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# Electrochemical nucleic acids biosensor on a microfluidic chip to understand the coupling between electrochemistry and microfluidics

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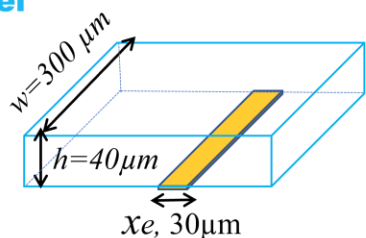
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## ABSTRACT

Teaching graduate students the entanglements of the interdisciplinary field of electrochemical DNA biosensors in microfluidics can be challenging as it involves multiple fields of research strongly  
15 interacting with each other. Yet, electrochemical biosensors coupled with the improved hydrodynamics of microfluidics allow for simpler, more affordable, more performant, and portable point of care devices. Here, we present a laboratory made electrochemical biosensor and a series of simple experiments with a two electrodes setup integrated on a microfluidic channel, that allows graduate students to understand the influence of microfluidics on key parameters and how microfluidics and electrochemistry are closely  
20 intricately intertwined to allow enhanced biosensors functionalities. For each biosensors' features, a theoretical background is provided enabling a comprehensive report for students and teachers.

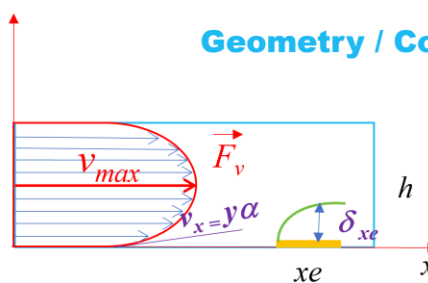
## Electrochemistry in microvolumes

### Ultramicroelectrode in rectangular channel



3D/2D

### Geometry / Confinement



Mass transfer / Reactivity

*Laminar flow conditions*

**Hydrodynamic / Fluidic**

*Poiseuille profile*

### KEYWORDS

25 Graduates, analytical chemistry, microfluidics, electrochemistry, biosensors, molecular diagnosis, limit of detection, sensitivity, laboratory instruction

### INTRODUCTION

Graduate students following a research master in nanosciences in general usually continue their career  
 30 into a research setting. They should develop the ability to integrate, transfer, couple several learned skills like photonics, electrochemistry, reactions at interfaces together and/or within confined spaces/a working device. Here, with the example of an electrochemical nucleic acid biosensor on a microfluidic chip, we aim at showing the coupling, integration of electrochemistry into a microfluidic chip thanks to two techniques which are cyclic voltammetry and Electrochemical Impedance Spectroscopy allowing to  
 35 characterize charge transfer and/or mass transfer.

The working principle of an electrochemical nucleic acids' biosensor is the identification of a target molecule by a probe sequence, complementary to the target in terms of Watson and Cricks pairing. This identification is translated into a measurable change. In electrochemistry, the change in current is directly measurable without further signal transformation (compared to an optical or

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40 fluorescent signal) easing their integration into portable devices. DNA electrochemical biosensors are converting the pairing of base pairs into an electrical signal as depicted on Figure 1.

Biosensors are devices composed of an active biological layer on top of a transducer playing the role of recognizing element and converting the biological interaction in a measurable signal. For a DNA electrochemical biosensor, the biological layer is composed of immobilized DNA on the surface of an  
45 electrode. Design and analysis of these sensors are asking for knowledge in surface functionalization, molecular biology, and microfluidics. Usually, in the graduates or undergraduate's curriculum, these courses are taught independently. Yet, it is the coupling between these domains, especially electrochemistry in confined, fluidic volumes that allow for high precision (specificity and sensitivity), simplicity of use, rapidity and devices that are sufficiently reduced in size (compatible with  
50 microfabrication techniques and materials) making electrochemical DNA biosensors excellent candidates for Point of Care diagnosis in a clinical setting. Fluidic channels of micrometric dimensions usually yield a sample volume of a few hundred of nanoliters, drastically reducing sample volumes and improving the overall hydrodynamics <sup>1-3</sup>.

There are many reports of biosensing's teaching<sup>4</sup> discussing extensively their main features  
55 such as molecular transport, binding kinetics<sup>5</sup>, miniaturization, selectivity, specificity, calibration curve<sup>6</sup>. This laboratory experiment uses the example of a DNA biosensor to explain the coupling between electrochemistry and microfluidics through two main electrochemical techniques CV and EIS.

<sup>1-3</sup>.

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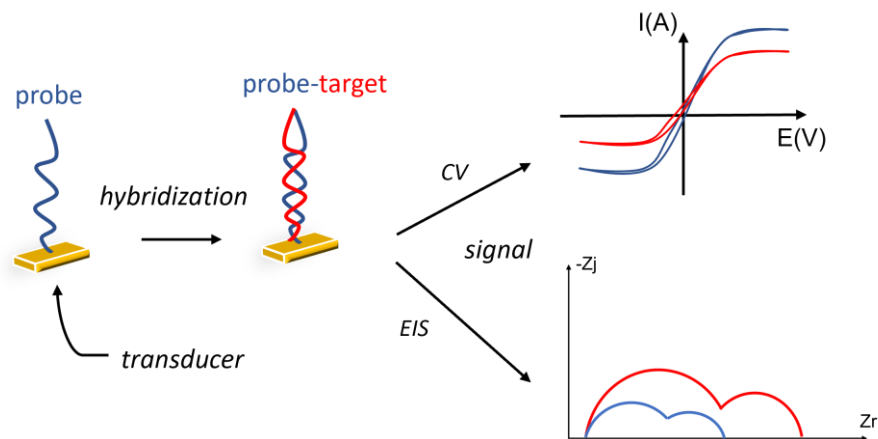
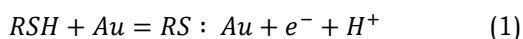


Figure 1. Schematic of DNA hybridization detection and resulting signal in Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS). Blue curves reflect the probe signal, immobilised on the gold electrode (the transducer). Red curves reflect the signal from hybridized target and probe increasing crowding compared to probe signal resulting in decreased faradic current and increased capacitive signal.

