Miniaturized electrochemical sensors as a tool to get real-time chemical information

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Laboratório de Sensores Eletroquímicos e Métodos Eletroanalíticos

Analytical Chemistry

The science of inventing and applying the concepts, principles, and instrumental strategies for measuring the characteristics of chemical systems and species

R. Murray, Analytical Chemistry, 63 (1991) 271A

Tutorial Outline – Research interests

- **Introduction on sensors**
- **Surface modification (pre-treatment)**
- **Electrodes with micrometric dimensions**
- **Wearable sensors**
- **Conclusions**

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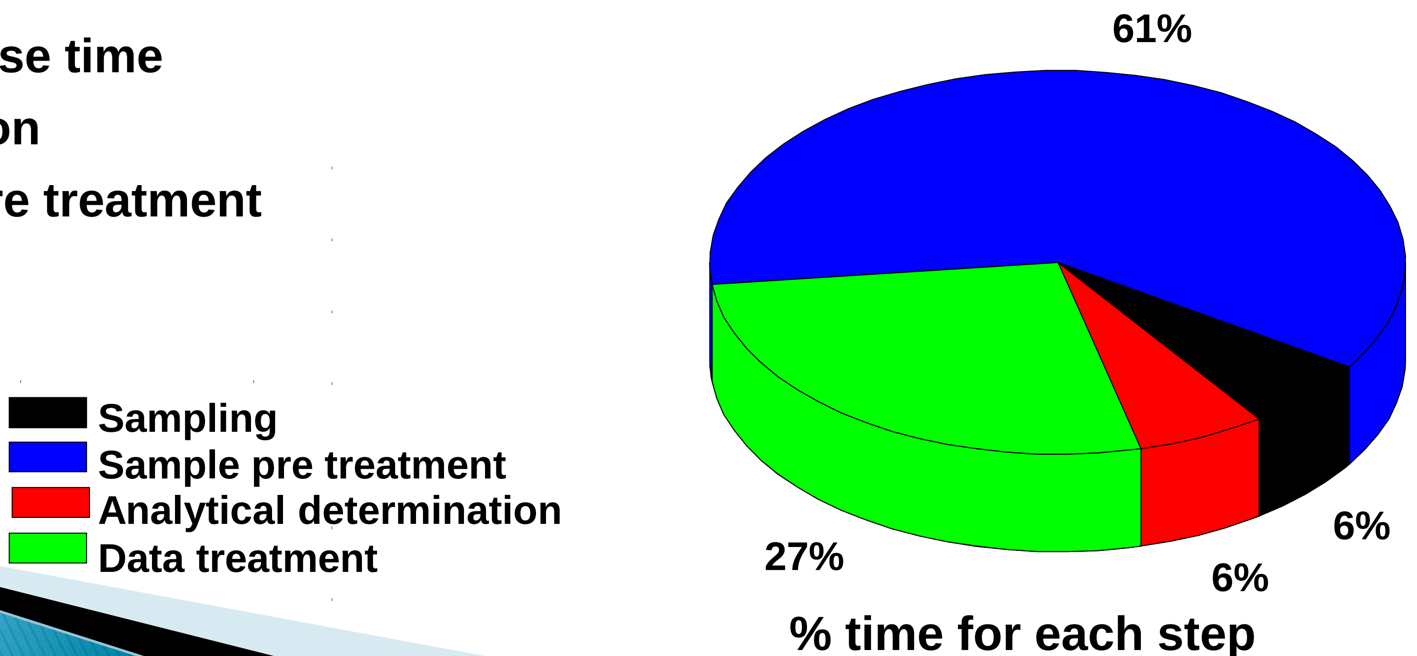
Sensors X Instrumental methods

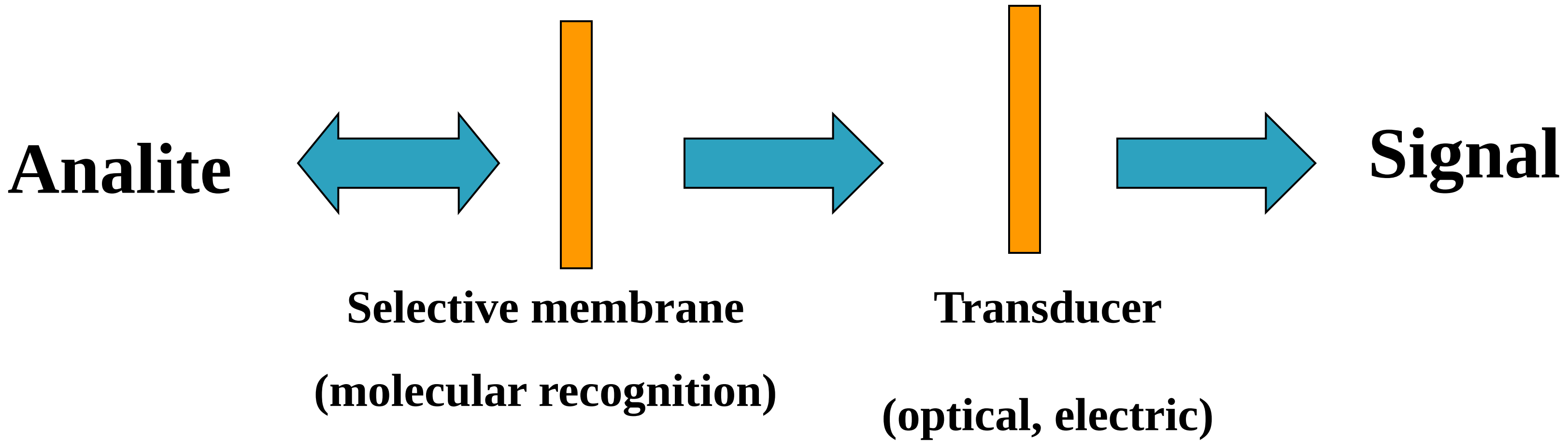
Sensors X Instrumental methods

- Real time field measurements (they are not so precise, but they can give an information on the concentration level)
- Information can be obtained at remote locations or with not easy (*in vivo monitoring,* ocean bottom, etc)
- Low cost
- Automation
- Portability
- Short response time
- Miniaturisation
- No sample pre treatment



Sensors: Definition

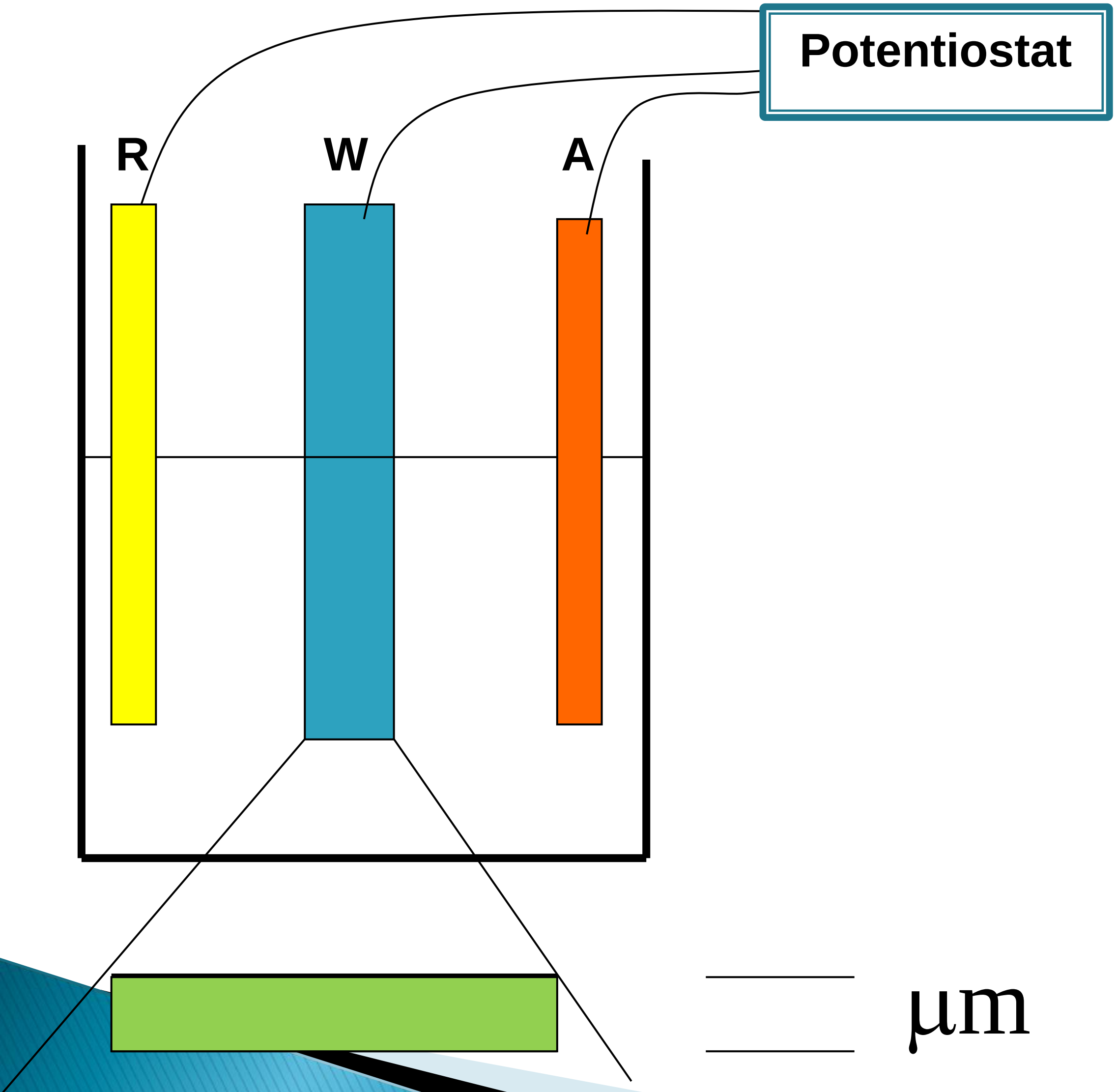
- Miniaturised transducers that operate in a selective and reversible way to give information on chemical compounds through electric signals dependent on concentration.



Sensors: Detected phenomenum = electric current

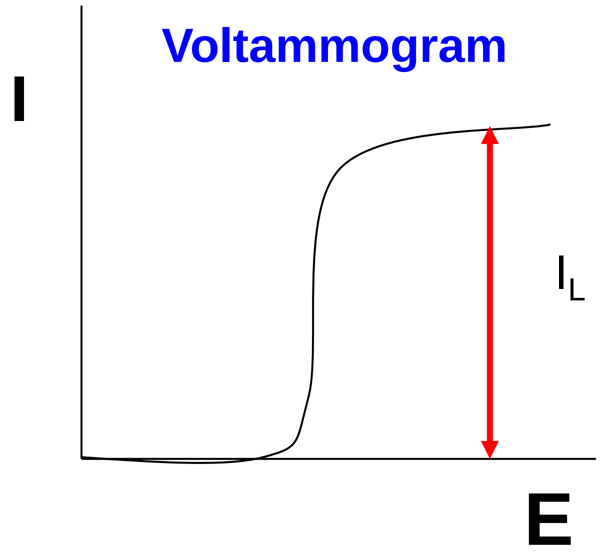
$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

An electrochemical experiment: basic configuration



1. Excitation
2. System (electrodes/solution)
3. Response

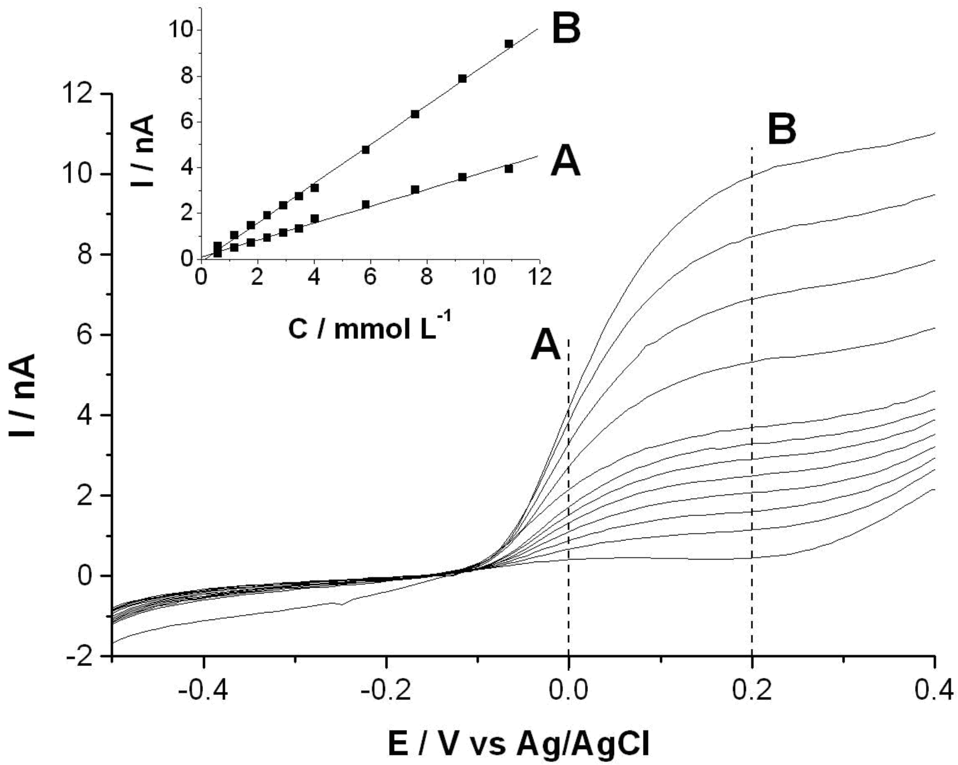
OX + e^- ⟶ RED



$$I_L = f\,(C)$$

Electrochemical experiments: analytical goal

<u>Electrochemical sensors</u> are evaluated both on their ability to detect the target compound in complex media (<u>selectivity</u>), and to detect a very small amount of material (<u>sensitivity</u>)



Voltammograms of <u>ascorbic acid</u> at different concentrations

Is it possible to get information at less positive (or negative) potentials with increased sensitivity?

Yes, by modifying the surface of the electrode!

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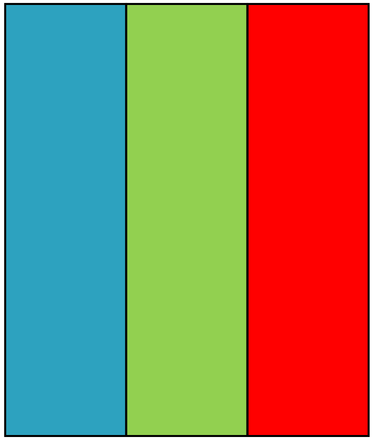
Modifying electrode surfaces

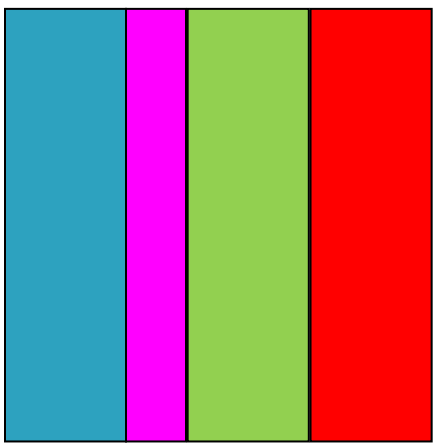
When the electrode material (Au, Pt, GC) does not allow the desired electron transfer reaction to occur under the selected experimental conditions, the surface can be tailored by activation approaches or immobilisation of mediators to make the investigated reaction more facile.

Electrode functionalisation (modified electrodes)

Electrode/Solution interface



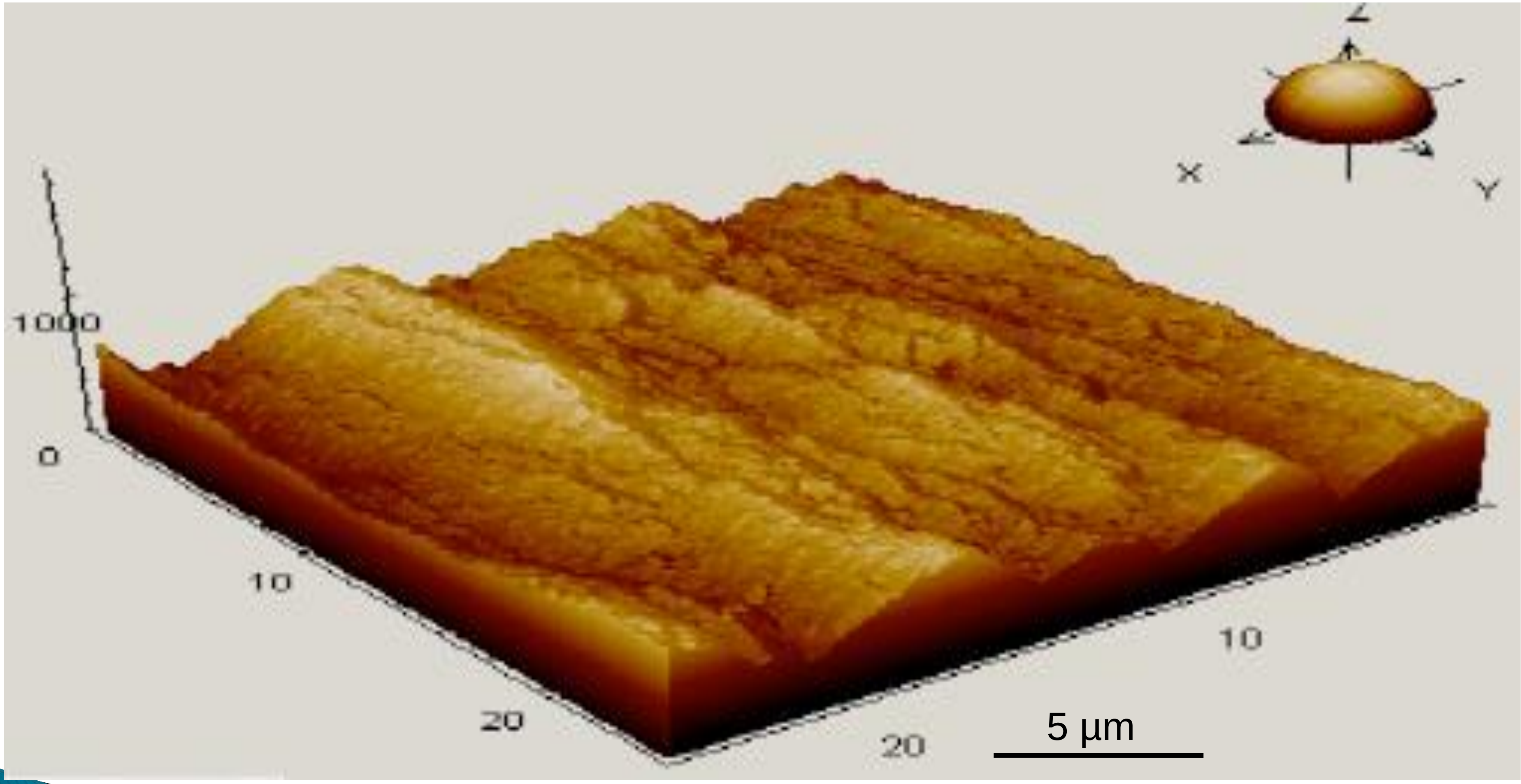
Electrode (conducting material)
Diffusion layer (µm)
Bulk solution



