Electrochemical Device for 96-Well Electrochemistry: Amperometry and Electrochemiluminescence

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Detection of redox species (**bio**)chemical reactions (or other applications) by electrochemical oxidation or reduction. ***In theory*** possible for any chemical species.

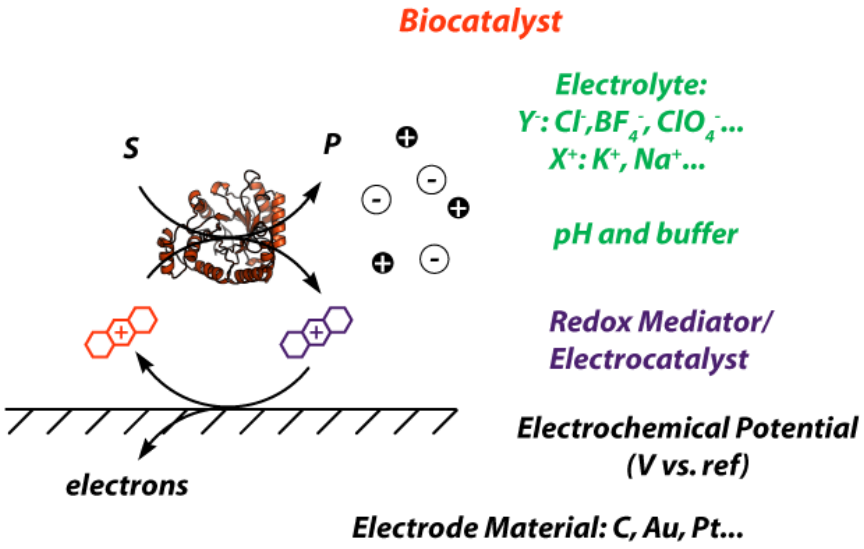
$$Ox + n\,e^- \underset{\text{Oxidation}}{\overset{\text{Reduction}}{\rightleftarrows}} Red$$

Several well established methods
Cyclic voltammetry, chronoamperometry, square wave voltammetry…

What is needed for electroanalysis ?

- **Electrochemical reaction interface: Working electrode (+ counter + reference electrode)**
- **Devices: potentiostats (1 to 8 ways)**

Multicomponent/multiparameter interface



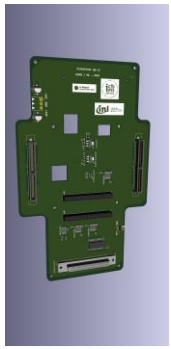
Optimization is time-consuming, mostly empirical or experience based

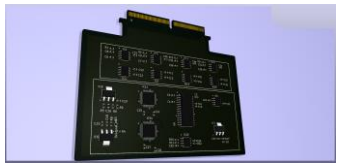
High Throughput Electrochemistry Needed

The CEITOP Project

Software for data acquisition and control

+





Hardware: 1 + 2 Cards

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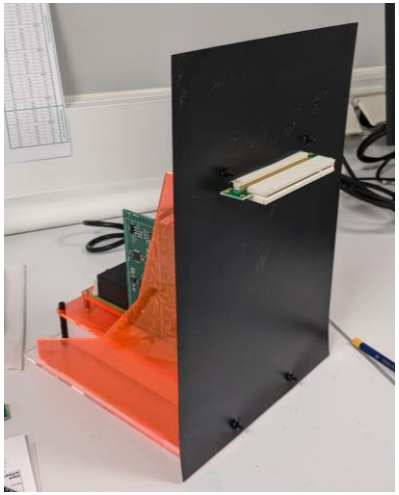