65

Among all electrode's material, gold is an easy and smart way to reversibly immobilize a monolayer of DNA probes. The self-assembly via thiol termination of DNA monolayer<sup>7</sup> allows, for example, to obtain a dense, well defined, and organized layer according to the following reaction 1:

70



Such a layer can self-assemble in less than two hours making this material relevant in a home-made microfluidic chip with educational purpose.

75

While previous published work focuses mainly on microfluidic chip fabrication<sup>8-14</sup>, this article aims at providing in-depth theoretical and practical knowledge of electrochemistry coupled with microfluidics. This laboratory experiment not only show how flow rates impact electrochemical measurements but explain the phenomenon behind it (e.g. the impact of forced convection on the diffusion layer and on the biosensor's detection limit). How the diffusion layer shrinks under forced convection and the consequences on biosensor's detection. More molecules can reach the electrode, the rate of collision between probes and targets is increased improving the LOD<sup>15</sup>. In addition, the overall hydrodynamics of the redox tracker are improved in terms of charge/mass transport. Chip fabrication can be done beforehand. Not many recent publications have addressed advanced electrochemistry that goes hereafter

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standard analysis of CV curves<sup>16-18</sup>. To our knowledge, this report is the first one implementing  
85 Electrochemical Impedance Spectroscopy in a laboratory experiment.

The first objective of this practical workshop is to provide students with a specialized theoretical  
background on all aspects of electrochemical DNA biosensors in microfluidics: diffusion/convection,  
chemical reaction, detection system with the presentation of two complementary analysis techniques  
90 Cyclic Voltammetry and Electrochemical Spectroscopy Impedance under flow hydrodynamics. The  
second objective is to provide, thanks to the example of an electrochemical nucleic acids biosensor,  
hands-on experiments to understand the coupling between microfluidics and electrochemistry:  
improved hydrodynamics, diffusion and charge transfer parameters.

General principles of electrochemistry<sup>19</sup> and microfluidics<sup>12,20,21</sup> (mass transport, chemical  
95 kinetics) should be acquired prior to this laboratory experiment. This practical workshop could be  
addressed to high level undergraduates or postgraduates.

## TEACHING MATERIAL

100 This article has been implemented in a class of Master level students. SI – 1 materials detail  
theoretical background concept valuable to offer preliminary context, detailed list of reagents and  
equipment.

## DESIGN AND CHARACTERIZATION OF THE MICROFLUIDIC CHIP

105 Simple and well-known microfabrication techniques are used for sensor fabrication to reduce complexity  
and cost. The 2-electrodes configuration in the electrochemical cell reduces the number of connections  
and therefore the size of the device. Nevertheless, sensor fabrication asks for basic cleanroom facilities.  
Electrochemical devices can be incorporated onto disposable substrates (plastic, paper, textile,...) but  
PDMS is the easiest way to mould microfluidic channels and is available in most laboratories  
110 compared to polymer photoablation, glass etching or 3D printing<sup>19</sup> for example.

All details about chemicals, materials and detailed experimental procedures including student's handout are provided in the SI-1

### A Design of the microfluidic chip

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This microfluidic device was made in the clean room facilities of the Centre of Nanosciences and Nanotechnologies (C2N, Palaiseau, France). The key steps of the microfabrication are extensively detailed in <sup>22,23</sup> and sum up on Figure 2. The counter electrode (CE) is positioned after the working electrode (WE), following the direction of the flow, to favor DNA immobilization and capture on the WE. The CE is 66-fold larger than the WE. Thus current density variations are lower than on the WE and it can be considered as a pseudo reference. Hydrodynamic conditions related to the fluidic channel and mass transport towards the electrodes are described in the SI-1.

120

#### Substrate



#### PDMS cover



Figure 2. Microfabrication key steps: inverted optical lithography, Ti/Au metallization, PDMS fabrication and bounding (not shown here).

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The lithographic masks were designed using the L-Edit or KLayout software (Figure 3 gives an example with the KLayout software).

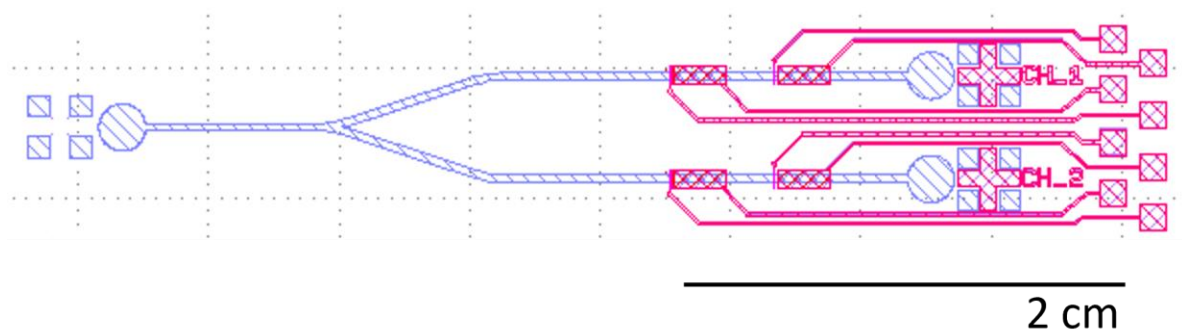


Figure 3. KLayout drawing of the channel mask (blue) aligned with the electrode mask (pink).