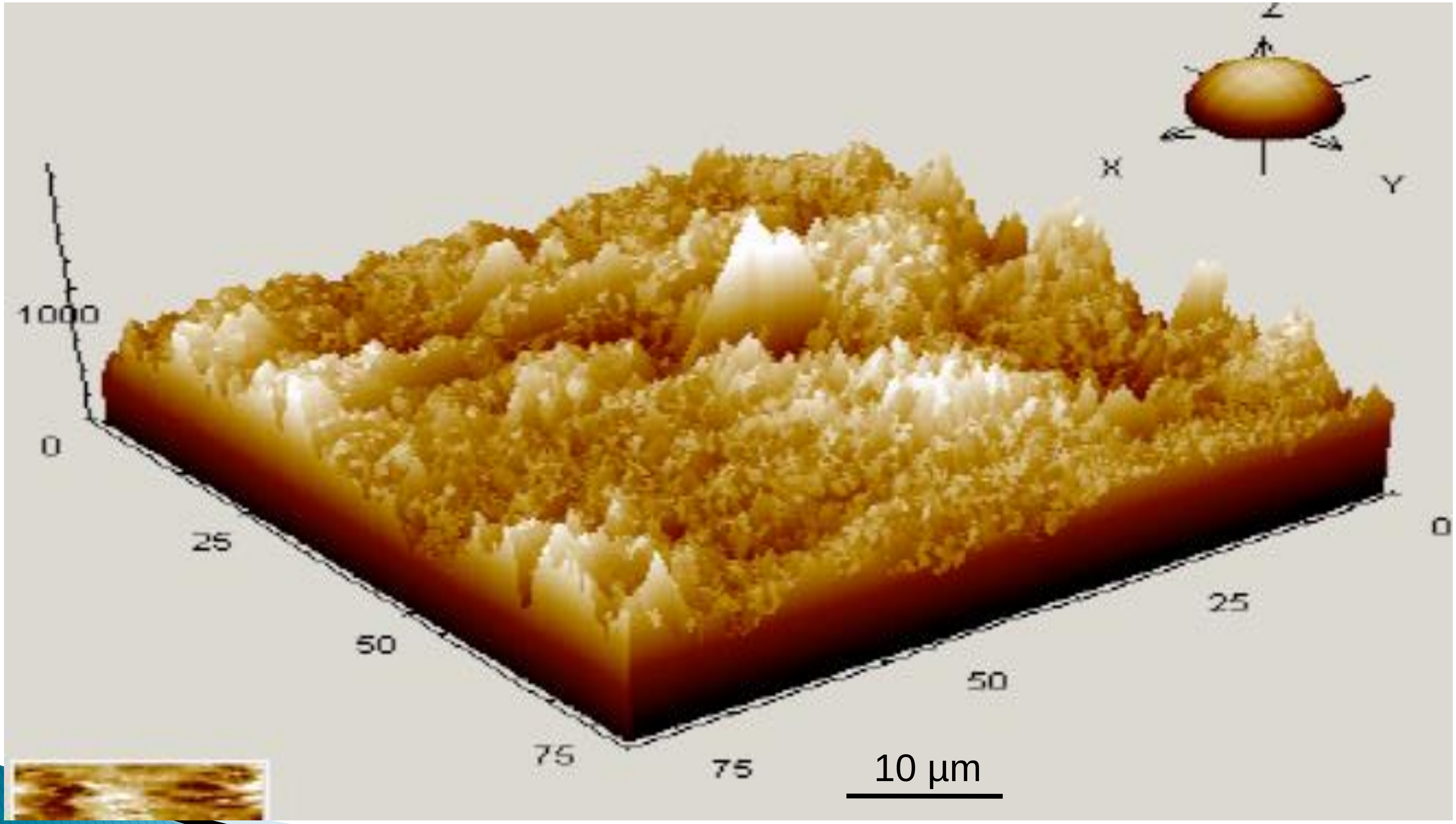
Electrode (conducting material)
Immobilised material (thickness = monolayer up to a few µm)
Diffusion layer (µm)
Bulk solution

Surface functionalisation: adsorption, covalent bonding, polymer film coating, electrochemical activation

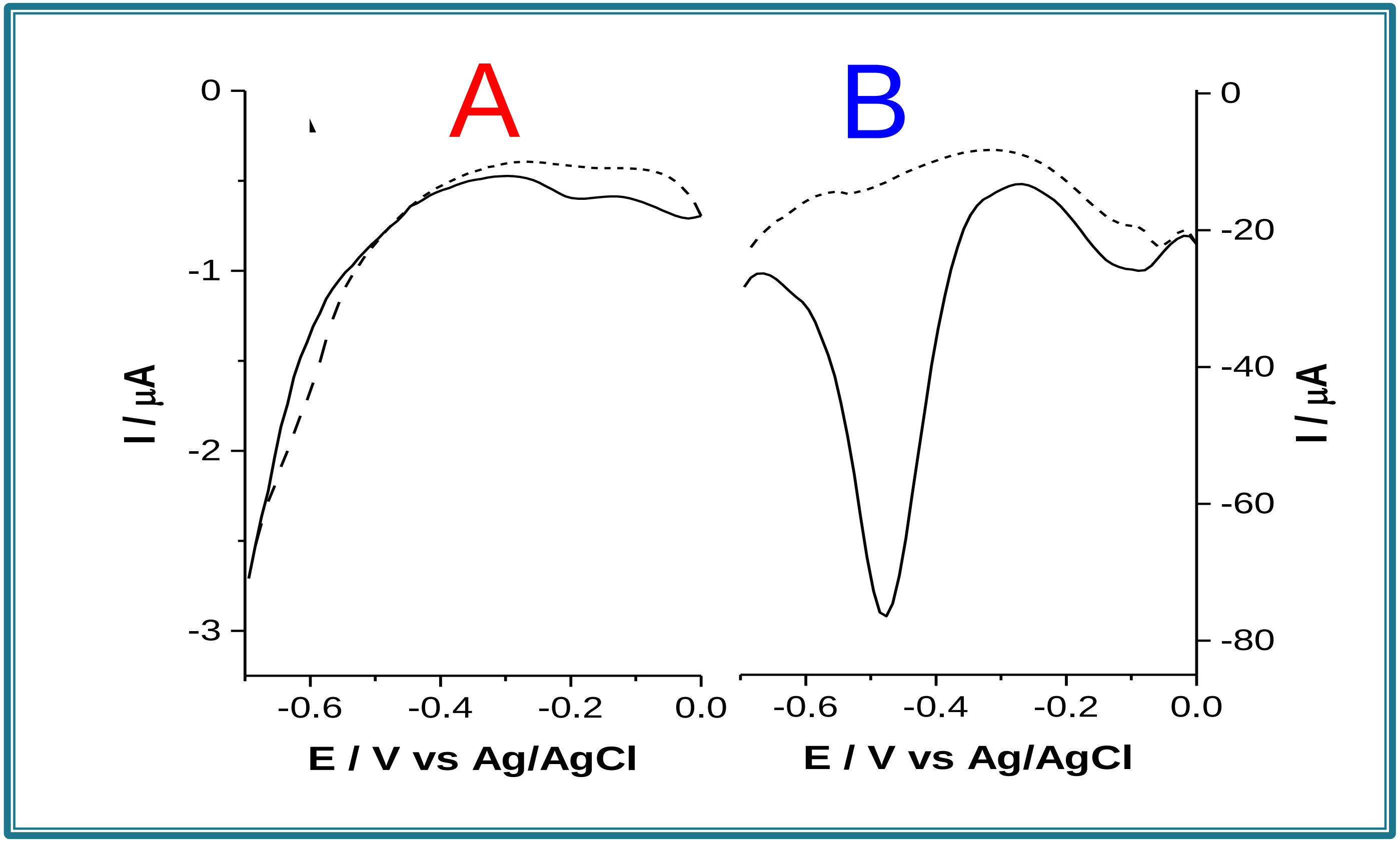
Bare copper surface



Activated copper surface



Cathodic reduction of nitrate at copper surfaces



- - - $0.1\ mol\ L^{-1}\ Na_2SO_4$, pH = 2

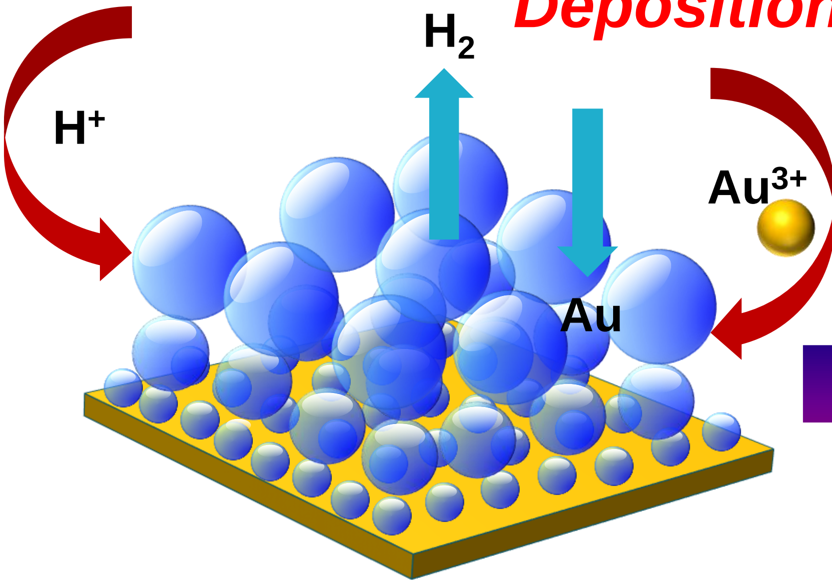
—— $0.1\ mol\ L^{-1}\ Na_2SO_4$ + **$1\ mmol\ L^{-1}\ NO_3^-$**, pH = 2

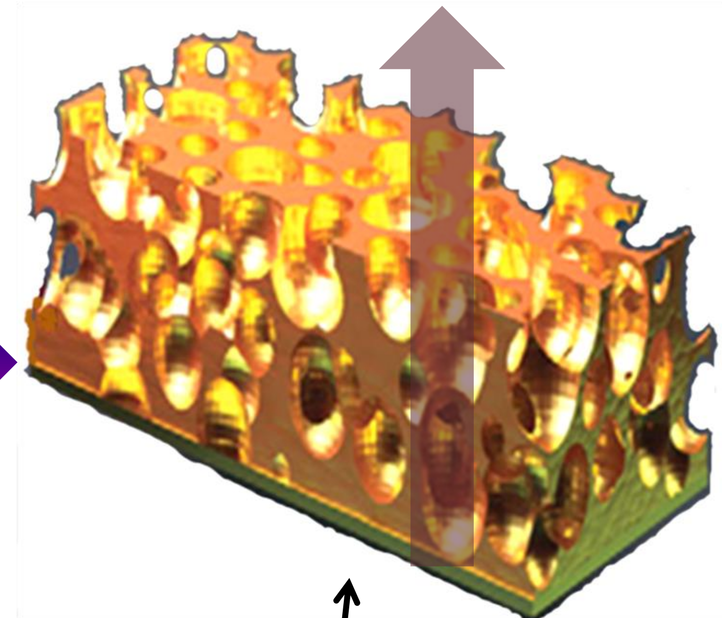
A: before activation

B: after activation

NPG sensor fabrication

Dynamic Hydrogen Bubble Template Deposition



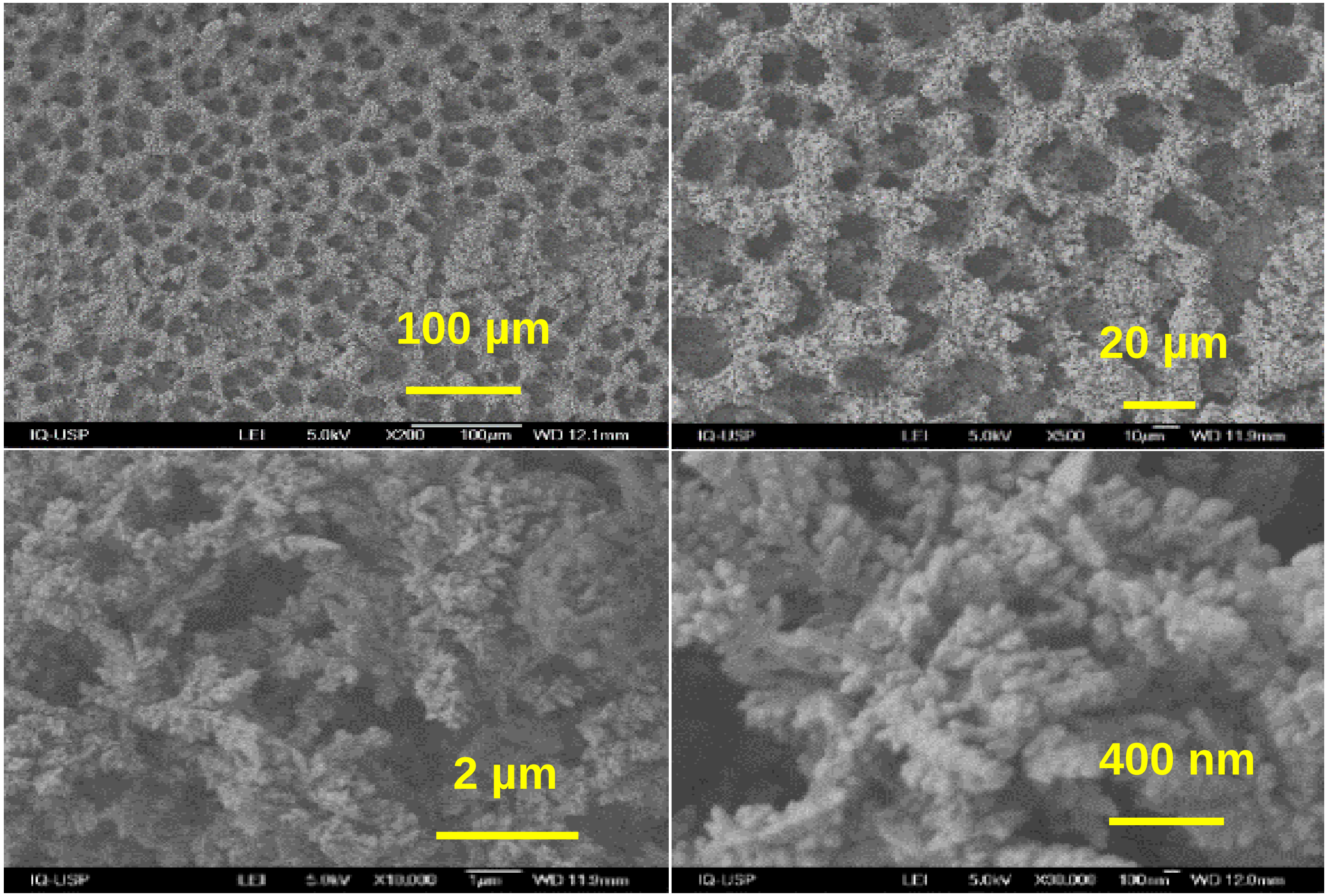


- Potentiostatic electrodeposition
- Hydrogen bubbles act as a template
- Control of rate and size of hydrogen bubbles by changing E_d and t_d

Tailoring morphology, structure and properties

Fabrication of HoneyComb-Like Dendritic porous gold surface

Au electrodeposition onto a Au electrode



FE-SEM images of the electrodeposited honeycomb-like dendritic porous Au film at different magnifications

NPG film characterisation

The thickness of the NPG film can be controlled by changing E_d and **t_d**

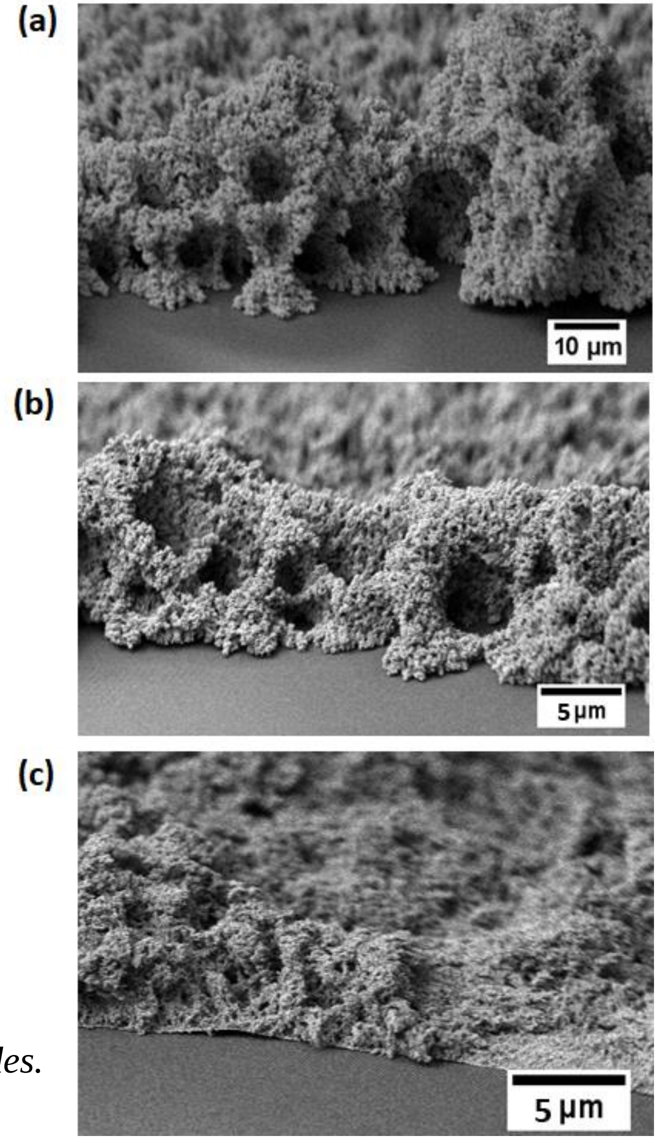
The higher **t_d**, the higher the thickness

NPG **3** > NPG **2** > NPG **1** > bare electrode (**0**)

NPG 3

NPG 2

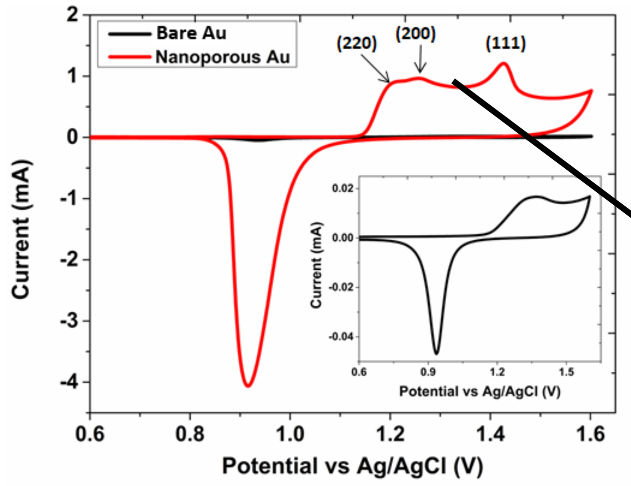
NPG 1



Cross-sectional SEM images of NPG3 (a), NPG2 (b) and NPG1 (c) films electrodeposited on gold electrodes.