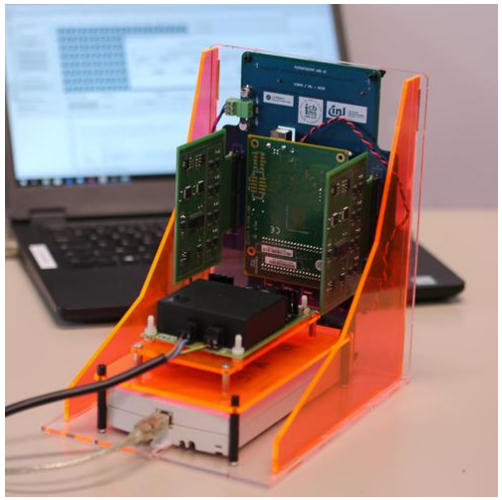

CEITOP Project
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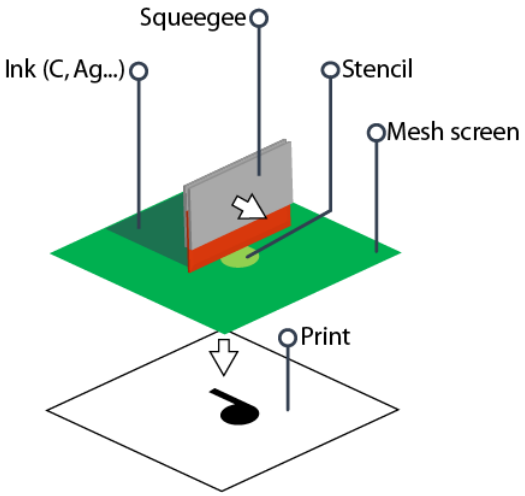
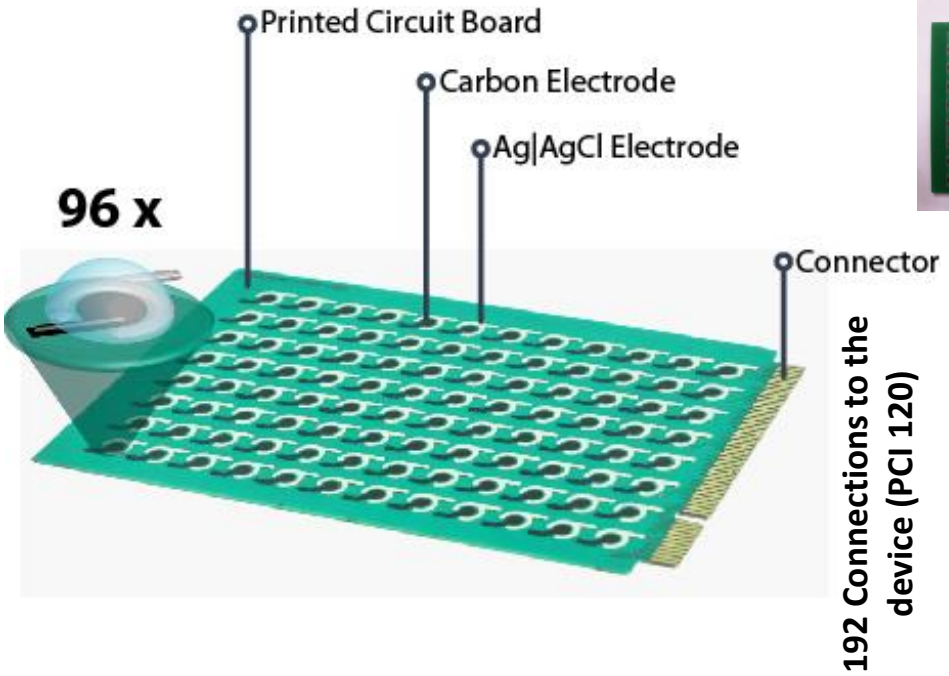
La Région
Auvergne-Rhône-Alpes