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The device consists of two parts, as depicted on Figure 4. The lower section bears the gold microelectrodes patterned on a glass substrate. The upper part contains the fluidic channel in PDMS.

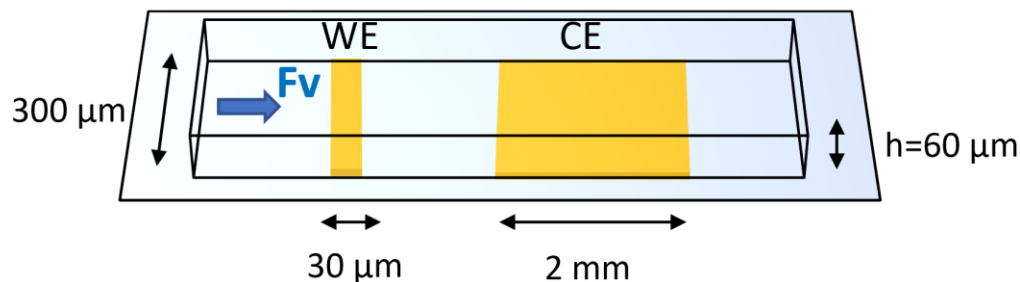


Figure 4. Schematic representation of the microchannel electrodes (not to scale). Detection area and fluidic channel microelectrodes with their dimensions. WE stands for working electrode (width  $w=300\mu\text{m}$ , length  $x_e=30\mu\text{m}$ ). CE stands for counter electrode (width  $w=300\mu\text{m}$ ,  $x_e=2\text{mm}$ ).  $h$  corresponds to the fluidic channel height.

135

Complete procedure to manufacture the electrochemical microfluidic device is described in SI-1.

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## B Microfluidic chip characterization

### Hazards

The chemicals used are not hazardous, and the use of PPE (lab coat, goggles, and gloves) is sufficient.

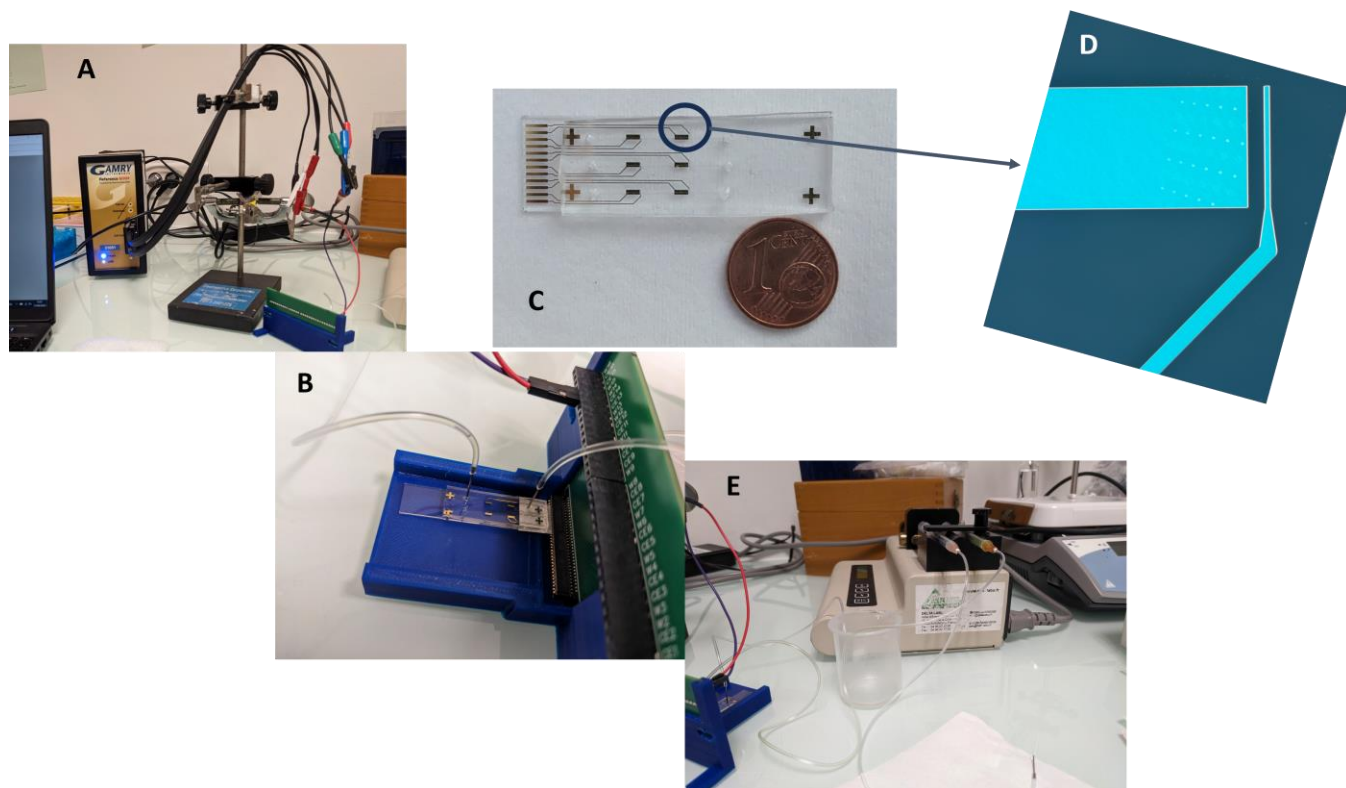
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Although ferricyanide/ferrocyanide redox couple exhibit low toxicity, care must be taken while disposal of potassium ferricyanide as it reacts with acids to form cyanides.