NPG film characterisation

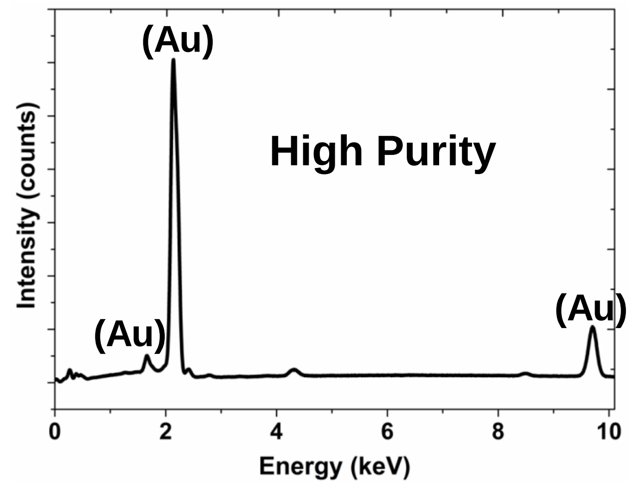
Cyclic Voltammetry in H_2SO_4



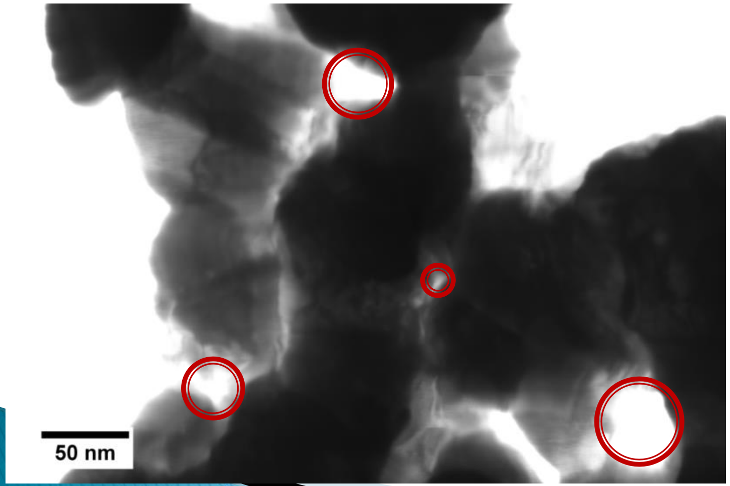
Increased current because of the enhanced area

Evolution of new low-indexed planes in NPG

Energy Dispersive Spectroscopy

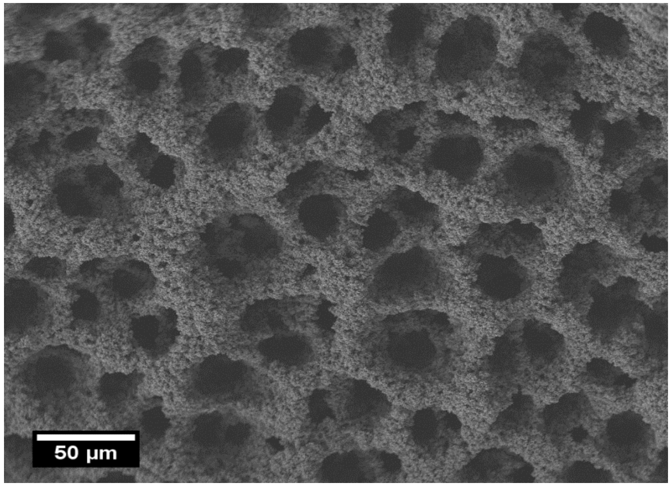


Transmission Electron microscopy

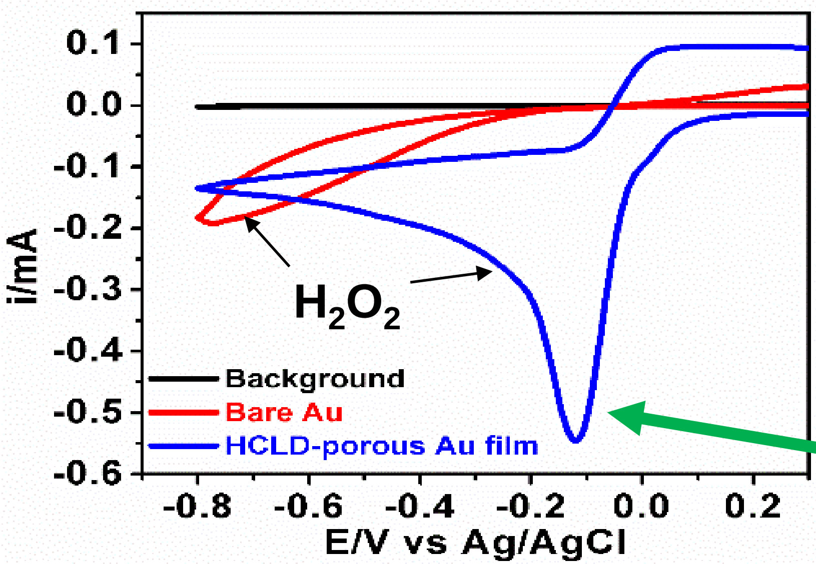


Nanometric &micrometric pores

Scanning Electron microscopy

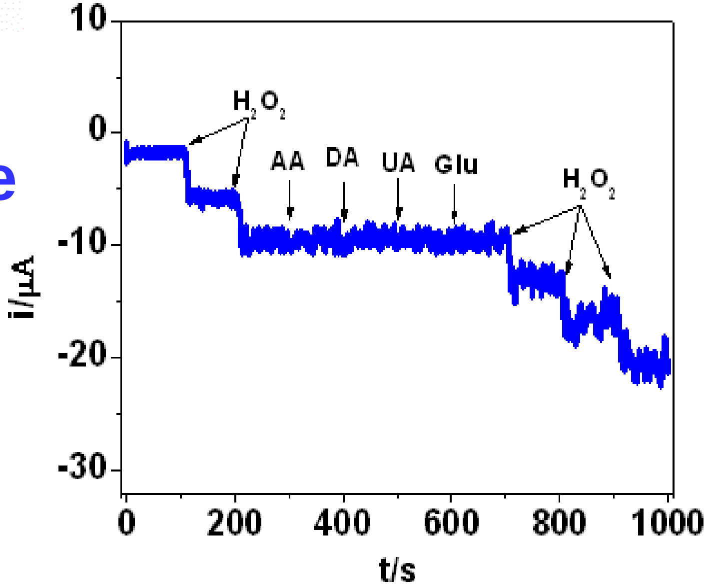


NPG modified electrode: reduction of H_2O_2



Potential shift has no relationship with the increased surface area of NPG3

E = -0.1 V



Bare gold electrode

NPG3 modified electrode

Fabrication of HoneyComb-Like Dendritic porous gold surface

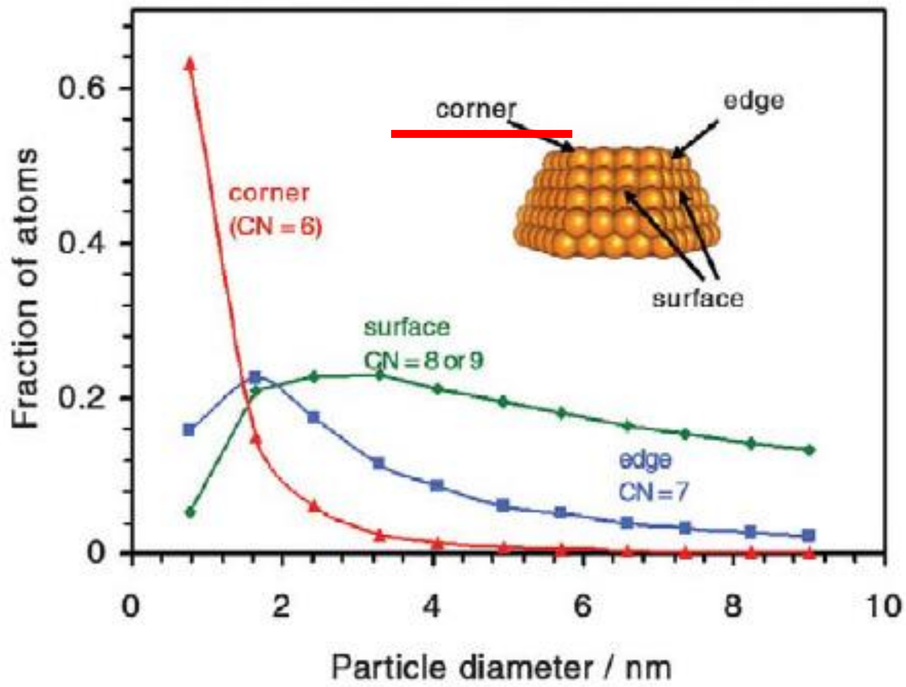


Fig. 5 Calculated fractions of Au atoms at corners (red), edges (blue), and crystal faces (green) in uniform nanoparticles consisting of the top half of a truncated octahedron as a function of Au particle diameter. The insert shows a truncated octahedron and the position of representative corner, edge, and surface atoms. (Reproduced with permission from[34]. © 2007 Springer.)

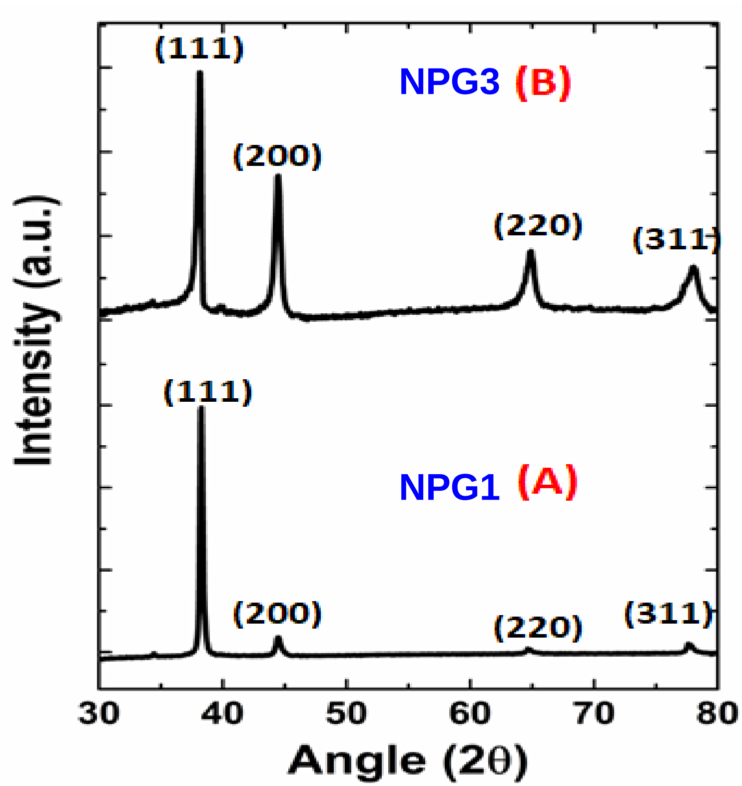
Electrocatalytic effect???

nanotoday AUGUST 2007 | VOLUME 2 | NUMBER 4

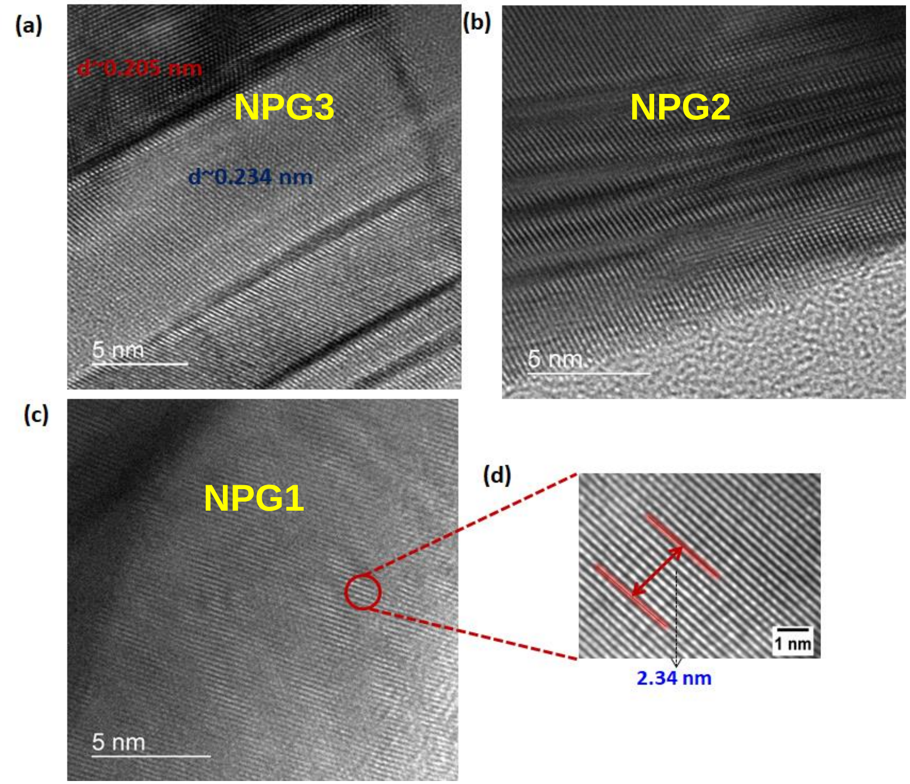
Low-coordinated atoms are more catalytically active!

but in this review we have pointed to one very important effect: the increased reactivity of low-coordinated Au atoms. Such atoms are particularly abundant on the smallest nanometer-sized nanoparticles, and this may go a long way toward explaining the catalytic activity of such Au nanoparticles.

NPG film characterisation



XRD pattern *of NPG1 (A) and NPG3 (B) films electrodeposited on a gold substrate using Cu K-α X-ray (1.54 Å).*



High resolution ***TEM images*** *of NPG3 (a), NPG2 (b) and NPG1 (c) samples showing different defects. Zoomed view of the lattice fringes and representation of interplanar distance (d).*

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Electrochemical sensors for real time, in situ detection in microenvironments

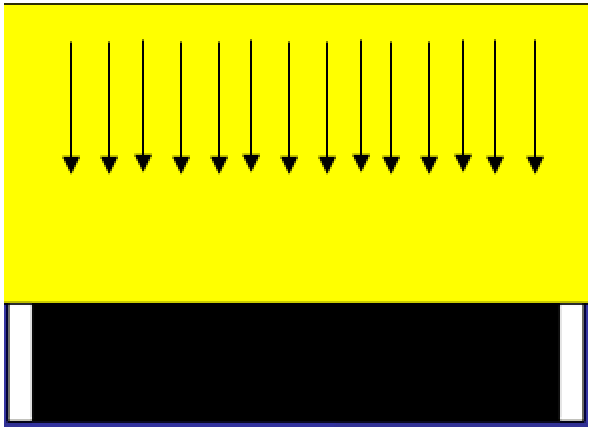
Advantages of using microelectrodes:

- Diffusion to the electrode surface is very efficient, hence current reflects the **real time** concentration at a specific solution region with no temporal dependence.
- They can be fabricated with very thin glass capillaries, so inspection can be performed in **very small environments**.

Diffusion at Disc Microelectrodes

Macroelectrodes

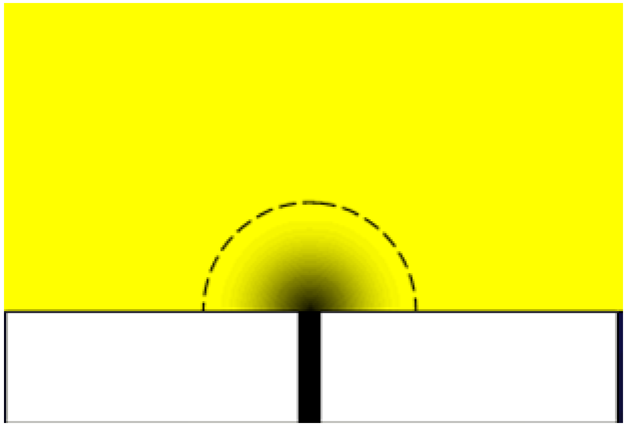
$$i(t) = \frac{nFAD^{1/2}C^{\infty}}{\pi^{1/2}t^{1/2}}$$ → Cottrell equation



$$\delta = \sqrt{\pi D t}$$

Microelectrodes

$$i_{ss} = \frac{nFADC^{\infty}}{r_s}$$

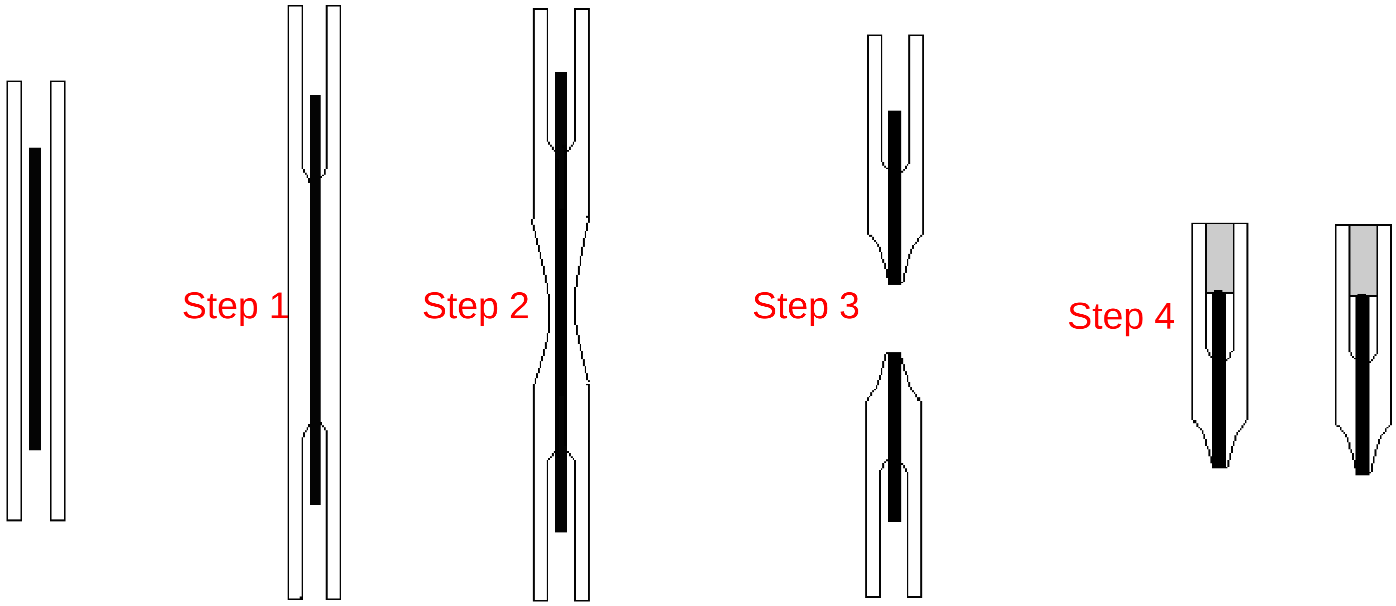


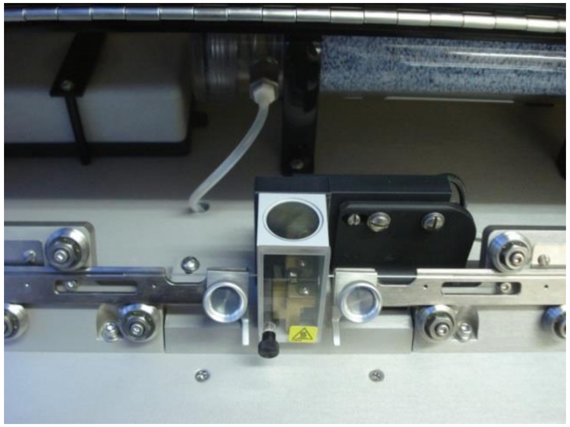
$$i(t) = \frac{nFADC^{\infty}}{r_s} + \frac{nFAD^{1/2}C^{\infty}}{\pi^{1/2}t^{1/2}}$$

Two limiting regimes;