Electrodes Architecture: Classical 96 well format

12 columns × 8 raws



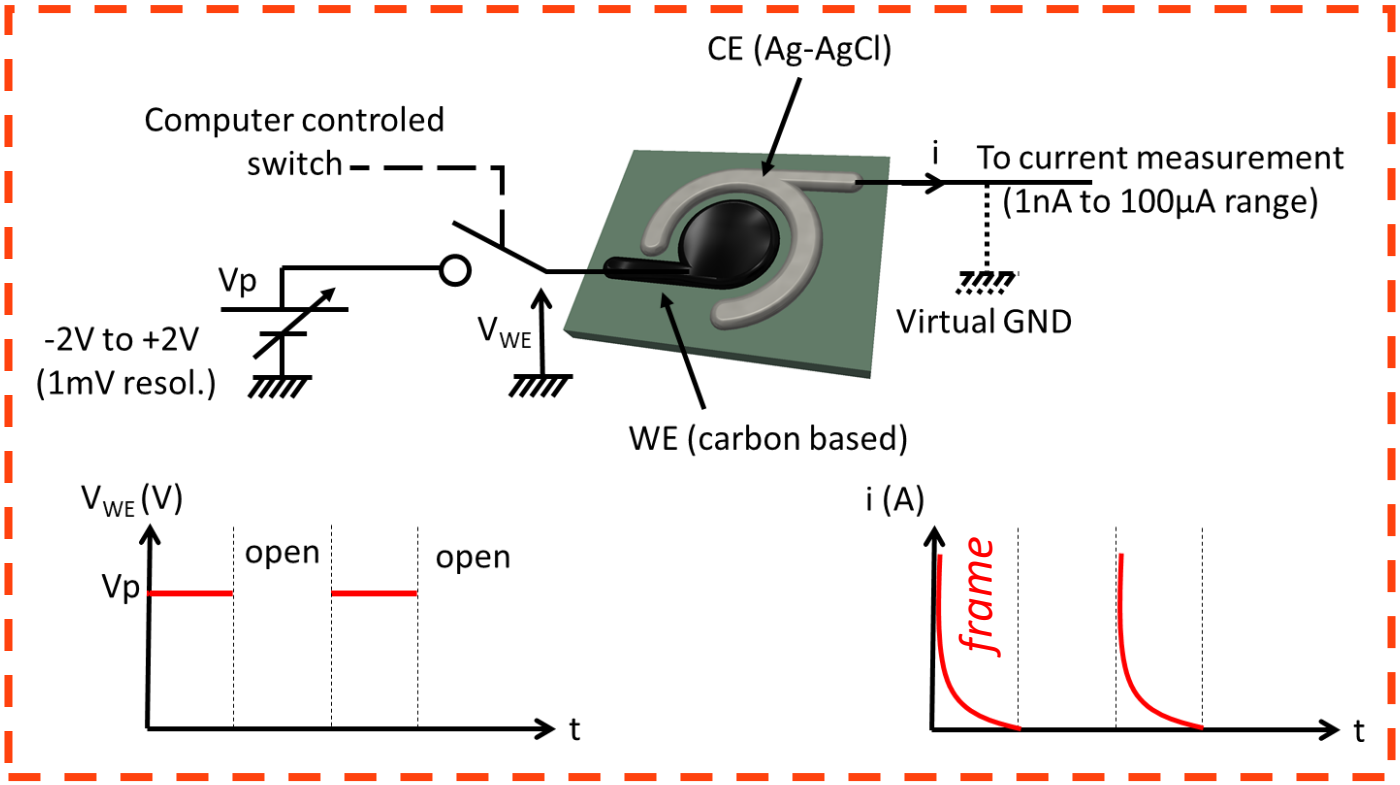


Electrochemical experiments are performed on 96 multiplexed electrodes screen-printed on a PCB. Working electrodes are made of carbon and counter/ref are Ag|AgCl electrodes.

PCB is only a connector between the device and the 96 electrodes (no electronics)

Samples are 96 drops of 35-50 µL covering each independent electrodes

Electrochemical method: Intermittent Pulsed Amperometry (IPA)

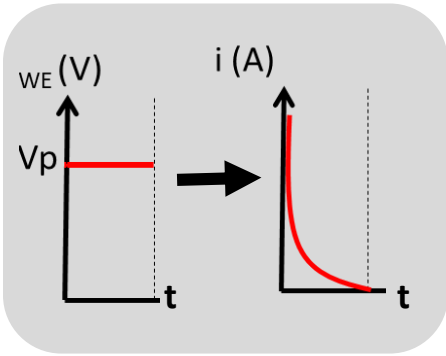


x 96

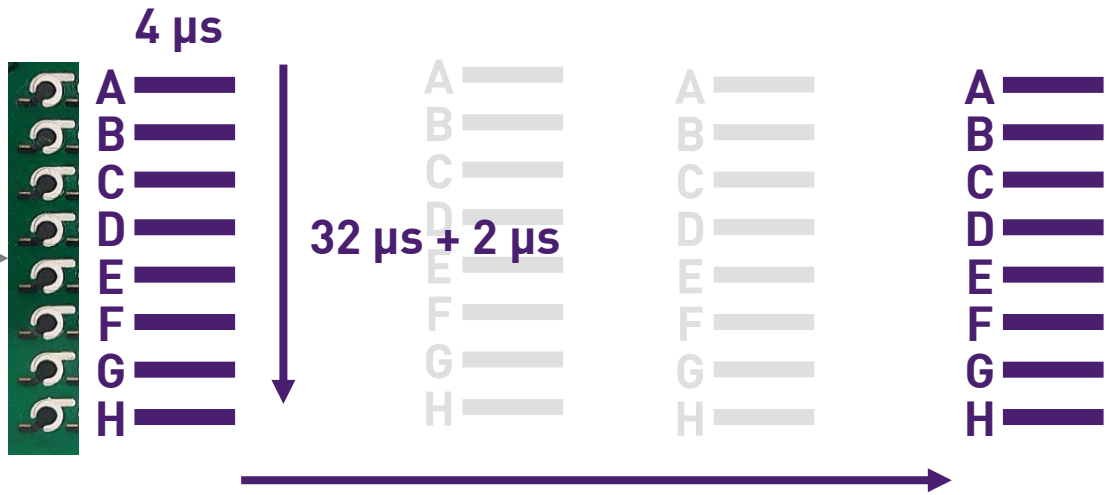
2 electrodes systems: Working (Carbon) and Counter/reference (Ag/AgCl)
WE is switched from a polarized state to an open state (≠Ø) periodically.
During polarization, current is measured: **one I *vs* time plots per frame**
(Chronoamperometry-like)