150 **OVERVIEW OF LABORATORY PROCEDURE**

The complete set up is shown on Figure 5. The electrochemical workstation controlling the potential and frequency is connected to the microfluidic chip thanks to an in-house 3D printed chip holder. The solution is inserted into the chip thanks to tubing and controlled by a syringe pump.



155

Figure 5. Set up including A. The Gamry 600+ electrochemical workstation. B. The chip holder 3D printed in-house. C. The microfluidic chip bearing 3 microfluidic channels and 2 couples of WE, CE as seen on D. and E. the syringe pump for flow control.

160 Characterization of the microfluidic chip is done with the redox couple  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}/[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$  on a gold working /counter electrode couple in cyclic voltammetry and electrochemical impedance spectroscopy investigating the surface properties, as the surface modification resulting from DNA functionalization.

The electroactive redox couple  $[\text{Fe}^{\text{III}}(\text{CN})_6]^{3-}/[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$  is associated to a one electron transferred reaction, as shown by the following equation 2:



Supplies and equipment are summarized in the SI-1

In the experimental section detailed with the students' handout in the SI-2, the steps to characterize the sensor are described by the drawing and analysis of cyclic voltammetry curves (I/E curves) and Electrochemical Impedance Spectroscopy curves (EIS curves).

170

Then, by testing different flow rates the hydrodynamics in the fluidic channel microelectrodes can be analyzed both in CV by comparing the value of the diffusion plateau to the expected theoretical value (given by the Levich theory) and discussed the potential deviation and EIS

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After chip characterization, students were asked to functionalize the electrodes with nucleic acids and to characterize the biosensor in terms of faradic and capacitive current.

180 **LEARNING OUTCOMES**

At the end of the laboratory procedure, students will be able to refresh their knowledge of cyclic voltammetry, gain a first approach of electrochemical impedance spectroscopy and characterize the electrochemistry/microfluidics coupling:

185

- Select a relevant scan rate for CV
- Identify diffusion / faradic phenomenon
- Identify different characteristics of the EIS curve
- Differentiate a static from a dynamic regime in EIS
- Assess flow impact on CV and EIS curves and link it to the theory

190 Assess the success of electrodes functionalization with short nucleic acids based on CV curves

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## RESULTS AND DISCUSSION

195 Examples of CV and EIS curves obtained under flow, on gold ultramicroelectrodes are given on Figure 6. To assess the hydrodynamics of the fluidic channel, increasing flow rates are introduced in the device with a syringe pump.

Figure 6A depicts sigmoid curves with an equilibrium potential set at 0 V as both electrodes are made of gold and are immersed in the same electrolyte. These characteristic S-shaped curves are typical of a  
200 fast redox system under laminar flow.

Electron transfer can be characterized qualitatively by comparing slopes at different flow rates. Indeed, the classical equation as reported in this excellent teaching material from Gonzalez et al.<sup>24</sup> cannot be applied in this case as diffusion is not strictly planar on ultramicroelectrodes and not semi-infinite in confined spaces<sup>25</sup>.

205 The diffusion can be characterized by measuring the limiting current plateau. The limiting current plateau characteristic of the diffusion phenomenon is flow dependent. Indeed, the diffusion layer near the ultramicroelectrode shrinks, under the influence of the forced convection<sup>15</sup>.

The limiting diffusion current of a thin rectangular microelectrode of width  $\omega$  and length  $x_e$ , placed perpendicularly to the flow direction is given by the limiting current or Levich current, Equation 3:

210 
$$I_{\text{lim}} = 0.925 \cdot n \cdot F \cdot c_{\infty} \cdot D^{2/3} \cdot x_e^{2/3} \cdot \omega \cdot \left( \frac{4Fv}{h^2 \cdot d} \right)^{1/3} \quad (3)$$

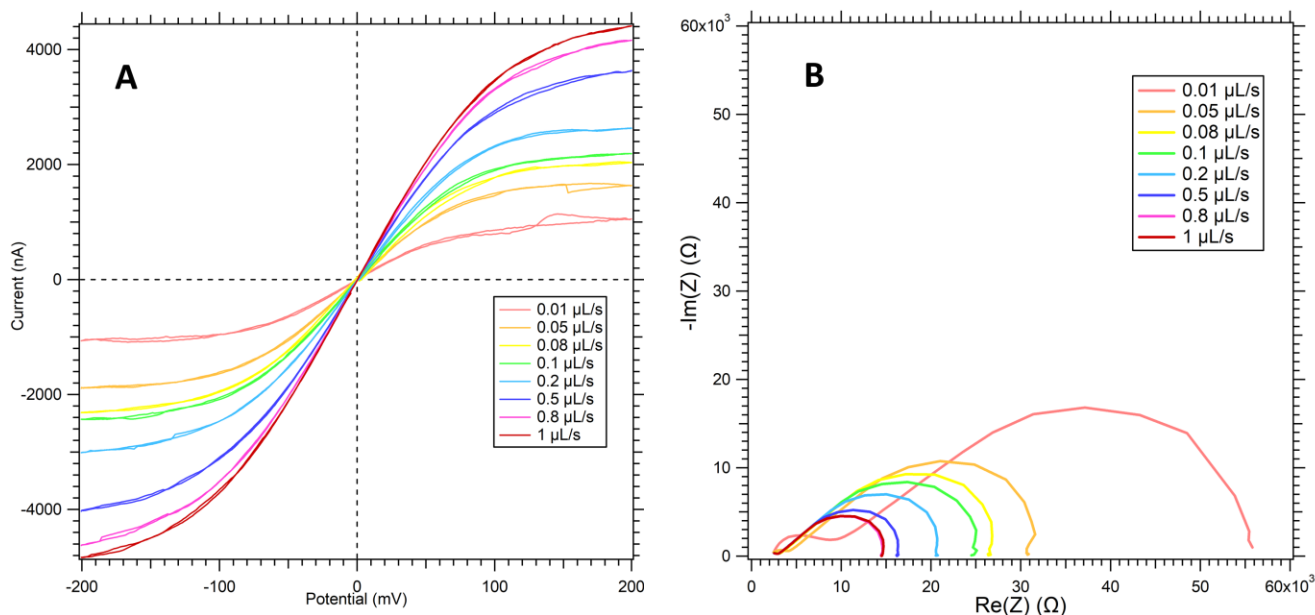
$n$  stands for the number of electrons exchanged,  $F$  the Faraday constant (96 485 C mol<sup>-1</sup>),  $c_{\infty}$  the concentration of electroactive species (mol cm<sup>-3</sup>) and  $D$  the diffusion coefficient of the electroactive species (cm<sup>2</sup> s<sup>-1</sup>).  $h$  and  $d$  refer to the channel height and width, respectively.

