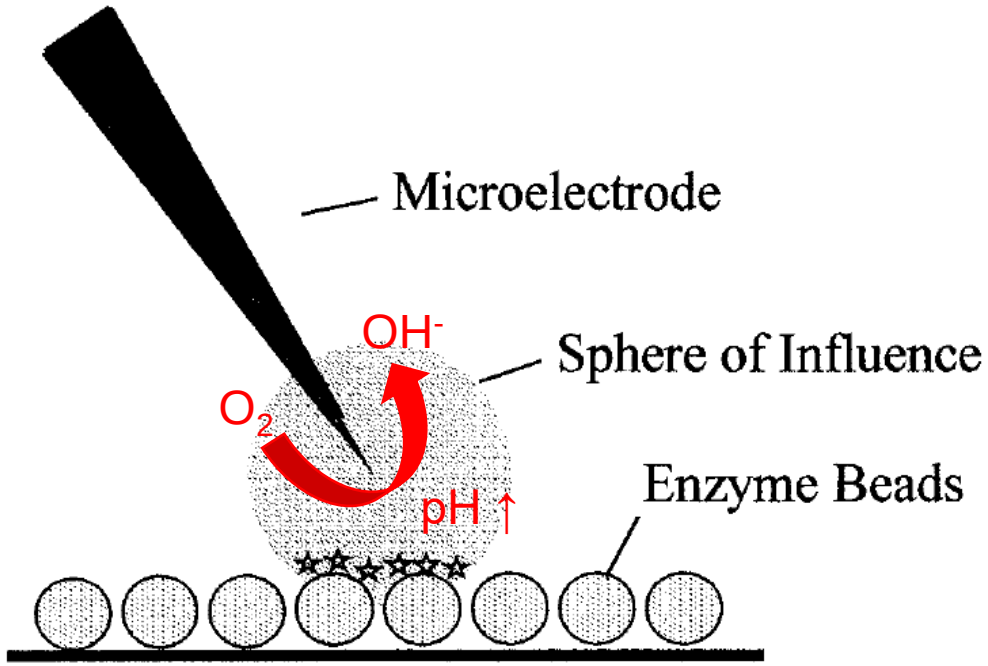


Positive feedback is hindered by the presence of DNA. The reduction of current is larger for dsDNA than for ssDNA.

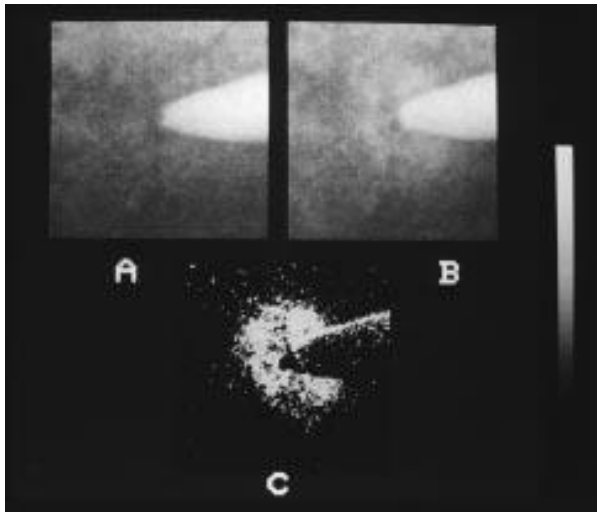
SECM can be used to detect and discriminate single stranded and double stranded DNA.

F. Turcu et al. / Biosensors and Bioelectronics 20 (2004) 925–932

Direct mode for local (de)activation of enzymes



A solution at pH 6 is used. The microelectrode is reducing O_2 into OH^- to locally produce a pH 9, at which the enzyme is activated.