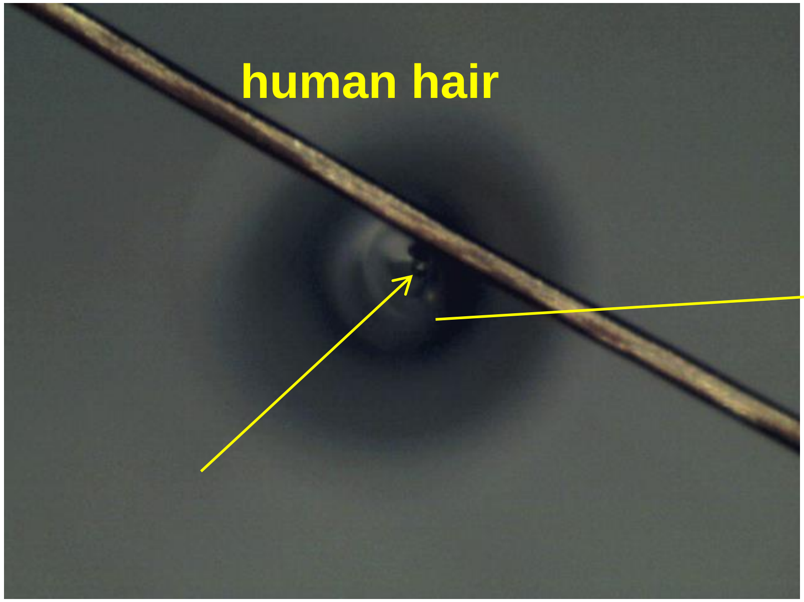
Long Times — Short Times

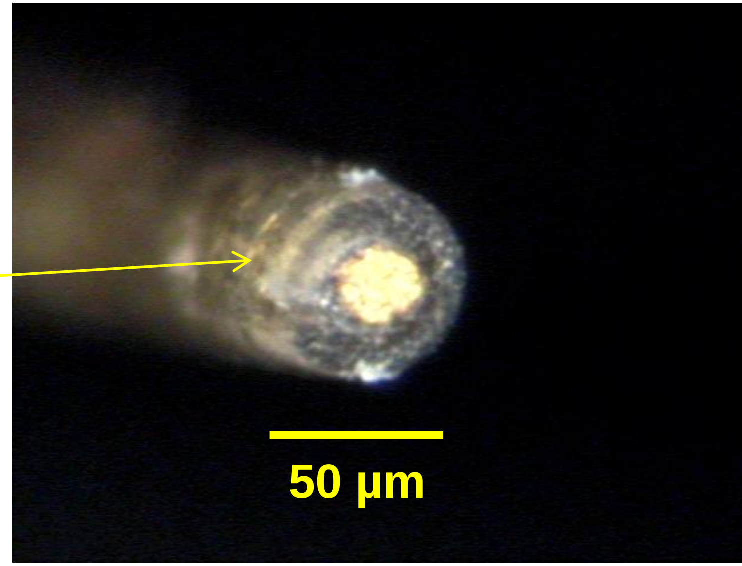
Fabrication methods – disc microelectrode

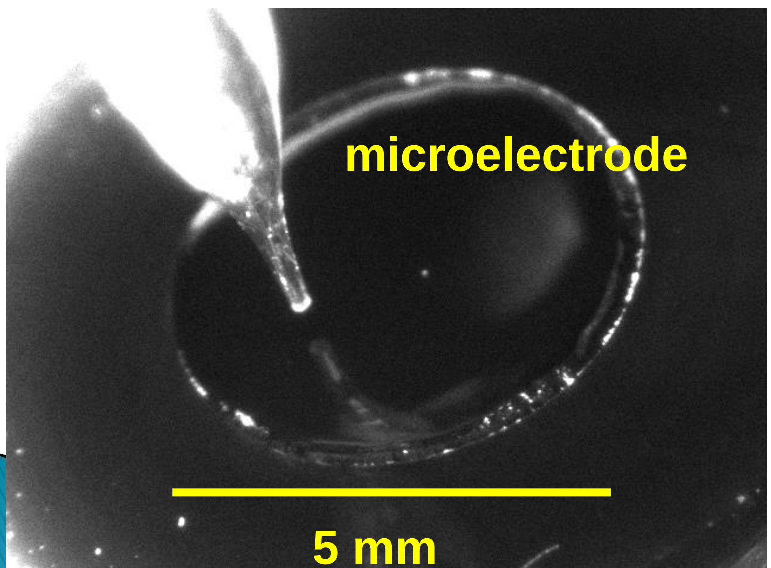


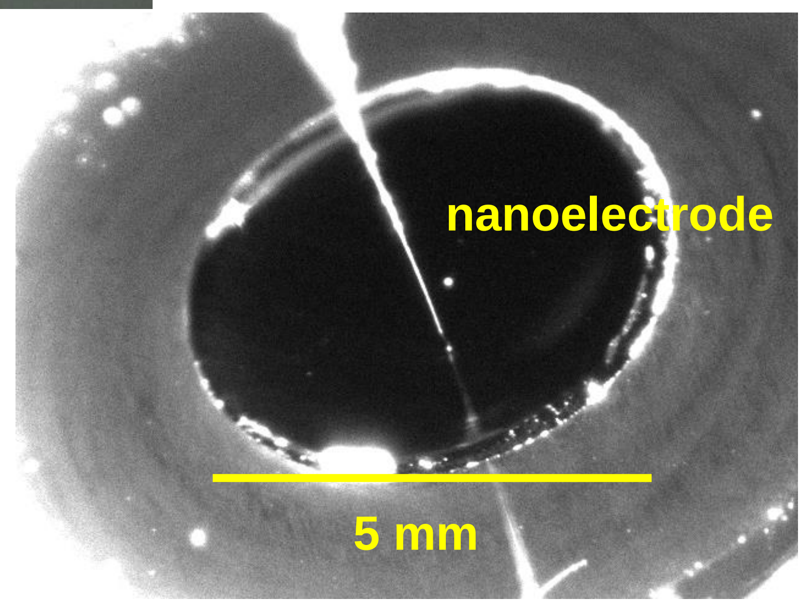


Fabrication methods – towards disc nanoelectrodes





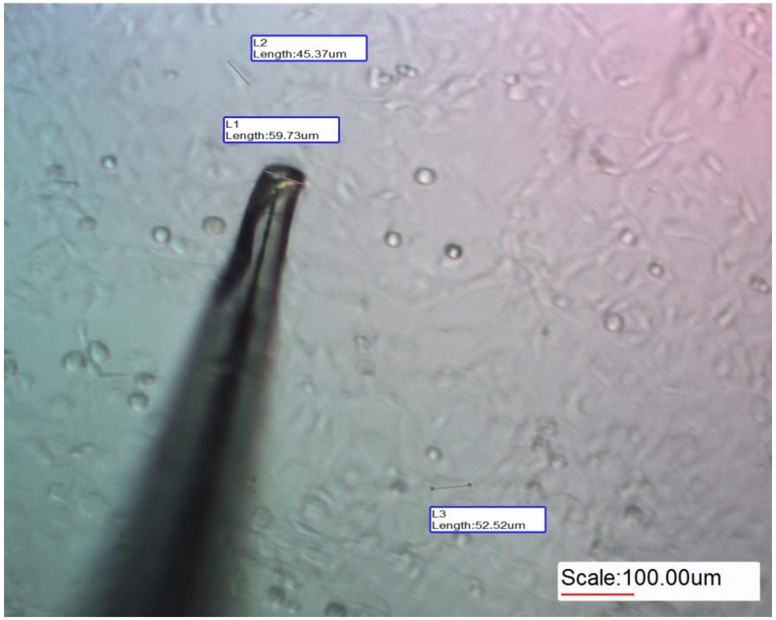




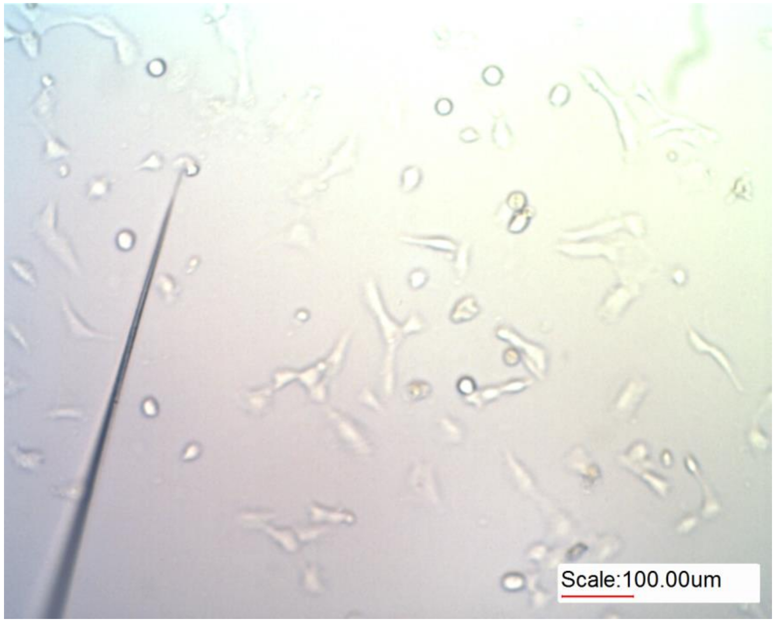
Enhanced resolution !!

Fabrication methods – towards disc nanoelectrodes

Microelectrode

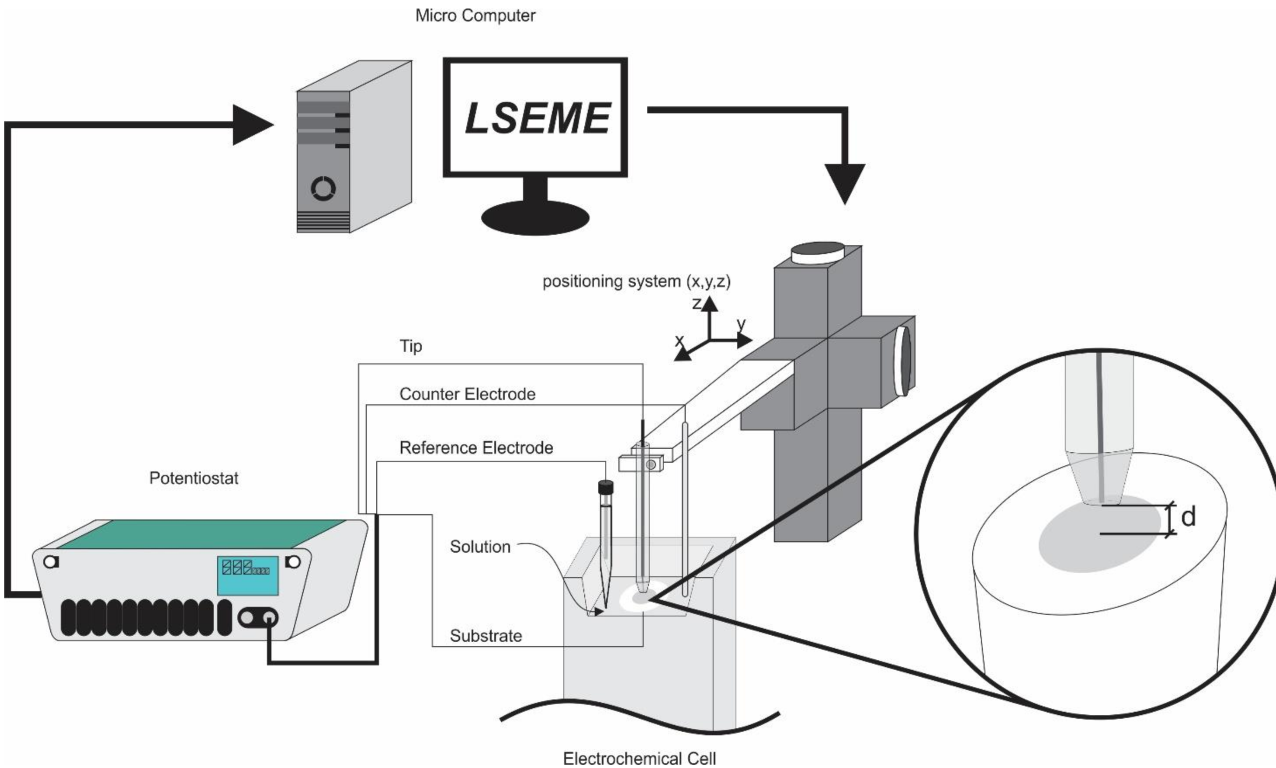


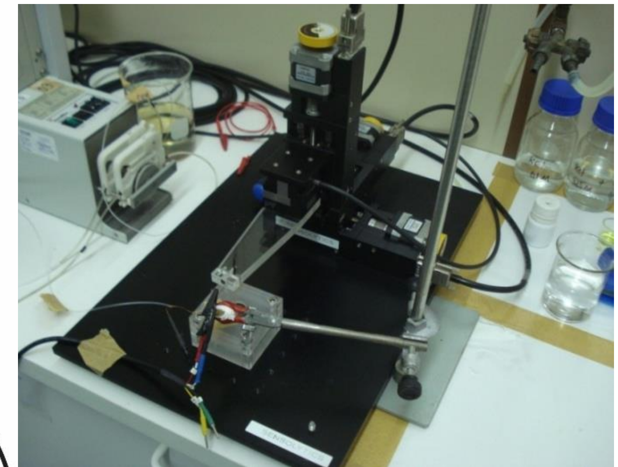
Nanoelectrode

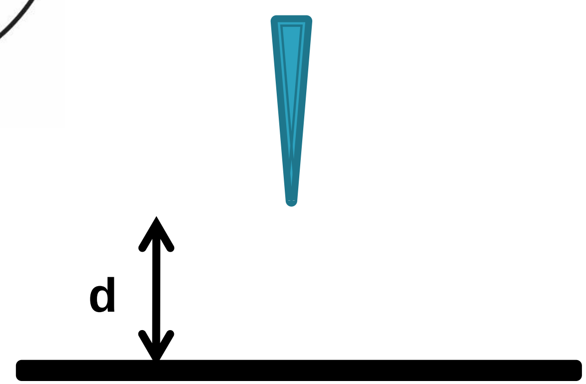


Breast cancer cells (HS578T)

A powerful tool: Scanning Electrochemical Microscopy (SECM)



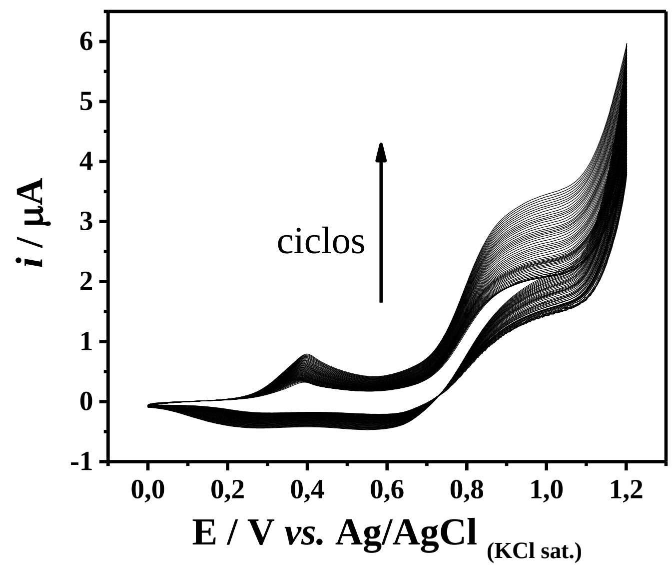




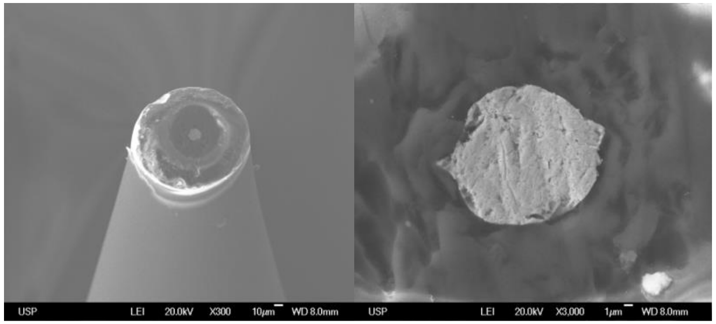
Substrate (conductive or insulating)

1. Proton consumption during nitrate reduction

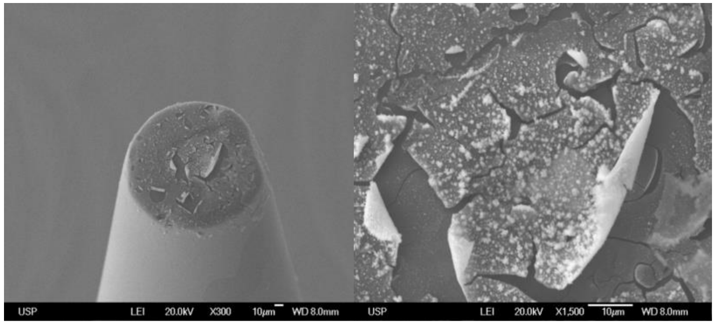
IrO_x film electrochemical deposition



Bare microelectrode



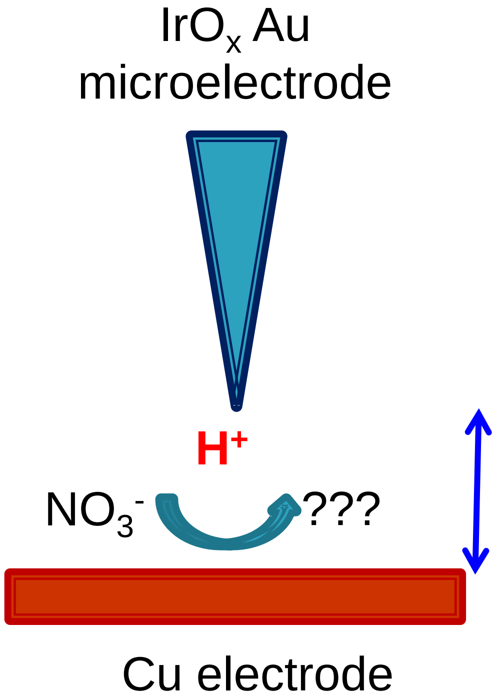
Modified microelectrode

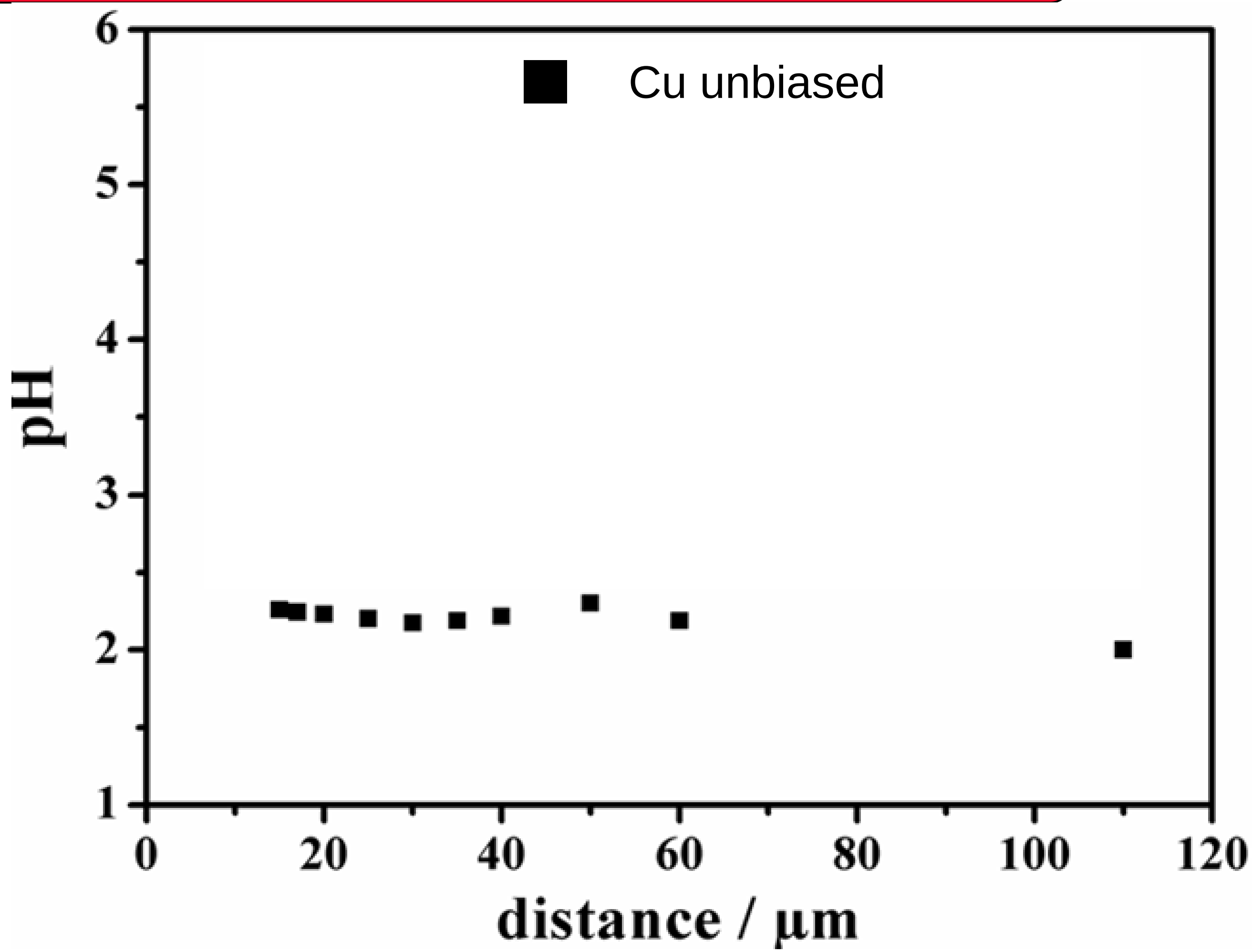


$$[Ir(COO)_2(OH)_4]^{2-}_{aq} \rightarrow IrO_{2(s)} + 2CO_2 + 2H_2O + 2e^-$$

C. S. Santos, et al., *Electroanalysis*, *28* (**2016**) 1441

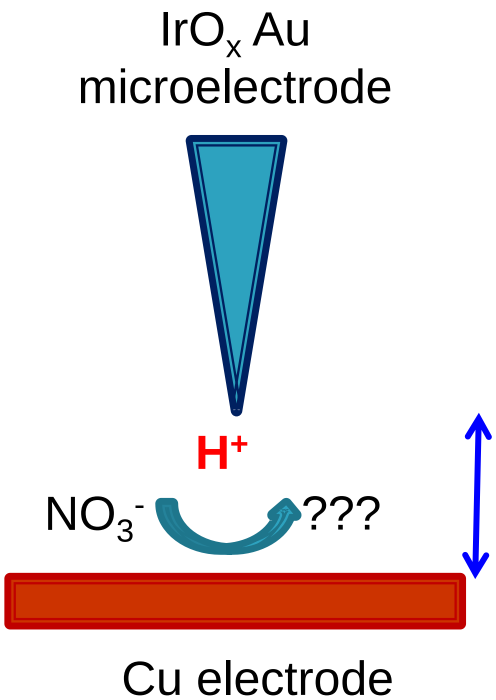
Proton consumption during nitrate reduction