215 The diffusion layer building from the microelectrode's leading edge ( $x=0$ ) must remain very small at its leaving edge ( $x=x_e$ ) for the Levich equation to remain valid.

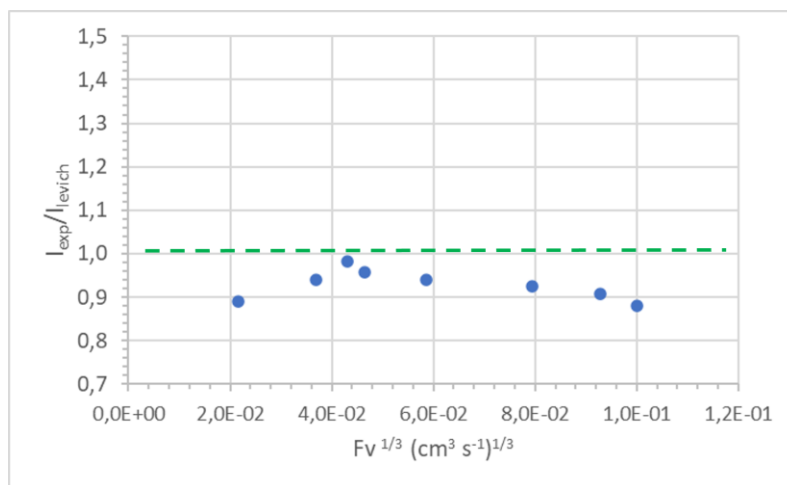
Details on the obtention of this equation can be found in the SI-1.

To compare with the theory, the current of the reduction or oxidation plateau current can be normalized with the expected Levich current and plotted against the volumetric flow rate as shown on  
220 Figure 6. Instructors can refer to the provided spreadsheet “I limit calculations” to implement flow rates and experimental currents.

Figure 6B displays high frequency electrode impedance spectroscopy with a frequency range of 1MHz to 0.1 Hz in potentiostatic mode allowing to describe the system in terms of capacitive (electrochemical  
225 double layer,  $C_{dl}$ ), faradic contribution (charge transfer resistance,  $R_{ct}$ ) and diffusion (diffusion resistance,  $R_D$ ). Characteristics ( $R_{ct}$ ,  $R_e$ ,  $R_D$ ,  $C_{dl}$ ) can be estimated from these curves. By taking the high frequency intersection,  $R_e$  can be estimated as the electrolyte resistance (ohmic drop) around 2 k $\Omega$ . By comparing the diameter of the second loop, flow impact can be estimated on the diffusion (as forced convection increases, the diffusion layer shrinks, and  $R_D$  decreases). The calculation of the  $R_{ct}$  and  $R_D$   
230 asks for a modelling using the modified-Randles equation (see SI-1). As an example, parameters with the same set up can be found in Poujouly et al.<sup>26</sup>, published by our group.  $R_D$  is the only parameter that should vary with the flow.

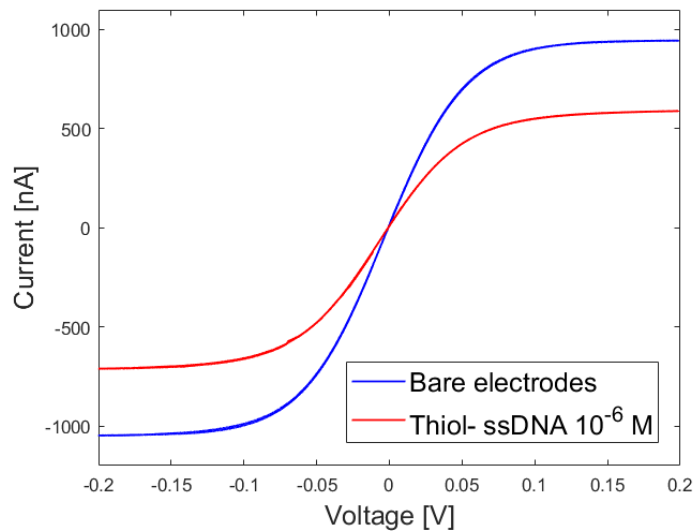


235 Figure 6. A. Example of limiting current intensity response of a WE microchannel electrode vs. flow rates. B. Example of potentiostatic electrochemical Impedance spectroscopy response vs. flow rates. Frequencies range from 1 MHz to 0.1 Hz. AC signal excitation = 100 mV. DC = 0 V. Eight flow rates are displayed here from 0.01  $\mu\text{L/s}$  to 1  $\mu\text{L/s}$ . The electrolyte is composed of 20 mM of an equimolar solution of  $[\text{Fe}(\text{III})(\text{CN})_6]^{3-}/[\text{Fe}(\text{II})(\text{CN})_6]^{4-}$  in 0.5 M NaCl. The selected CV scan rate is 10 mV/s.