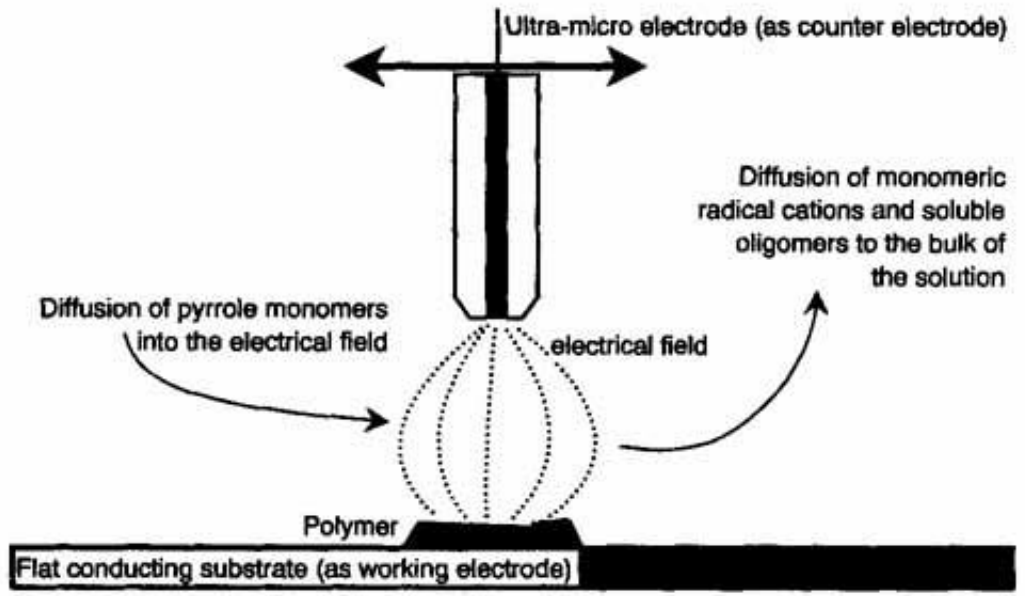


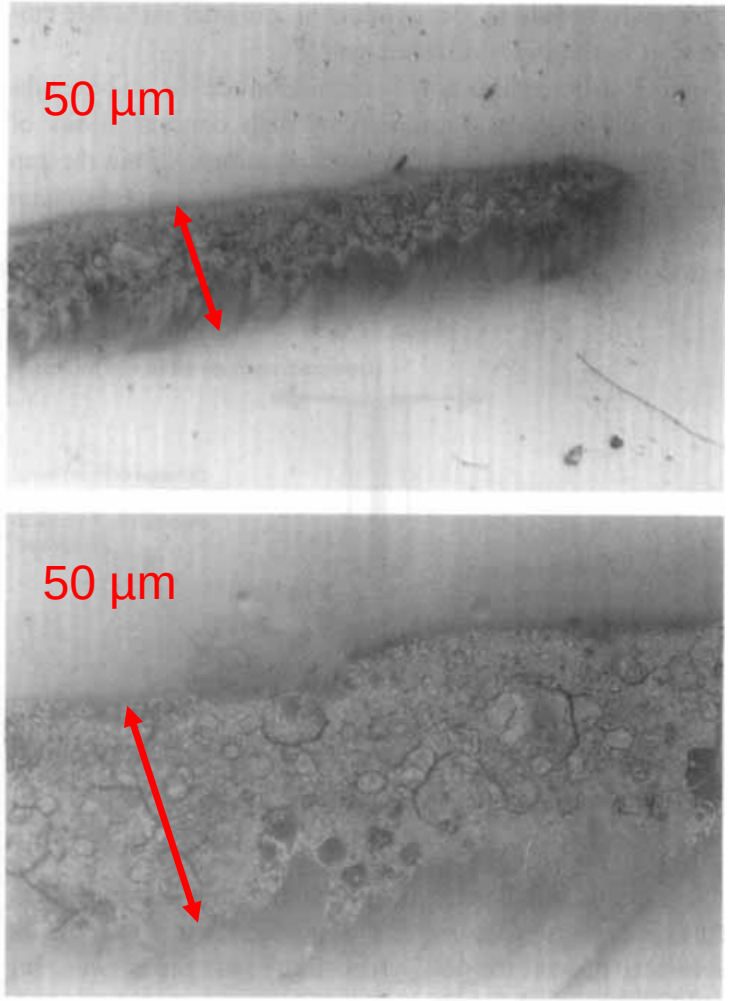
The activity of the enzyme is detected by fluorescence.

SECM can be used to locally change the environment of a receptor and study its response.