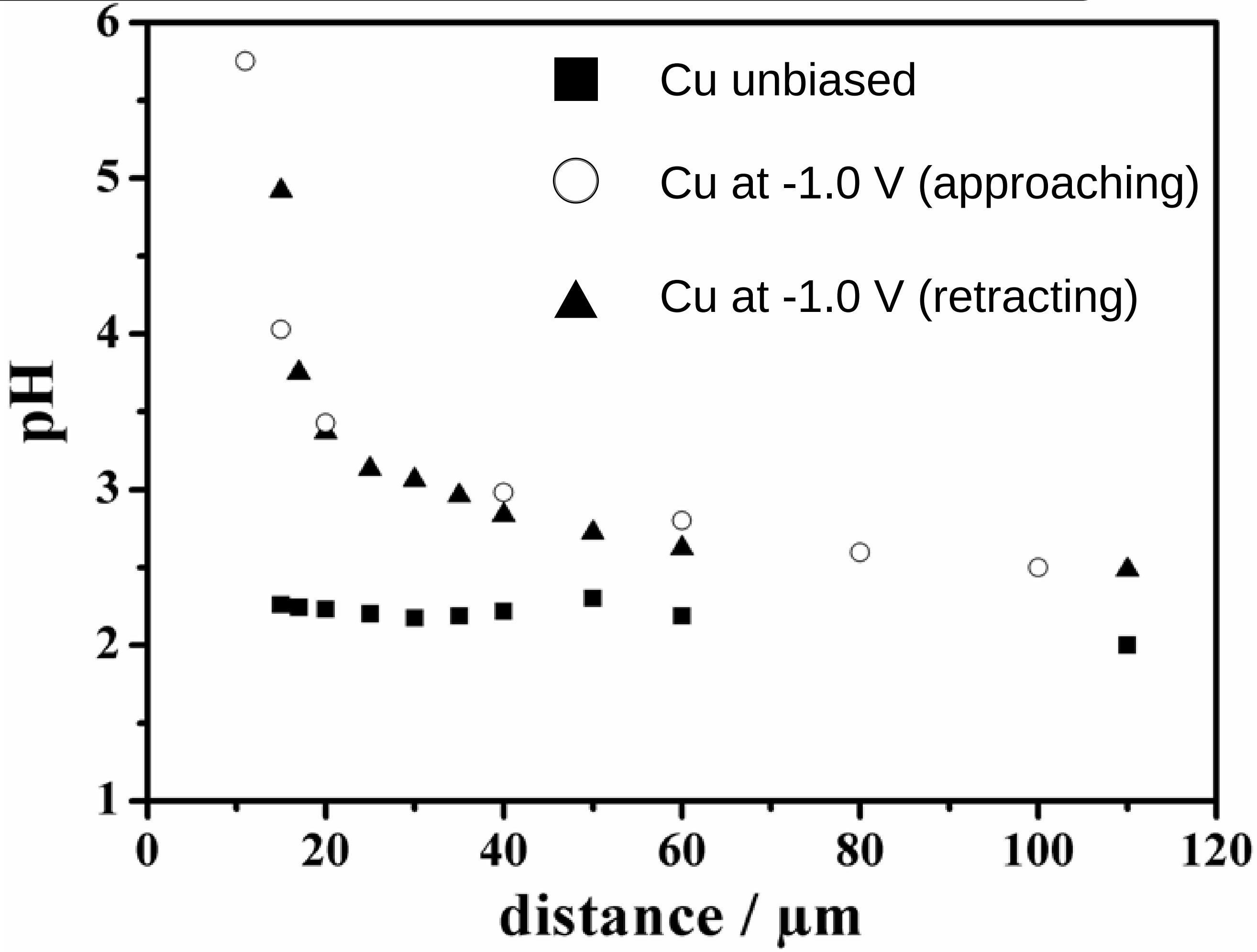




0.1 M Na_2SO_4 (pH = 2) + 0.5 mM NO_3^-

Proton consumption during the nitrate reduction





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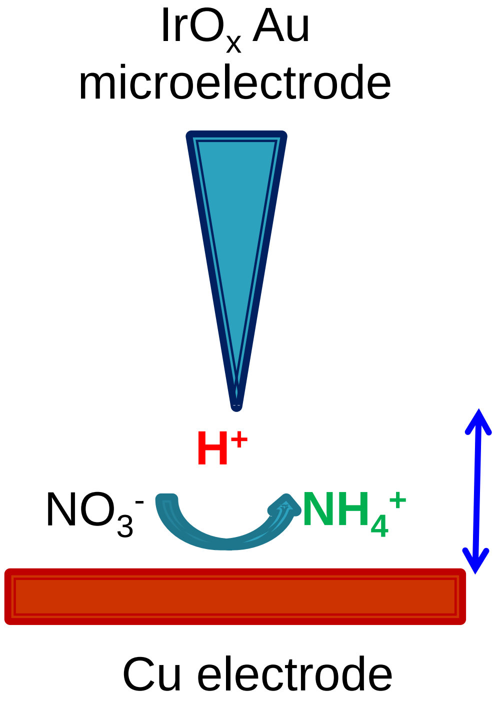
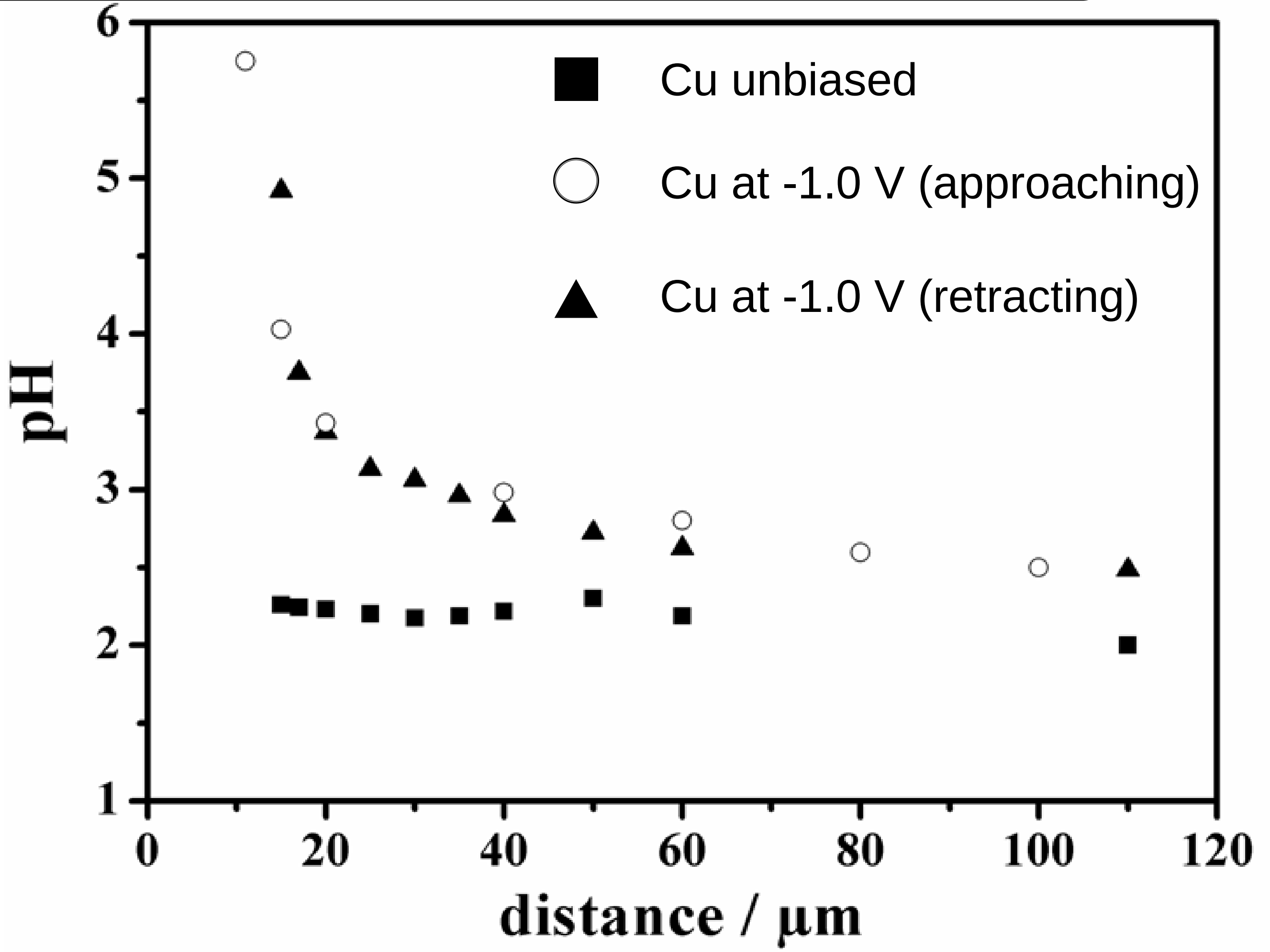




0.1 M Na_2SO_4 (pH = 2) + 0.5 mM NO_3^-

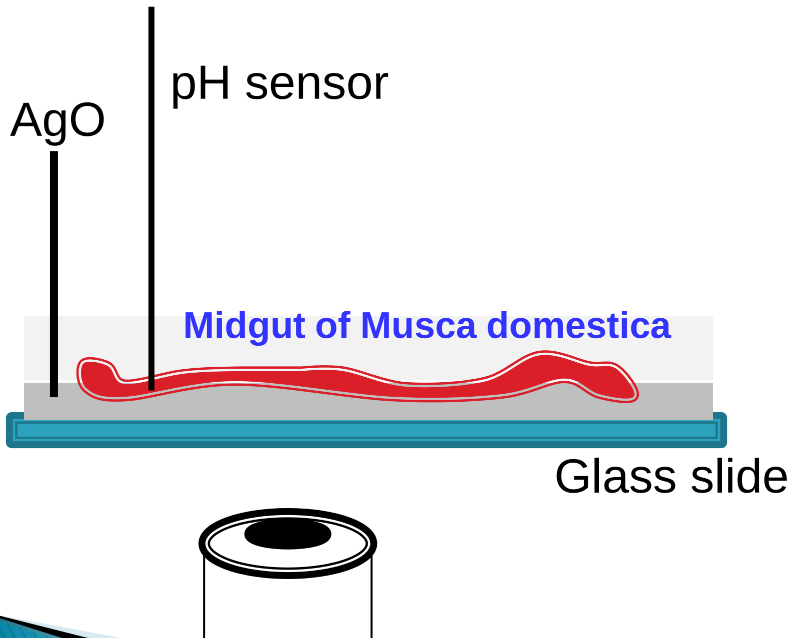
2. In vivo pH measurements

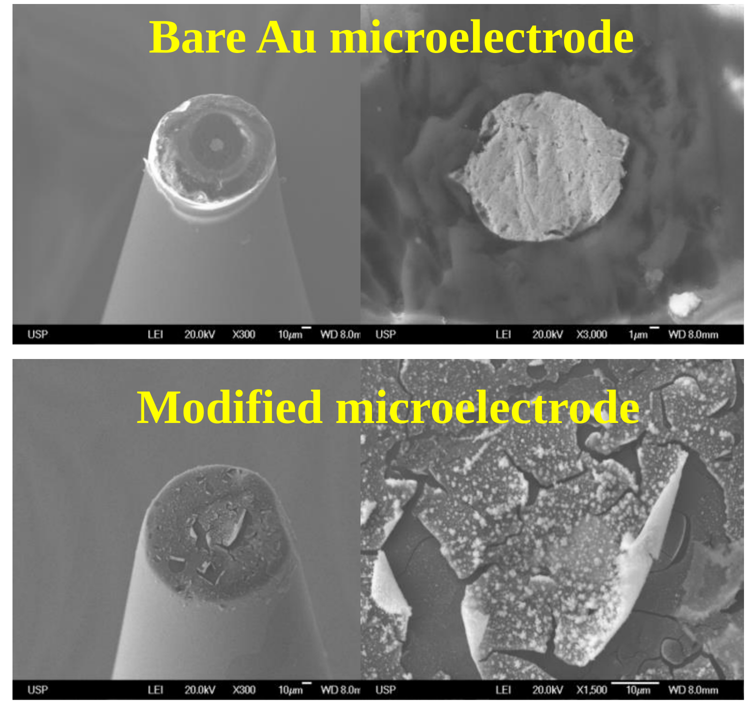
The pH of midgut contents affects digestive enzymes

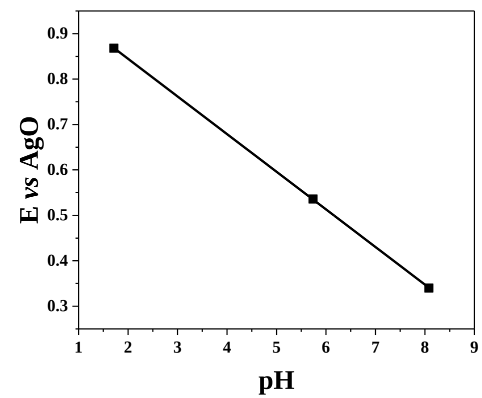
Laboratory of Insect Biochemistry

Coordinators: Prof. W. Terra and Clélia Ferreira

Biochemistry Department (USP)