240 Figure 7. Example of limiting current ratio between experimental current  $I_{\text{exp}}$  and calculated theoretical current obtained from the Levich equation  $I_{\text{Levich}}$ . Ratio is given as a function of the volumetric flow rate  $Fv$  to the power of  $1/3$ .

Figure 8 shows an example of electrodes functionalization with a sequence of 20 nucleotides modified with a thiol at the 5' end.



245 Figure 8. Example of cyclic voltammetry curves obtained before (blue – bare gold) and after (red) functionalization with a single-stranded DNA probe at  $10^{-6}$  M modified with a thiol end.

Thiols spontaneously absorb on gold substrates building self-assembled monolayers SAMs according to Equation (1). Currents can be compared before and after functionalization to assess its success. DNA is composed of a sugar phosphate backbone. The negatively charged backbone prevents the

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diffusion of the  $[\text{Fe}(\text{III})(\text{CN})_6]^{3-}/[\text{Fe}(\text{II})(\text{CN})_6]^{4-}$  negatively charged redox complex towards the electrode thus reducing current compared to a bare electrode<sup>27,28</sup>. CV curves show an apparent decrease in charge transfer (quantitative). The apparent decrease origin is still under investigation.

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## IMPLEMENTATION AND ANALYSIS OF THE COMPREHENSION OF THE STUDENTS

This practical workshop was successfully implemented during a 3-hour session with 21 graduate students from the Quantum, light, materials and nanosciences (QLMN) Master 2 of Paris-Saclay

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University (France). Students who follow this Master usually continue their career into research whether public research in academic settings or private research in R&D or research centers. The first hour was devoted to theoretical and technical aspects described in SI-1. Then experiments were presented, and students could handle the microfluidic chips. Beforehand, students had 2 weeks to prepare bibliography.

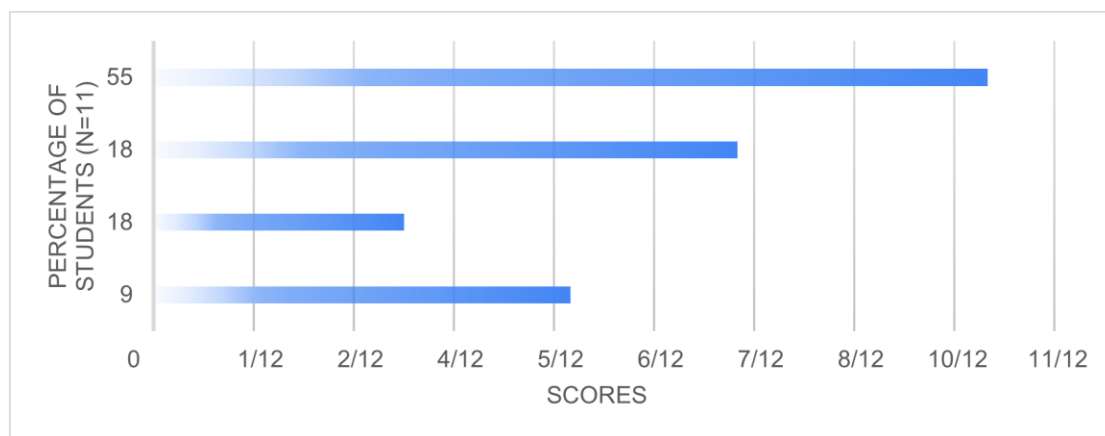
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Two strategies were followed to collect data on students' performance with a google form format. First students learning was assessed through open questions to analyze if students could grasp some of the main concepts developed in this teaching material (microfluidics, electrochemistry, their coupling and limiting currents). Then students were asked questions requiring calculations) A complete analysis of students' answers can be found on Figure 9. Evaluation criteria and list of

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questions are detailed in section "Assessment of students' learning" in SI-1. With the collected information and general observation from the practical work, following comments can be drawn:

- In total 73% of students achieved scores higher than the mean (See Figure 9). This exercise was optional and only half the students answered. Assessment could be improved by making the questionnaire mandatory.



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Figure 9. Overall scores.

- Numerical parameters are well understood apart from 3 students
- As shown on Figure 10, students who were able to answer in a concise manner (column “precision”, the column “sophistication” reports on the complexity of the answer) were usually the ones that understood the experiments the best. It seems electrochemistry was better understood as a concept than microfluidics generating more elusive answers. This point could be improved by showing students Comsol simulations that can be found in the “Notes for instructors” section from SI-1.

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Experiments with flow rates took a lot of time. Maybe this section can be improved by selecting fewer flow rates or dividing the work between students.