J.C. O'Brien, J. Shumaker-Parry, R. C. Engstrom, Anal. Chem. 1998, 70, 1307-1311

Direct mode for local polymer deposition



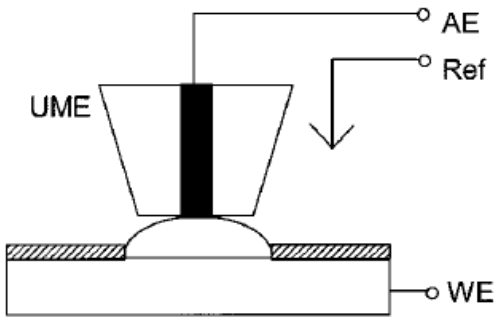


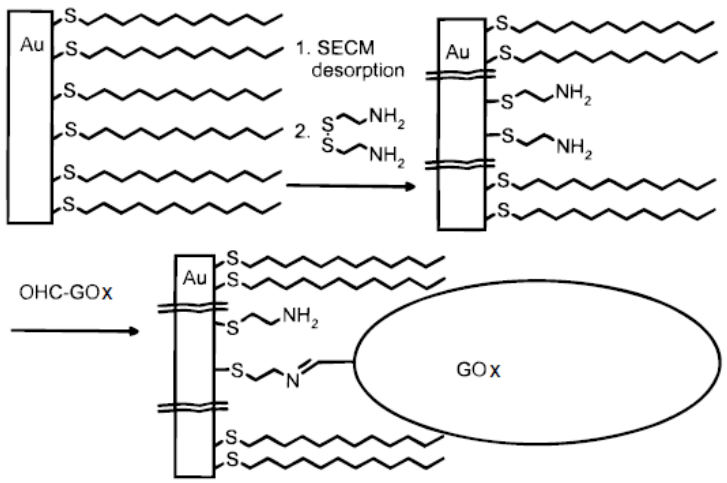
SECM was used in direct mode to deposit lines of polypyrrole on the surface of a gold substrate.
A pulse deposition protocol is established.

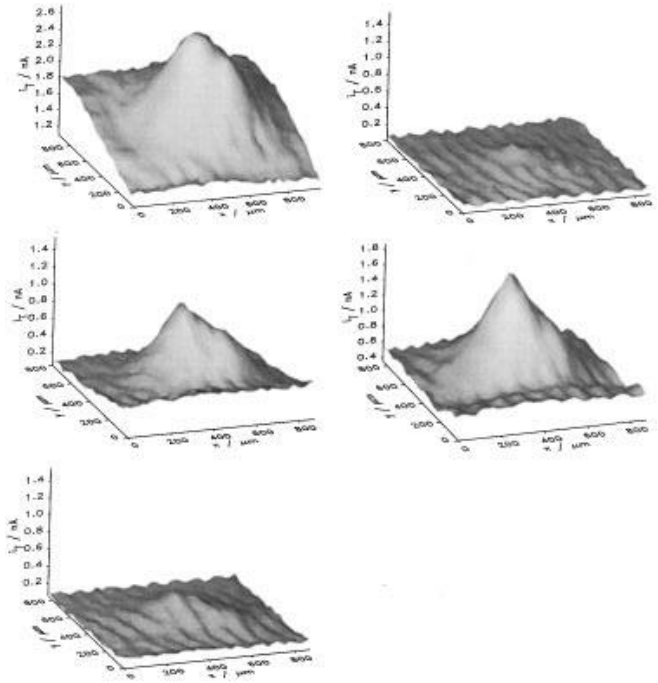
Note that monitoring of thickness and amount of deposited material could be done with a Quartz Crystal Microbalance.

C. Kranz, M. Ludwig, H. E. Gaub, W. Schuhmann, Adv Mater 1995, 7. Nº 1

Direct mode for local functionalization







1. SECM desorption of chemisorbed alkanethiolate monolayer performed in 5 or 50 mM KOH. Two cyclic voltammograms (CVs) between -1200 and +1200 mV were performed, starting and finishing at 0 mV.
2. Cystaminium dihydrochloride (Merck) was chemisorbed onto the renewed gold surface from a 100 mM solution in water during 30 min.
3. A suspension of periodate-oxidized glucose oxidase (GOx) is added.

Enzyme activity is then showed using SECM in generation /collection mode using O_2/H_2O_2 couple as mediator.

SECM can be used to locally modify the surface and investigate new immobilization methods.

G. Wittstock, W. Schuhmann, Anal. Chem. 1997, 69, 5059-5066