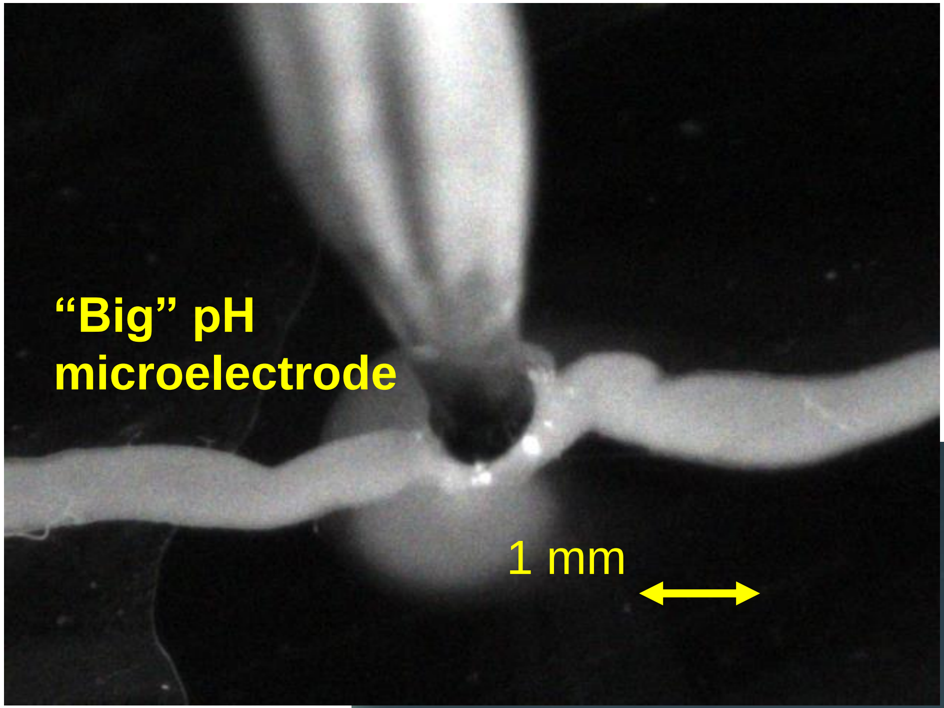


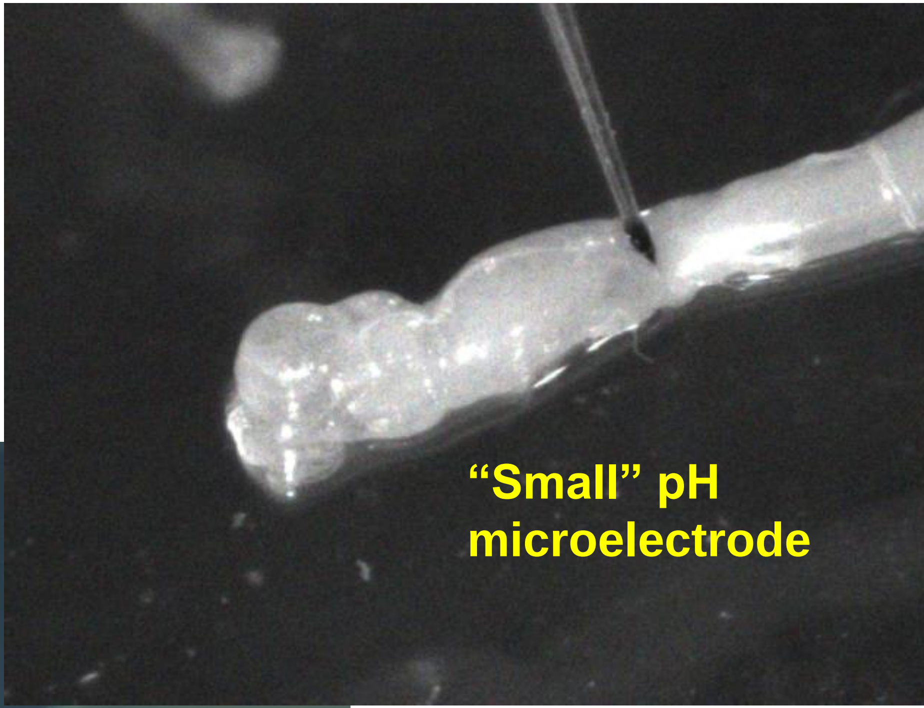


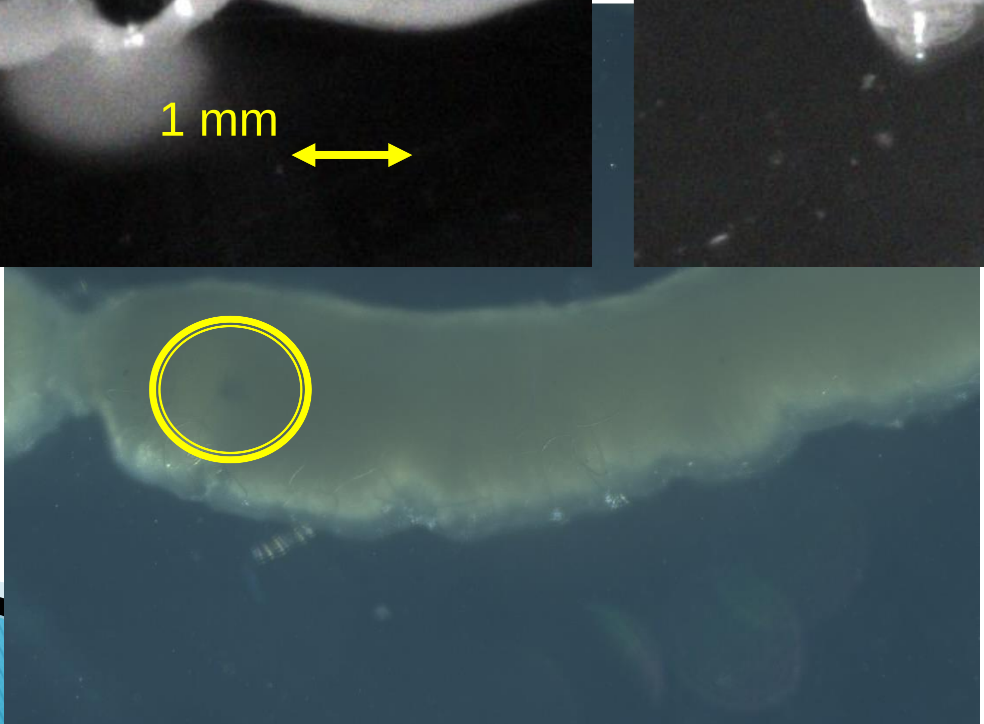


In vivo pH measurements

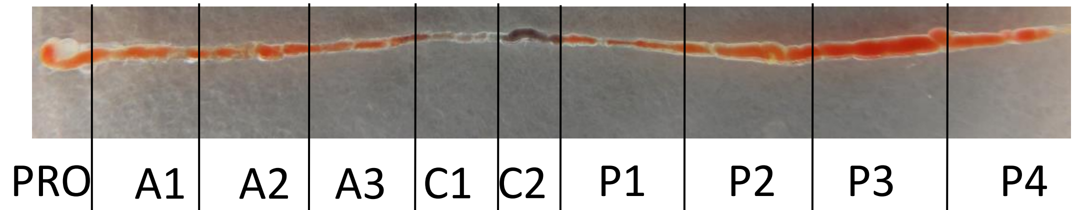
Musca domestica midgut: activity of enzymes depends on pH







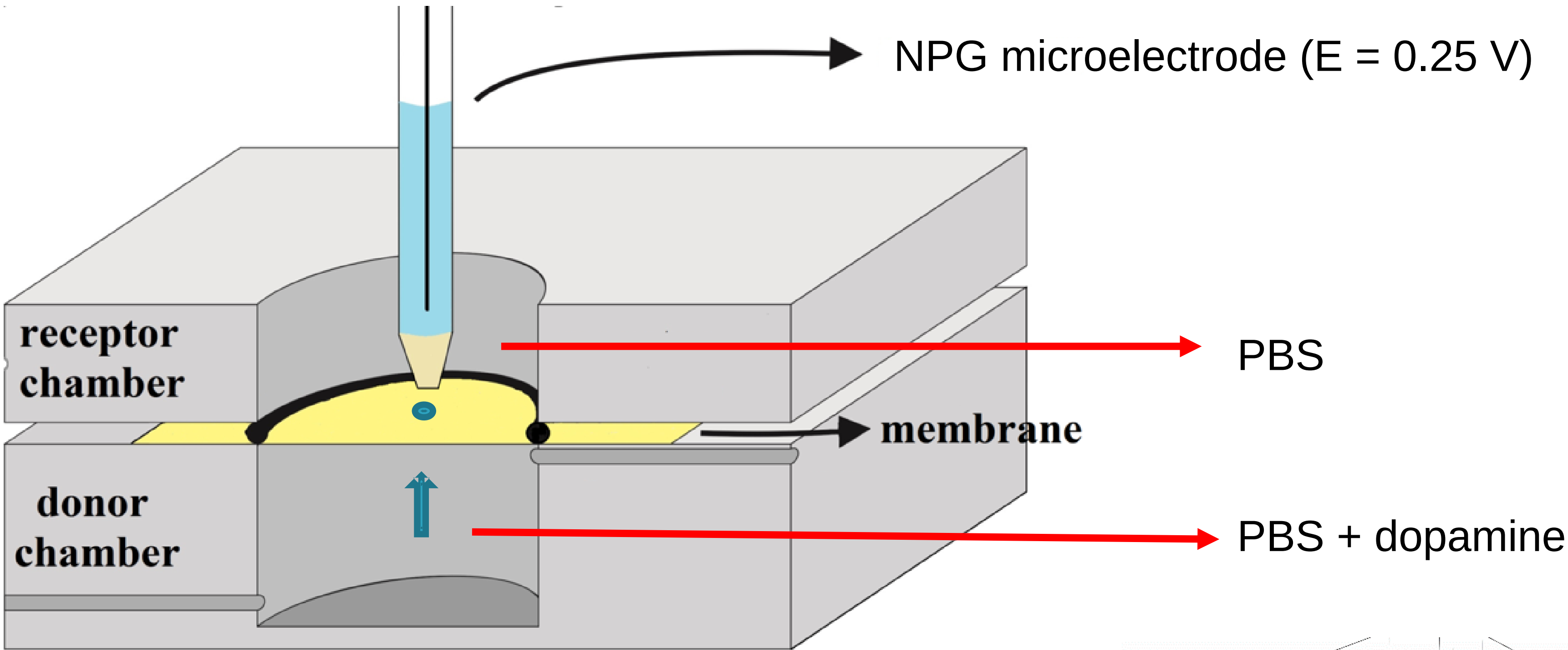
pH values from 7 to 2 were noticed along the midgut

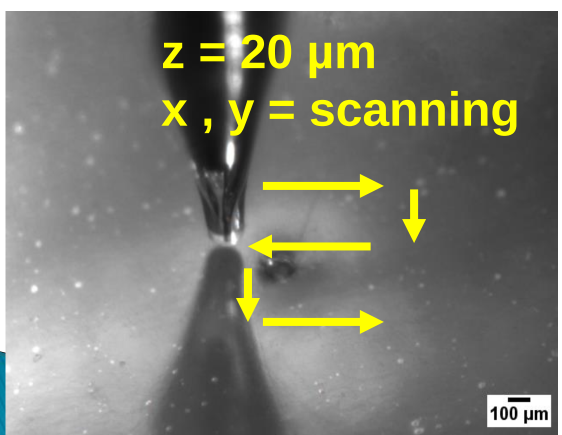


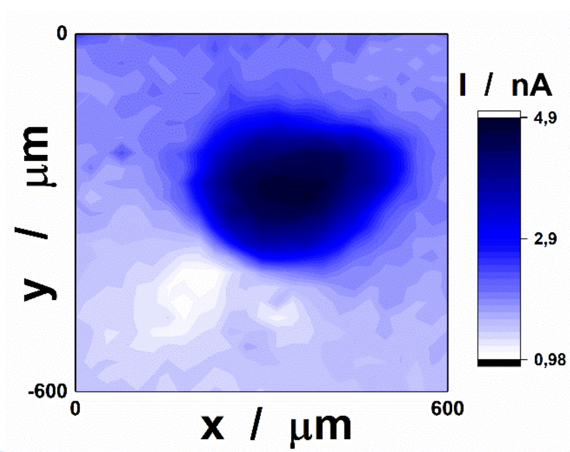
Food	Section	Section	Average pH (n=5)	Section average pH
Protein + carbohydrate	A	Pro	6,71	6.6
		A1	6,59	
		A2	6,77	
		A3	6,17	
	C	M1	5,82	5.1
		M2	4,45	
	P	P1	6,68	6.7
		P2	7,53	
		P3	7,47	
		P4a	6,56	
		P4b	5,37	
Starch	A	A1	5,34	5.4
		A2	5,62	
		A3	5,23	
	C	M1	4,29	3.4
		M2	2,49	
	P	P1	5,53	5.9
		P2	6,18	
		P3	6,44	
		P4b	5,56	
Hydrolysed protein	C	M2	2,54	2.5
	P	P1	7,24	7.7
		P2	7,87	
		P3	8,02	
		P4b	7,73	
Ovoalbumin	C	M2	2,82	2.8
	P	P1	5,33	5.7
		P2	5,89	
		P3	6,17	
		P4	5,30	

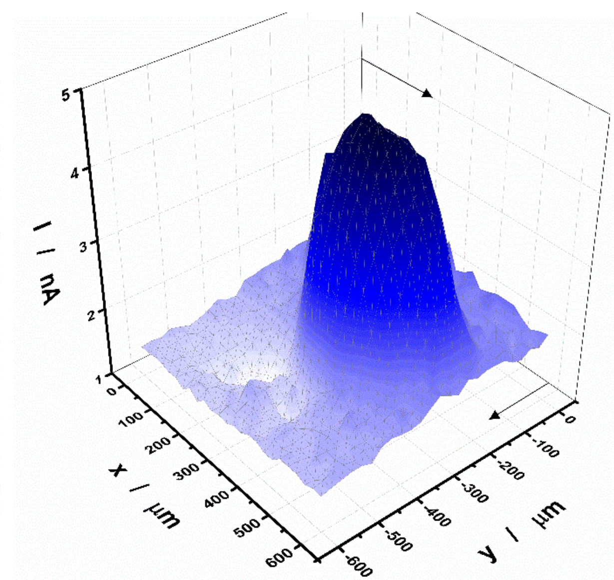
Acidification occurs at middle midgut through a mechanism involving a particular enzyme

3. Simulating dopamine release: transport through channels







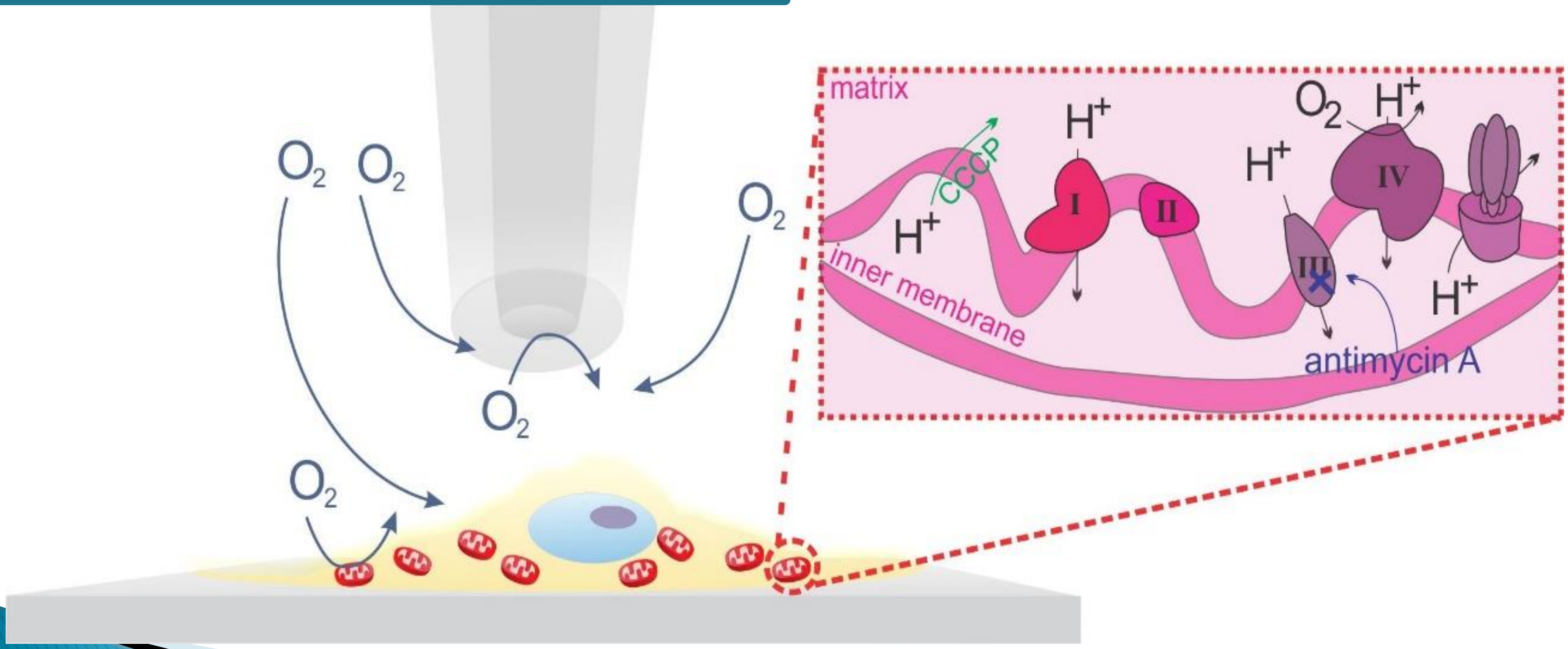


4. Localised monitoring of O_2 consumption

Energy and Metabolism Laboratory

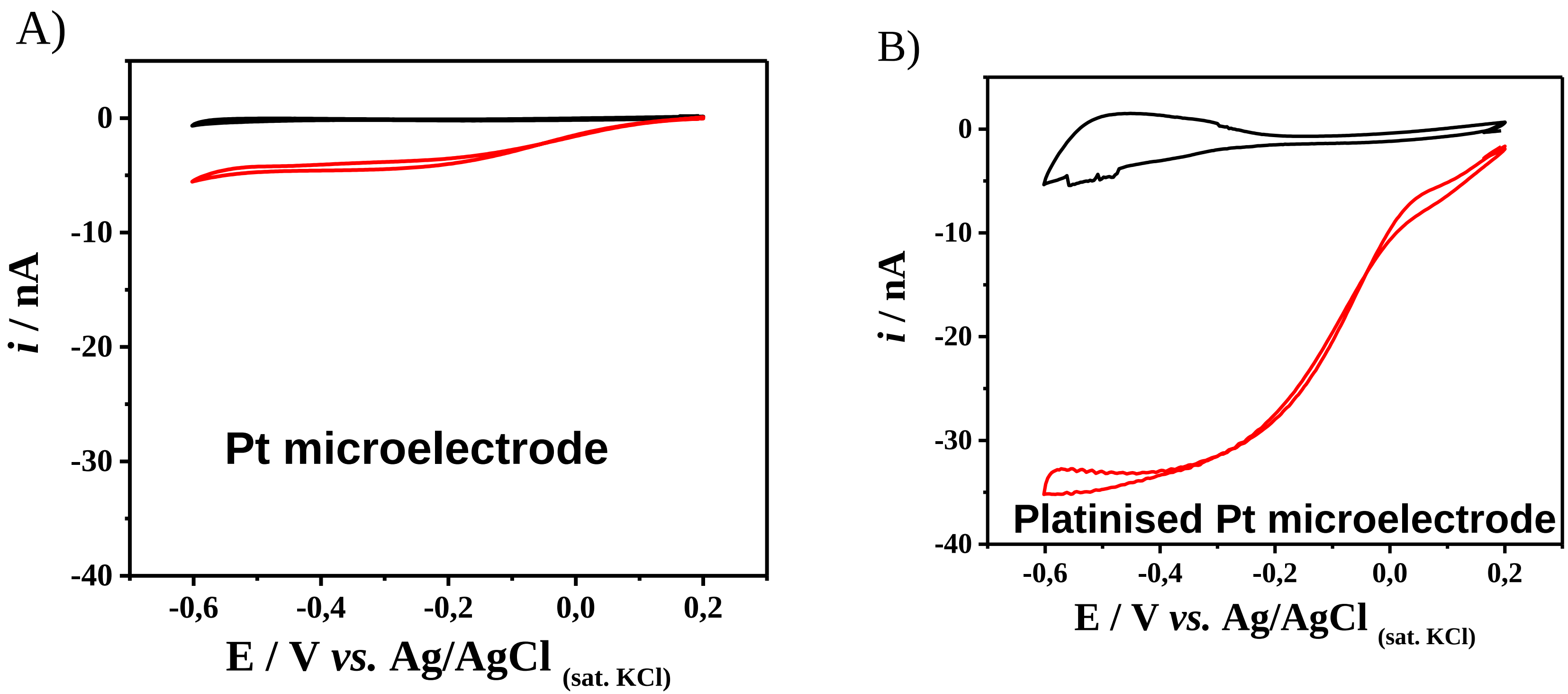
Coordinator: Prof. Alicia Kowaltowski

Biochemistry Department (USP)

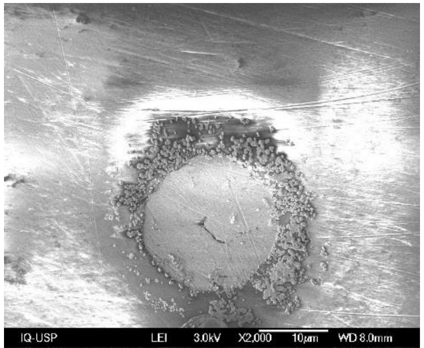


Platinisation of Pt microelectrodes

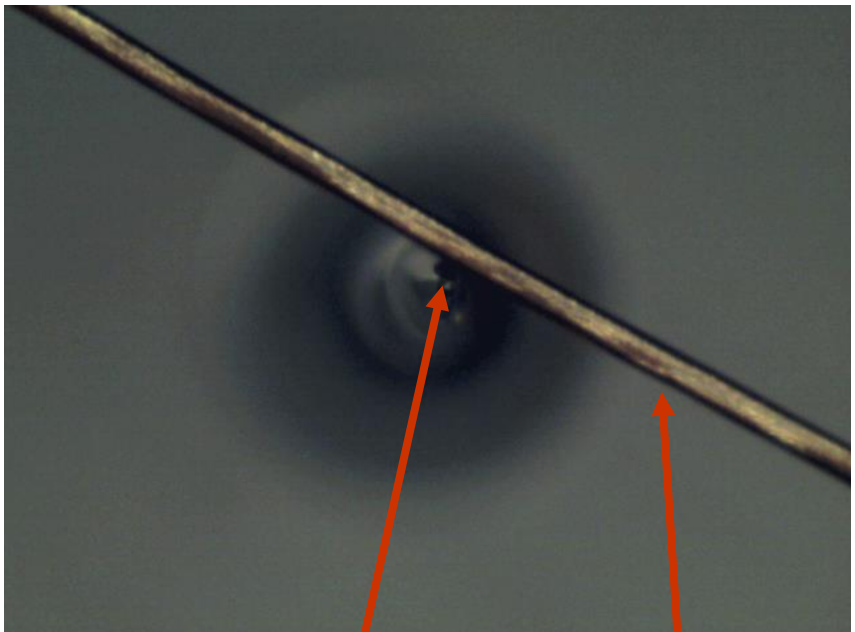
Sensor for O_2 detection

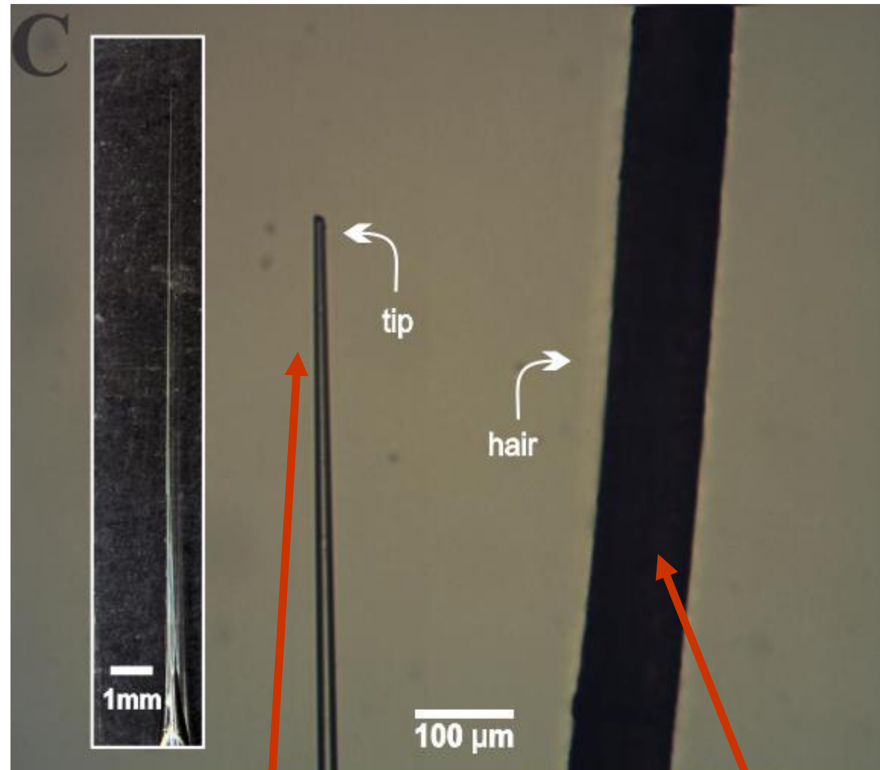


Voltammograms recorded with a bare (A) and a platinised (B) Pt microelectrode in culture medium in <u>absence</u> and <u>presence</u> of O_2 (saturated). ν: 100 mV s^{-1}.