I vs time plots × number of frame × 96

Column period: residence time on the electrode column



Delay | Polarization duration (-2/+2 V)



N aquisitions of 34 µs = 1 frame = I vs t plot

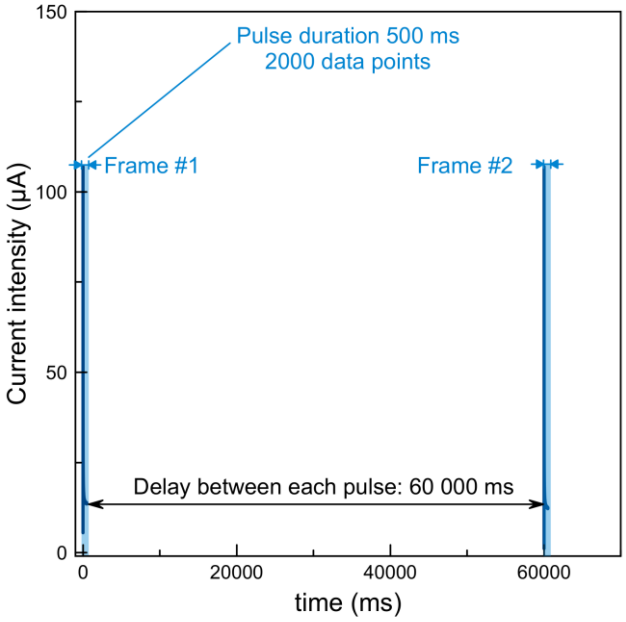
For each column, intensity is recorded N times with a maximum time resolution of 34 µs.

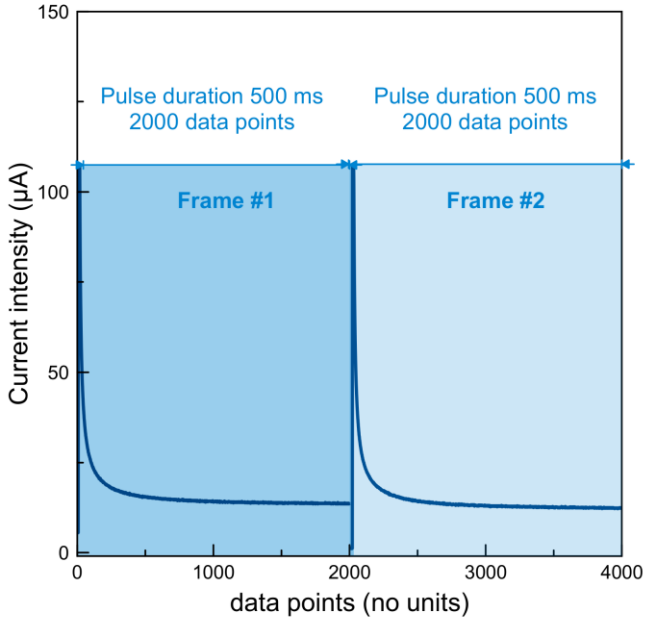
Ex: Polarization duration of 100 ms lead to 2940 data points per well (23520 per column)

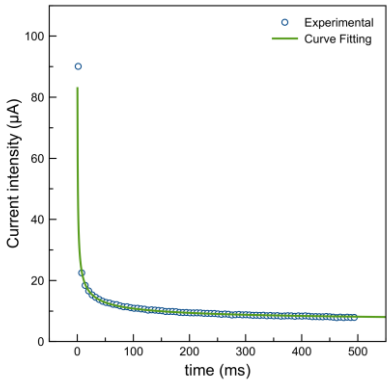
But could be less if necessary!