Students	Sophistication	precision	Sophistication	precision	Correct	Sophistication	precision	Correct answer to numerical questions	Overall score
	Microfluidics definition		Electrochemistry definition			limiting current parameters			
1	2	2	1	1	Y	1	2	4/4	10/12
2	3	3	3	2	N	3	1	1/4	3/12
3	1	3	3	1	Y	3	2	4/4	10/12
4	2	3	2	2	Y	2	1	4/4	10/12
5	3	3	2	2	Y	2	2	3/4	7/12
6	1	1	1	2	Y	1	1	3/4	7/12
7	1	1	1	1	N	1	1	2/4	5/12
8	1	1	3	2	Y	3	1	1/4	3/12
9	1	1	1	3	Y	1	1	4/4	10/12
10	1	1	1	3	Y	1	1	4/4	10/12
11	3	1	3	3	Y	3	2	4/4	10/12

Figure 10. Analysis of students answers to open and numerical questions.

## CONCLUSIONS

This practical workshop aimed at both provide core concepts and handling of key parameters that had visual impacts on the output signal thanks to an in-house microfluidic chip functionalized with DNA on microelectrodes. Students' comprehension was satisfactory both on theoretical concepts and practical considerations. This workshop sought to provide a model of how a series of simple experiments can help understand specialized topics that students usually don't learn together and subsequent students' comprehension evaluation.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available on the ACS Publications website at DOI:

10.1021/acs.jchemed.XXXXXXX. [ACS will fill this in.] Example brief descriptions with file formats indicated are shown below; customize for your material.

SI-1 Theory, note to instructors and examples of raw data (PDF, DOCX)

SI-2 Example of laboratory procedure (PDF, DOCX)

I limit calculations (XLS)

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## REFERENCES

- 320 (1) Compton, R.; Fisher, A.; Wellington, R. G.; Dobson, P.; Leigh, P. Hydrodynamic Voltammetry with Microelectrodes. Channel Microband Electrodes: Theory and Experiment. *The Journal of Physical Chemistry* **1993**, *97* (40), 10410–10415. <https://doi.org/10.1021/j100142a024>.
- (2) Amatore, C.; Sella, C.; Thouin, L. Electrochemical Time-of-Flight Responses at Double-Band Generator-Collector Devices under Pulsed Conditions. *Journal of Electroanalytical Chemistry* **2006**, *593*, 194–202. <https://doi.org/10.1016/j.jelechem.2006.04.002>.
- 325 (3) Faure, M.; Pallandre, A.; Chebil, S.; Le Potier, I.; Taverna, M.; Tribollet, B.; Deslouis, C.; Haghiri-Gosnet, A.-M.; Gamby, J. Improved Electrochemical Detection of a Transthyretin Synthetic Peptide in the Nanomolar Range with a Two-Electrode System Integrated in a Glass/PDMS Microchip. *Lab Chip* **2014**, *14* (15), 2800–2805. <https://doi.org/10.1039/c4lc00240g>.
- (4) Giri, B.; Peesara, R. R.; Yanagisawa, N.; Dutta, D. Undergraduate Laboratory Module for Implementing ELISA on the High Performance Microfluidic Platform. *Journal of Chemical Education* **2015**, *92* (4), 728–732. <https://doi.org/10.1021/ed4009107>.
- 330 (5) Prakash, P.; Varma, M. A Pedagogical Introduction to Biomarker Detection in Surface-Based Biosensors. *Journal of Chemical Education* **2022**, *99* (11), 3694–3701. <https://doi.org/10.1021/acs.jchemed.2c00606>.
- 335 (6) Amor-Gutierrez, O.; Rama, E. C.; Fernandez-Abedul, M. T.; Costa-Garcia, A. Bioelectroanalysis in a Drop: Construction of a Glucose Biosensor. *Journal of Chemical Education* **2017**, *94* (6), 806–812. <https://doi.org/10.1021/acs.jchemed.6b00948>.
- (7) Bain, C. D.; Evall, J.; Whitesides, G. M. Formation of Monolayers by the Coadsorption of Thiols on Gold: Variation in the Head Group, Tail Group, and Solvent. *Journal of American Chemical Society* **1989**, *111*, 7155–7164.
- 340 (8) Hemling, M.; Crooks, J. A.; Oliver, P. M.; Brenner, K.; Jennifer, G.; Lisensky, G. C.; Weibel, D. B. Microfluidics for High School Chemistry Students. *Journal of Chemical Education* **2014**, *91* (1), 112–115. <https://doi.org/10.1021/ed4003018>.
- (9) Vangunten, M. T.; Walker, U. J.; Do, H. G.; Knust, K. N. 3D-Printed Microfluidics for Hands-On Undergraduate Laboratory Experiments. *Journal of Chemical Education* **2020**, *97* (1), 178–183. <https://doi.org/10.1021/acs.jchemed.9b00620>.
- 345 (10) Lin, X.; Jin, X.; Xu, C.; Lai, H.; Lin, M.; Ren, N.; Cai, L. Iodometric Titration on Microfluidic Paper-Based Analytical Devices for Determination of Ascorbic Acid: A Laboratory Experiment for Chemical Education Undergraduates. *Journal of Chemical Education* **2023**, *100* (5), 1997–2002. <https://doi.org/10.1021/acs.jchemed.2c01236>.
- 350 (11) Agustini, D.; Bergamini, M. F.; Marcolino-Junior, L. H. Simple and Inexpensive Microfluidic Thread Based Device for Teaching Microflow Injection Analysis and Electrochemistry. *Journal of Chemical Education* **2018**, *95* (8), 1411–1414. <https://doi.org/10.1021/acs.jchemed.8b00211>.
- (12) Shaffer, T. A.; Harrada, C. U.; Walker, A. M.; Casto-Bogges, L. D.; Holland, L. A.; Johnson, T. R.; Jones, M. E.; Elshamy, Y. S. A Cost-Effective Microfluidic Device to Teach the Principles of Electrophoresis and Electroosmosis. *Journal of Chemical Education* **2023**, *100* (7), 2782–2788. <https://doi.org/10.1021/acs.jchemed.2c01028>.
- 355 (13) Wietsma, J. J.; van der Veen, J. T.; Buesink, W.; van den Berg, A.; Odijk, M. Lab-on-a-Chip: Frontier Science in the Classroom. *Journal of Chemical Education* **2018**, *92* (2), 267–275. <https://doi.org/10.1021/acs.jchemed.7b00506>.
- 360 (14) Teerasong, S.; McClain, R. L. A Student-Made Microfluidic Device for Electrophoretic Separation of Food. *Journal of Chemical Education* **2011**, *88* (4), 465–467. <https://doi.org/10.1021/ed100717m>.
- (15) Squires, T. M.; Messinger, R. J.; Manalis, S. R. Making It Stick: Convection, Reaction and Diffusion in Surface-Based Biosensors. *Nature Biotechnology* **2008**, *26* (4), 417–426. <https://doi.org/10.1038/nbt1388>.
- 365 (16) Kandahari, E.; Smith, E. J.; Goeltz. Beyond the Textbook: Introducing Undergraduates to Practical Electrochemistry. *Journal of Chemical Education* **2021**, *98* (10), 3263–3268. <https://doi.org/10.1021/acs.jchemed.1c00155>.