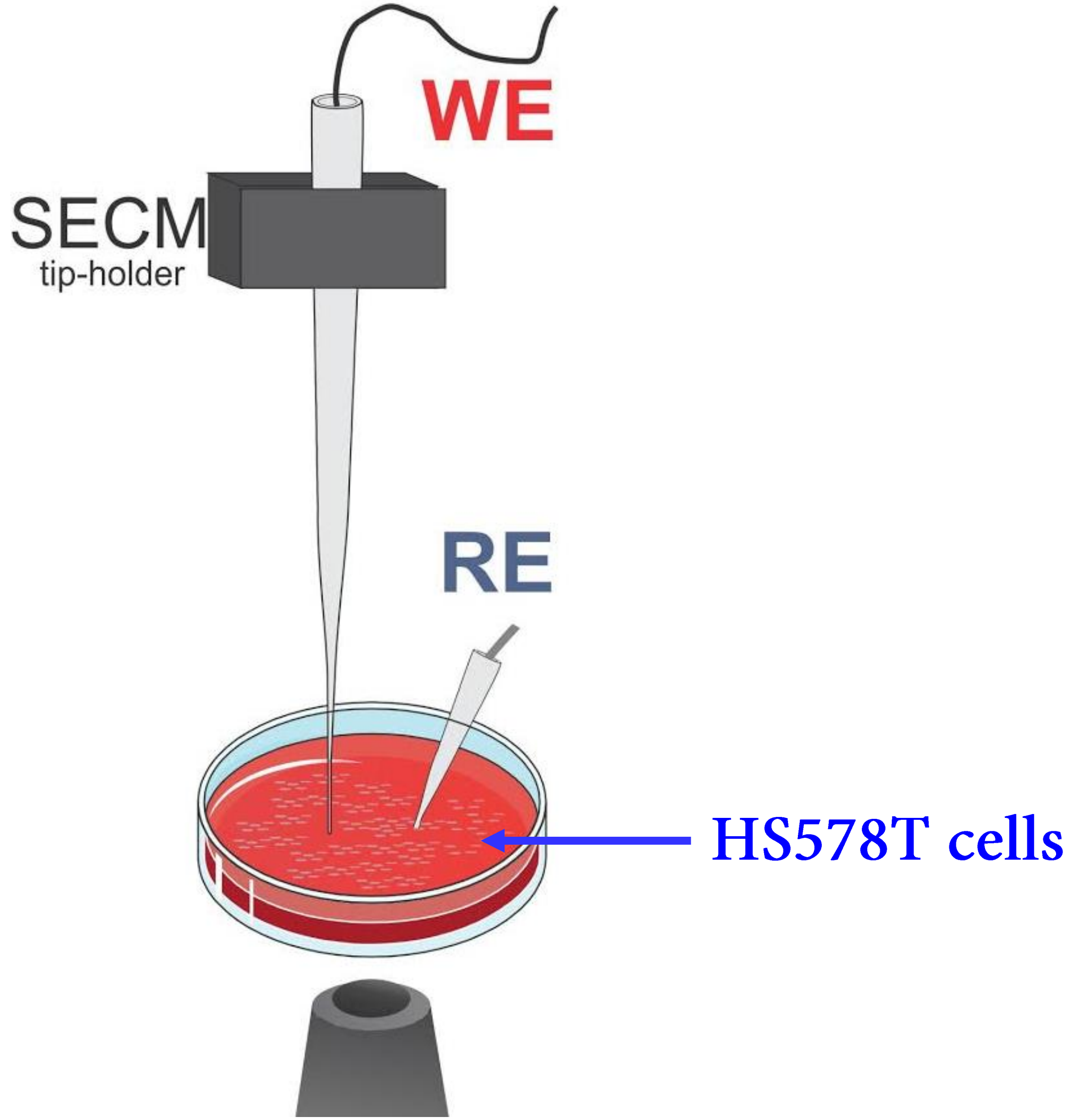


The size of the electrochemical sensor



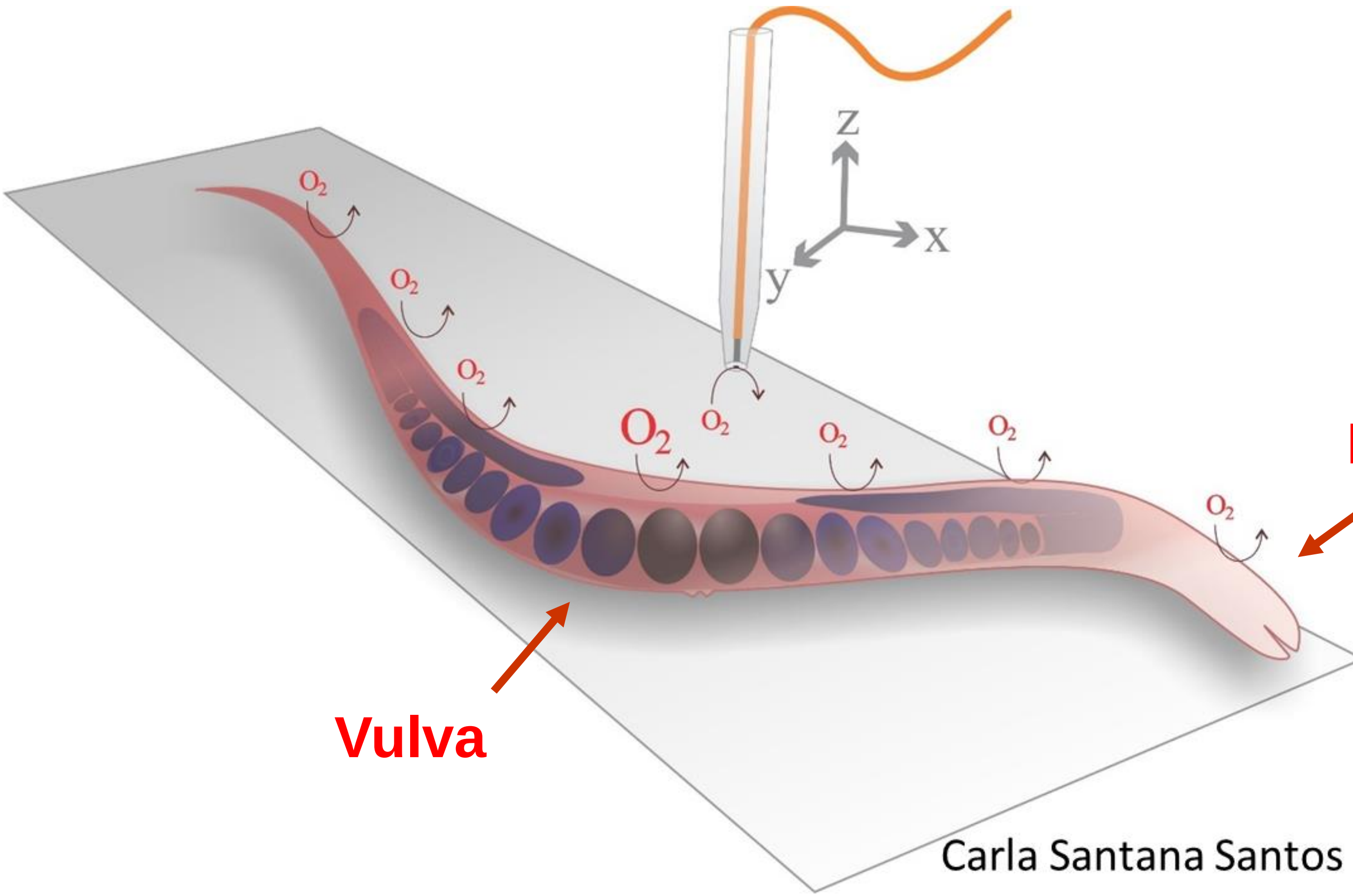


Monitoring respiration activity



Inverted microscope

Monitoring the respiration activity in C. Elegans



Is the oxygen consumption in a single C. Elegans animal homogeneous throughout the body?

Dr. Fernanda Marques da Cunha
Dep. Biochemistry - UNIFESP

Monitoring the respiration activity in C. Elegans

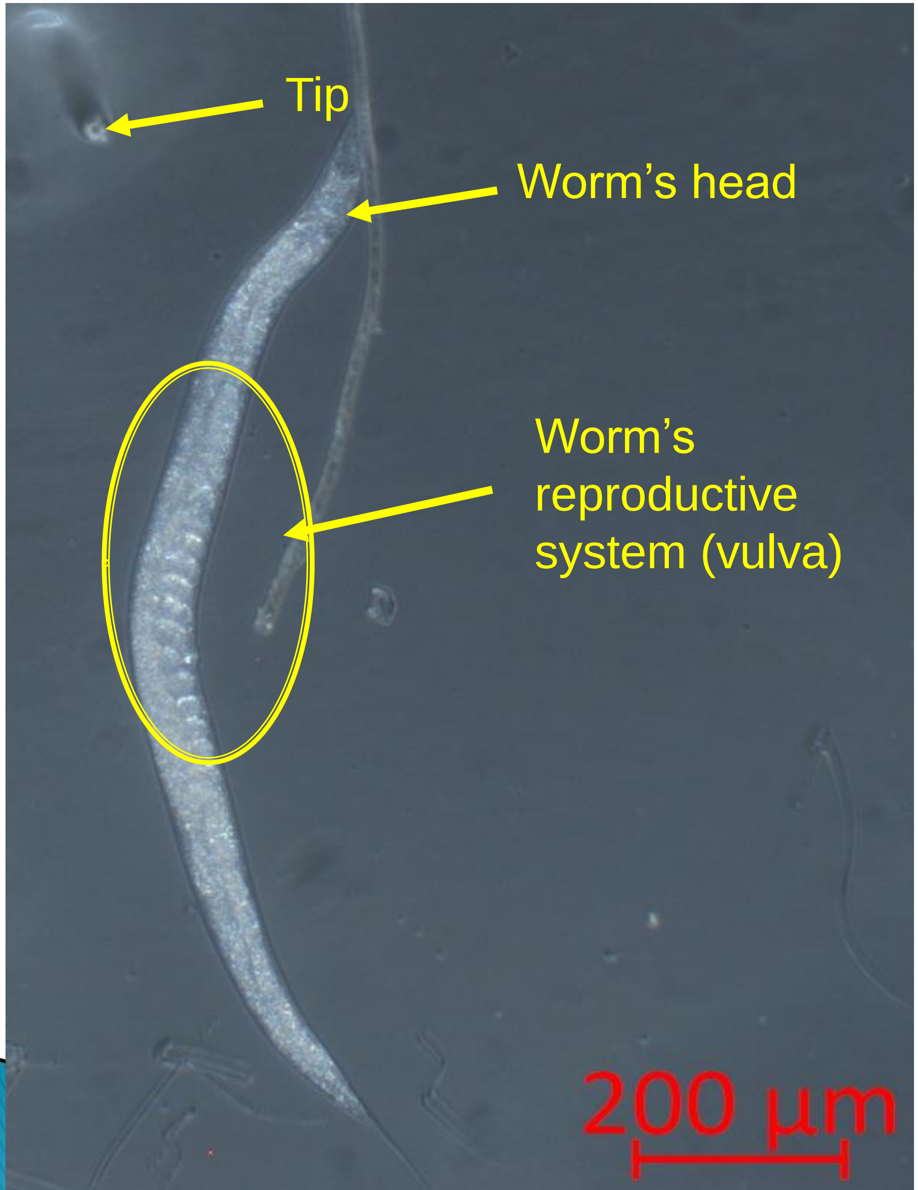
Experimental setup

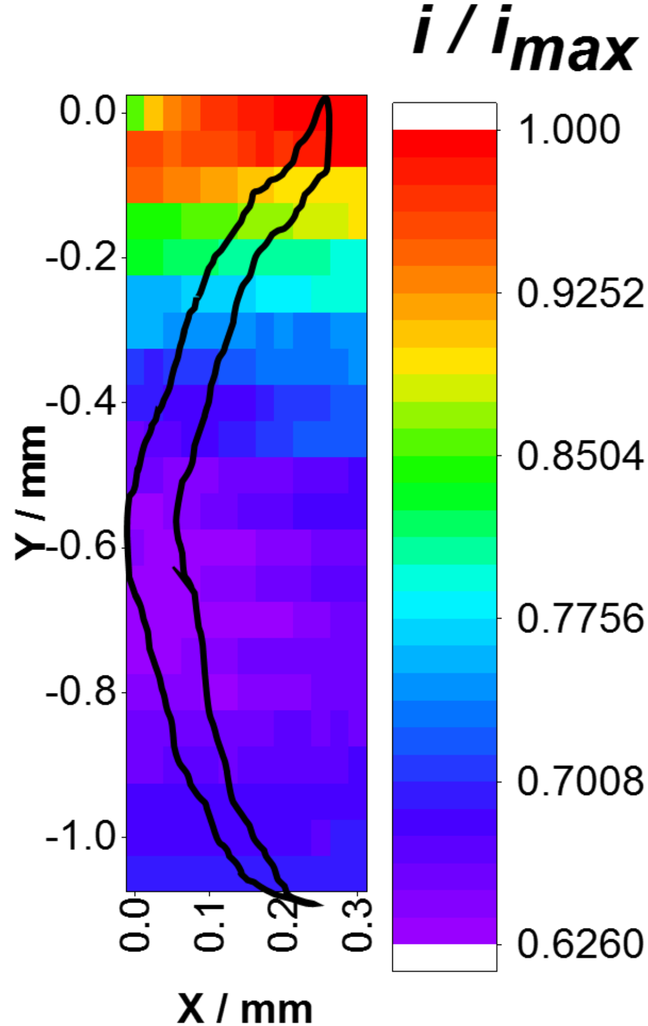
- Adults C. Elegan (N2 Bristol) were collected and **not attached** to the Petri dish (no food during the experiment).
- Experimements were carried out in M9 + 0.3 M 2,3-Butanedione monoxime solution **(anesthetic).** The animals took around 30-60 min to be steady. Respiration is not affected!
- The electrode was moved in *x* and *y* and the current was recorded at each *x* and *y* position.
- Experiments were performed with a platinised Pt microelectrode (**r ~ 400 nm**)

E = -0.4 V

d = 100 µm (from the glass)