Repeat indefinitely for the 12 columns

Results for a single electrode (should be mutipled by 96)



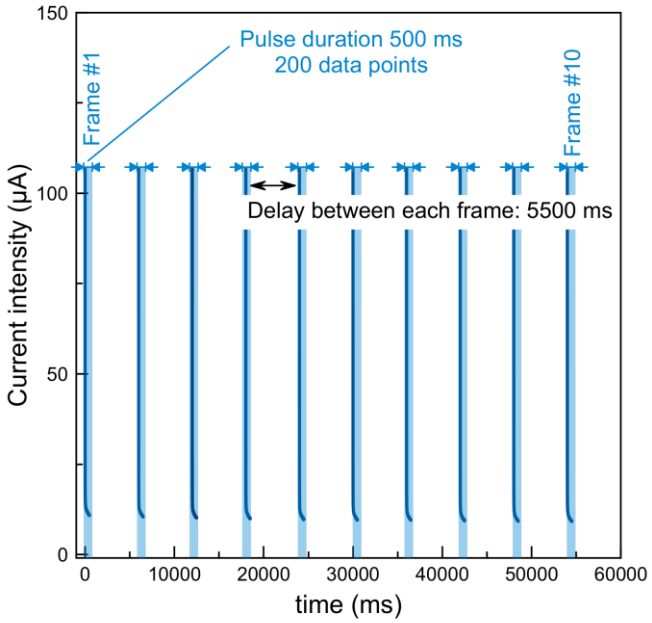


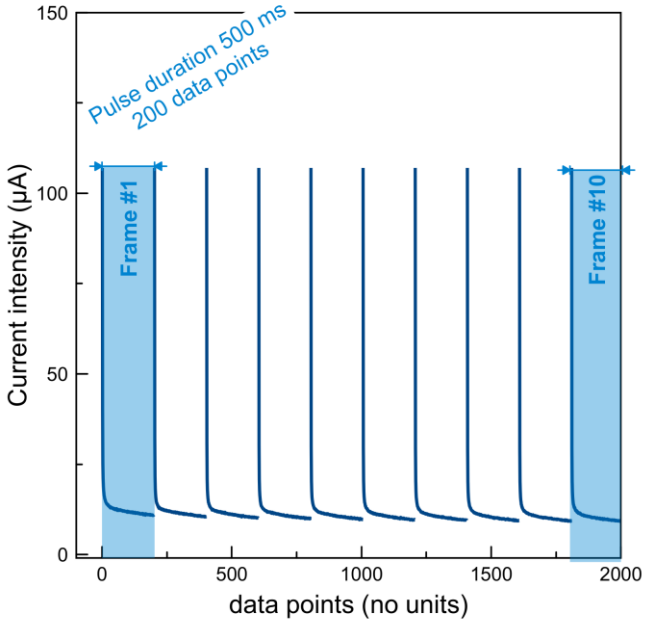


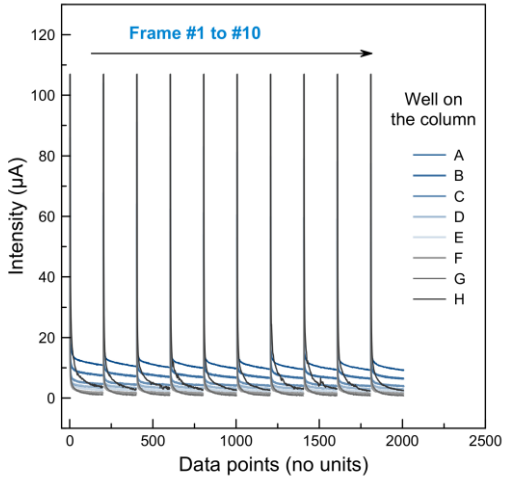
$$I = nFAC^* \sqrt{\frac{D}{\pi t}}$$

- Potential is applied for **500 ms** with one acquisition every 0.25 ms resulting in a **I vs. t frame of 2000 data points**.
- A second frame is acquired after 60 000 ms (1 min).
- The two frames are nearly identical (shown as function of data point number)
- As a **first approximation**, the **I vs. t plot was fitted to the Cottrell equation**.

Results for a single electrode (should be mutipled by 96)







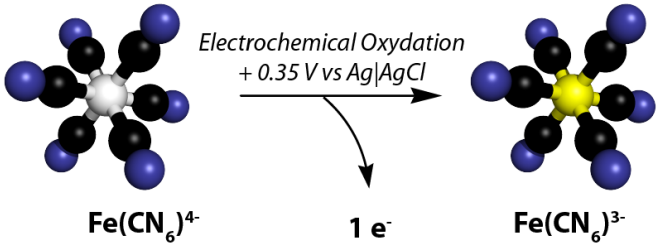
Inter electrode variability

- Potential is applied for **500 ms** with one sample every 2.5 ms resulting in a **I vs. t frame of 200 data points**.
- Ten nearly identical frames are acquired during 60 000 ms (1 min) (left).
- Inter electrode variability still occurs due to non-optimized screen-printing (right) (*work in progress*).