- 370 (17) Elgrishi, N.; Rountree, K. J.; McCarthy, B. D.; Rountree, E. S.; Eisenhart, T. T.; Dempsey, J. L. A Practical Beginners' Guide to Cyclic Voltammetry. *Journal of Chemical Education* **2018**, *95* (2), 197–206. <https://doi.org/10.1021/acs.jchemed.7b00361>.
- (18) Augustini, D.; Bergamini, M. F.; Marcolino-Junior, L. H. Simple and Inexpensive Microfluidic Thread Based Device for Teaching Microflow Injection Analysis and Electrochemistry. *Journal of Chemical Education* **2018**, *95* (8), 1411–1414. <https://doi.org/10.1021/acs.jchemed.8b00211>.
- 375 (19) DeLeon, C.; Tabibi, T.; Alagic, A. Characterization and Electrochemical Analysis of Microelectrodes and the Interface with a Fabricated 3D-Printed Microfluidic Chip in an Upper-Division Analytical Course. *Journal of Chemical Education* **2020**, *97* (12), 4453–4461. <https://doi.org/10.1021/acs.jchemed.0c00427>.
- 380 (20) Piunno, P. A. E.; Zetina, A.; Chu, N.; Tavares, A. J.; Omain Noor, M.; Petryayeva, E.; Uddayasankar, U.; Veglio, A. A Comprehensive Microfluidics Device Construction and Characterization Module for the Advanced Undergraduate Analytical. *Journal of Chemical Education* **2014**, *91* (6), 902–907. <https://doi.org/10.1021/ed400728a>.
- (21) Chao, T.-C.; Bhattacharya, S.; Ros, A. Microfluidic Gel Electrophoresis in the Undergraduate Laboratory Applied to Food Analysis. *Journal of Chemical Education* **2012**, *89* (1), 125–129. <https://doi.org/10.1021/ed101064p>.
- 385 (22) Horny, M.-C. Electrochimie et Nanoparticules Magnétiques Sur Laboratoire-Sur-Puce Microfluidique Pour Le Diagnostic Médical. Détection Du microARN-122., Université Pierre et Marie Curie, Chimie analytique, Paris, 2017.
- 390 (23) Horny, M.-C.; Lazerges, M.; Siaugue, J.-M.; Pallandre, A.; Rose, D.; Bedioui, F.; Deslouis, C.; Haghiri-Gosnet, A.-M.; Gamby, J. Electrochemical DNA Biosensors Based on Long-Range Electron Transfer: Investigating the Efficiency of a Fluidic Channel Microelectrode Compared to an Ultramicroelectrode in a Two Electrode Set Up. *Lab On Chip* **2016**, *16* (22), 4373–4381. <https://doi.org/10.1039/C6LC00869K>.
- 395 (24) Gonzalez, J.; Laborda, E.; Molina, A. Voltammetric Kinetic Studies of Electrode Reactions: Guidelines for Detailed Understanding of Their Fundamentals. *Journal of Chemical Education* **2023**, *100* (2), 697–706. <https://doi.org/10.1021/acs.jchemed.2c00944>.
- (25) Bellagha-Chenchah, W.; Stella, C.; Thouin, L. Understanding Mass Transport at Channel Microband Electrodes: Influence of Confined Space under Stagnant Conditions. *Electrochimica Acta* **2016**, *202*, 122–130. <http://dx.doi.org/10.1016/j.electacta.2016.04.011>.
- 400 (26) Poujouly, C.; Gonzalez Losada, P.; Boukraa, R.; Freisa, M.; Le Gall, J.; Bouville, D.; Deslouis, C.; Gamby, J. Diffusion-Convection Impedance for a Micro-Band Electrode under Microfluidic Conditions. *Electrochemistry Communications* **2022**, *137*, 107262. <https://doi.org/10.1016/j.elecom.2022.107262>.
- 405 (27) Kelley, S. O.; Jackson, N. M.; Hill, M. G.; Barton, J. K. Long-Range Electron Transfer through DNA Films. *Angew. Chem. Int. Ed.* *38* (7). 1999, pp 941–945. 10.1002/(SICI)1521-3773(19990401)38:7<941::AID-ANIE941>3.0.CO;2-7.
- (28) Kelly; Barton. Electrochemistry of Methylene Blue Bound to a DNA-Modified Electrode. *Bioconjugate Chem.* 1997, pp 31–37.

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