Monitoring the respiration activity in C. Elegans



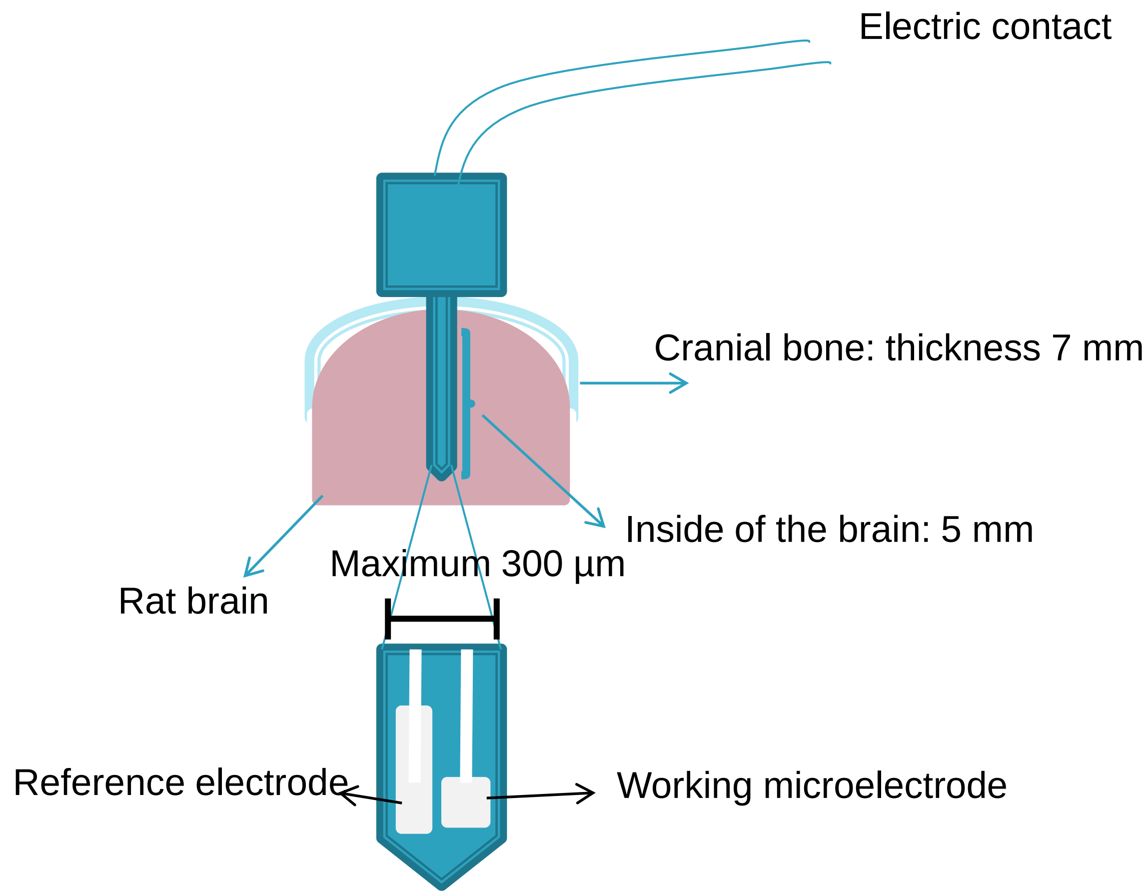


CONTROLS

- Modulation of the respiration activity
- Removal of the vulva

5. In vivo NO detection

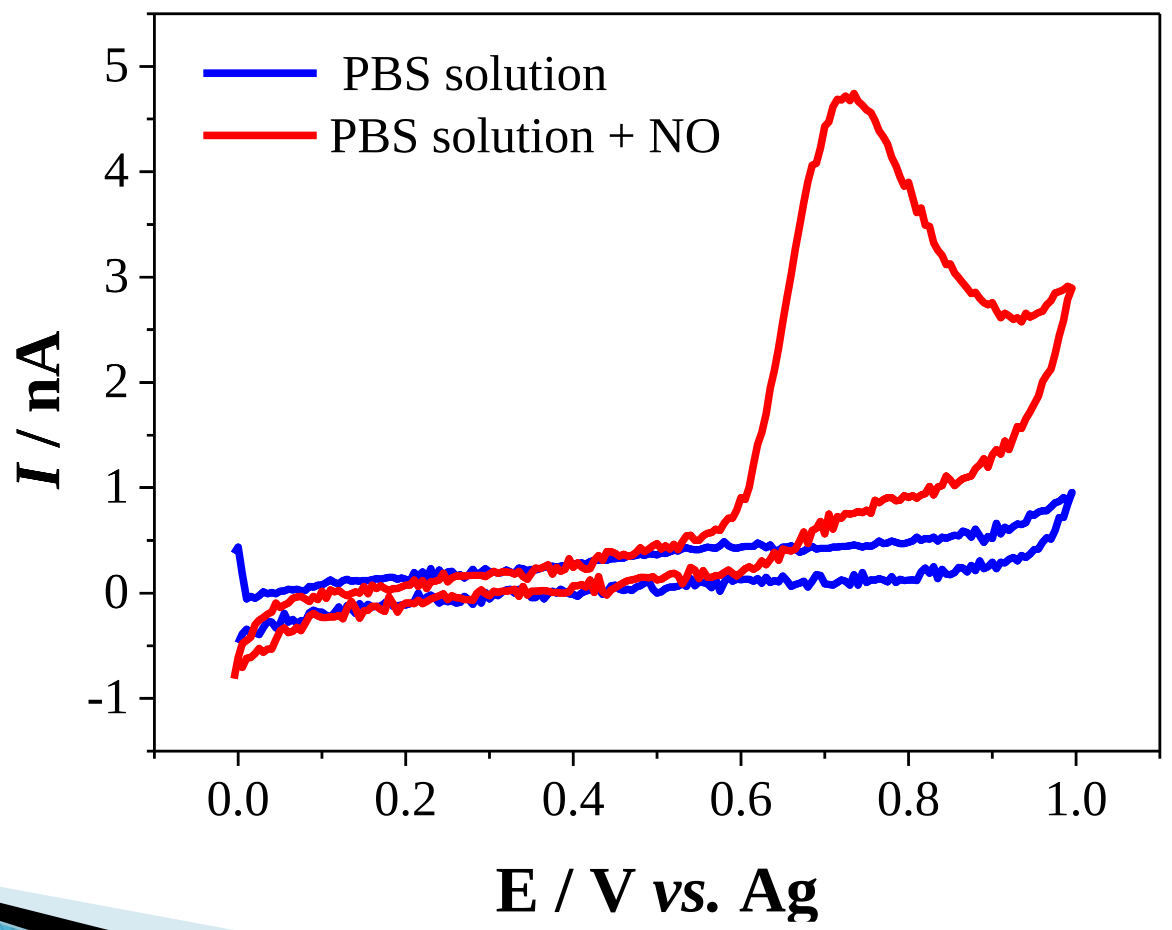
Prof. Newton S. Canteras
Prof. Simone Cristina Motta
Institute of Biomedical Sciences (USP)



In vivo NO detection

In vivo NO detection

NO electrochemical response at a Pt microelectrode



Tutorial Outline – Research interests

- Introduction on sensors
- Surface modification (pre-treatment)
- Electrodes with micrometric dimensions
- **Wearable sensors**
- Conclusions

nature biotechnology

FOCUS | REVIEW ARTICLE
https://doi.org/10.1038/s41587-019-0045-y

Wearable biosensors for healthcare monitoring

Jayoung Kim, Alan S. Campbell, Berta Esteban-Fernández de Ávila and Joseph Wang*

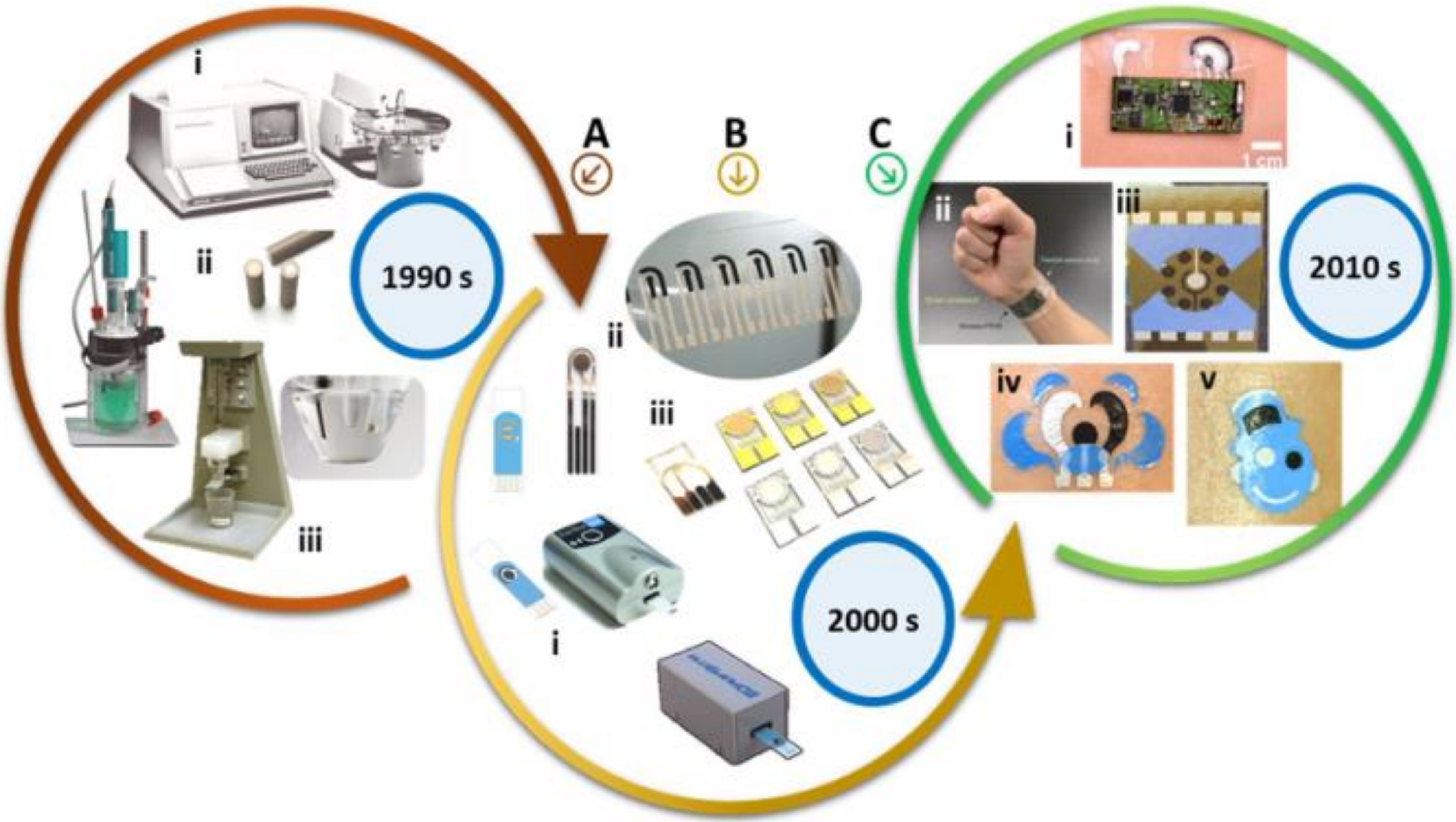
NATURE BIOTECHNOLOGY | VOL 37 | APRIL 2019 | 389–406 | www.nature.com/naturebiotechnology

K. Mahato and J. Wang

Sensors and Actuators: B. Chemical 344 (2021) 130178

Fig. 4. Miniaturization of glucose analyzers: from the bench onto the skin. (A) Benchtop YSI glucose analyzer (https://www.ysi.com); (B) hand-held meeting for self-testing of blood glucose (https://www.diabetes.co.uk); (C) The G6 Dexcom CGM system (https://www.dexcom.com).

The evolution of analytical sensing



Sensors & Actuators: B. Chemical 344 (2021) 130178

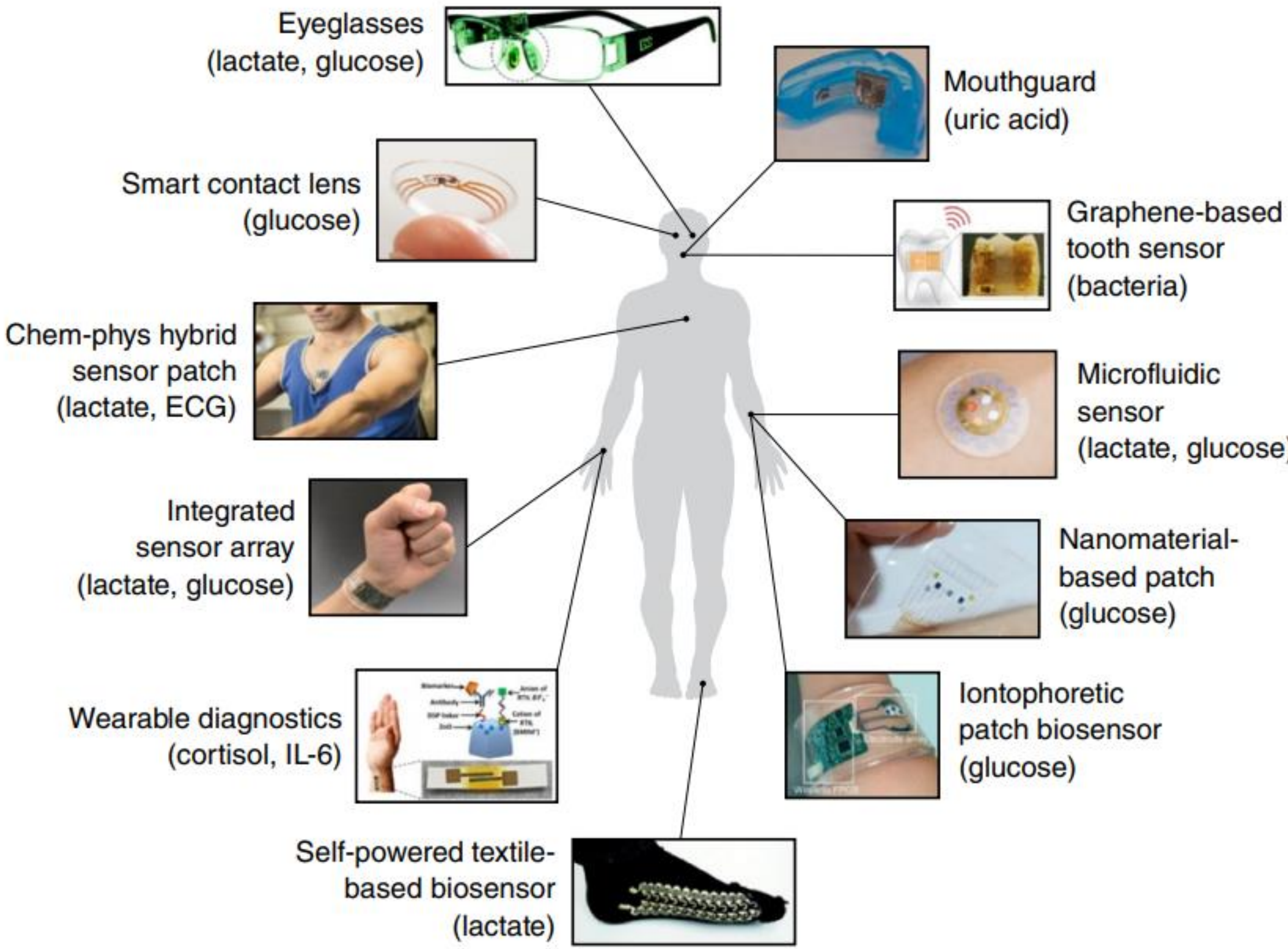
Wearable sensors: Biological fluids

- Sweat
- Tears
- Saliva

Typical target analytes

- Glucose, lactate, urea, uric acid, creatinine
- K^+, Na^+, Ca^{2+}, Cl^-, pH
- Alcohol
- Drugs (cocaine)

Wearable sensors: Representative examples



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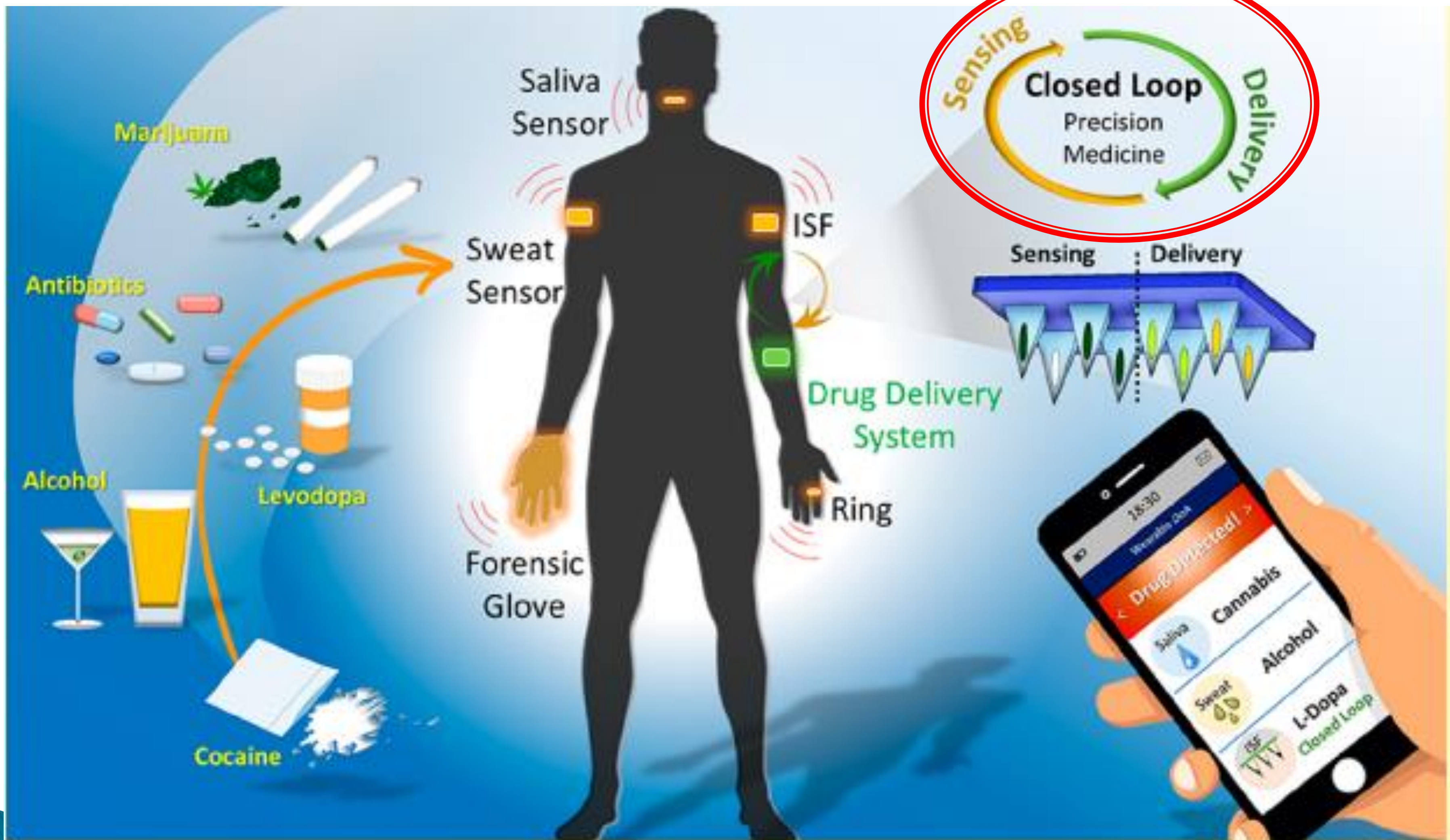
Sensing - Delivery



DOI: 10.1021/acs.analchem.9b04668
Anal. Chem. 2020, 92, 378–396

Wearable sensors: The revolution

- **Chemically modified electrodes**
- **Miniaturized instrumentation**
- **Printed planar electrodes**
- **Flexible materials**
- **Lab-on-chip detection microsystems**
- **Advances in digital communication**

Wearable sensors: Challenges

- **New materials for electrode surface coating (membranes)**
- **Novel mediators and platforms to facilitate the electron transfer process**
- **Fabrication of fully integrated and user-friendly analytical methods**
- **Biofluids x Blood**
- **Prolonged operation (power…) under uncontrolled conditions**
- **Sensors: Biocompatibility, calibration, robustness**
- **Successful translation to the commercial market has been lacking**

The acceptance of these noninvasive biosensors by the medical community will require extensive and successful validation in human testing and improved understanding of the clinical relevancy of sensor information

- Introduction on sensors
- Surface modification (pre-treatment)
- Electrodes with micrometric dimensions
- Wearable sensors
- **Conclusions**

Conclusions

1. More selective and sensitive responses can be obtained by modifying the surface of an electrode.

2. Electrochemical sensors are a powerful tool to get reliable, localised, rapid and costless information.

3. Real time and high resolution chemical information can be obtained by using microelectrodes.

4. Collaboration with researchers from bio-related areas is not easy, but the results can be exciting for both sides!

Curiosity's Capabilities

A Robotic Field Geologist

- Long life, ability to traverse many miles over rocky terrain
- Landscape and hand-lens imaging
- Ability to survey composition of bedrock and regolith

A Mobile Geochemical and Environmental Laboratory

- Ability to acquire and process dozens of rock and soil samples
- Instruments that analyze samples for chemistry, mineralogy, and organics
- Sensors to monitor water, weather, and natural high-energy radiation

Roger Murray, The Cost of Chemical Analysis in Space
Analytical Chemistry – Editorial - (109A) 2004

"Why go?"

Interesting first analytical steps were taken recently on Mars with the robotic conveyers Spirit and Opportunity. The **major analytical chemistry question** thus far addressed, "Was water there in the past?", appears to have been answered by data from an IR emission spectrometer, which provided information about mineralogy by detecting patterns of thermal radiation; a Moessbauer spectrometer, which detected jarosite, an iron sulfate mineral associated with possible previous water exposure; and an alpha particle X-ray spectrometer, which indicated the presence of sulfur and other elements, mounted on Spirit.

"If the colleges, universities, and research institutes are to meet the rapidly increasing demands of industry and Government for new scientific knowledge, their basic research should be strengthened by use of public funds."

The importance of a social contract between the scientific community and the public: "You support our scientific endeavors, and we will strive to make discoveries that will enrich your lives."

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- Chemistry of Biomolecules Laboratory (IQ-USP)
- Protein and Redox Biology laboratory (IB-USP)
- Energy Metabolism Laboratory (IQ-USP)
- LSEME members

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CLAQ 2022 CBQ

Instituto de Química

LSEME
Laboratório de Sensores Eletroquímicos e Métodos Eletroanalíticos

Thank you for your kind attention!