Empirical equation… $I = nFAC^* \sqrt{\frac{D}{\pi t}} + \frac{k}{t} + I_0$ … to be justified by diffusion-reaction

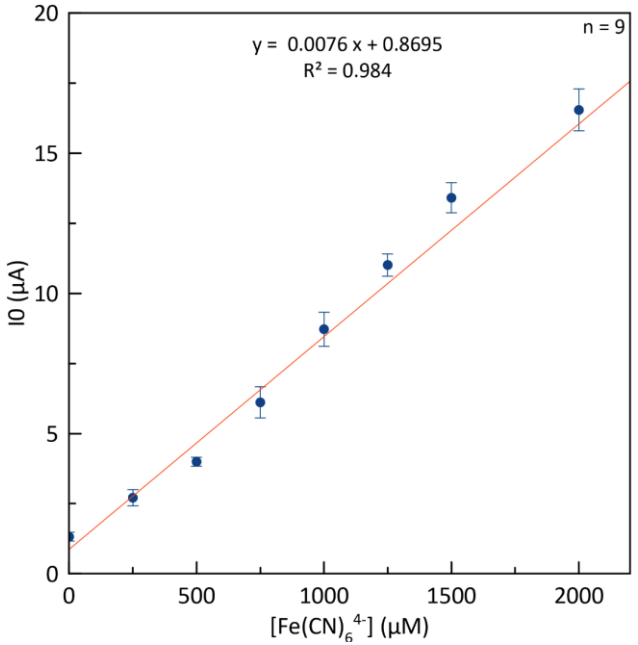
Home made software for automatic data analysis of all frames: ***POSTER S1-P026***
(part of Rainier-Numa GEORGES PhD work)

Model reaction



Metrics:

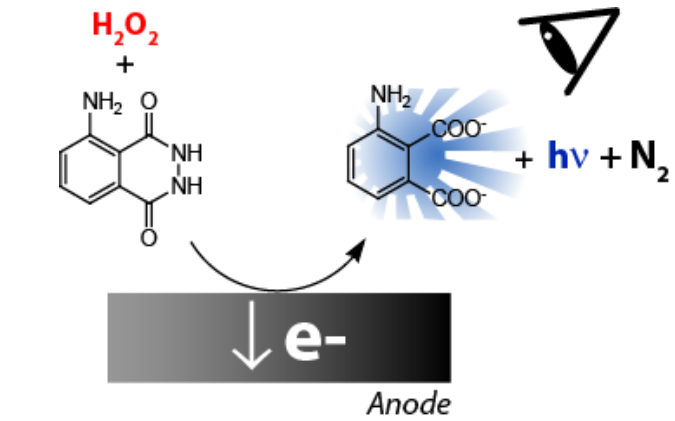
2 minutes – 96 electrodes
10 frames of 1sec /electrode
400 data point per frame = **384 10^3 data points**
Analysis duration: some sec !

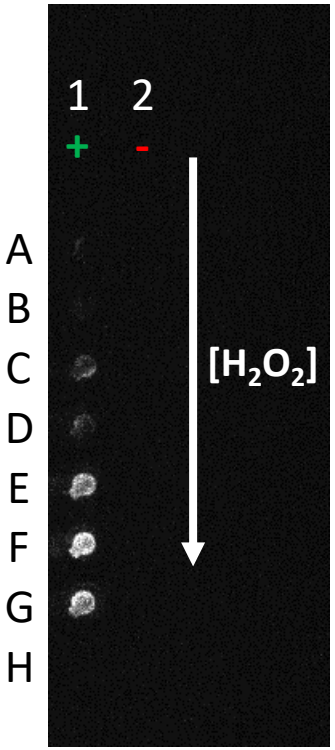


Sensitivity: 7.6 µA.nM^{-1}
LoD below 200 µM (Screen-printed electrodes)

Electrochemiluminescence is also possible

Model reaction





part of Nathan Montmailler Master work

Luminol oxidation in the presence of H_2O_2 illustrates the periodic polarization of the electrodes (still some defects due to screen printing)

Rapid ECL protocol optimization

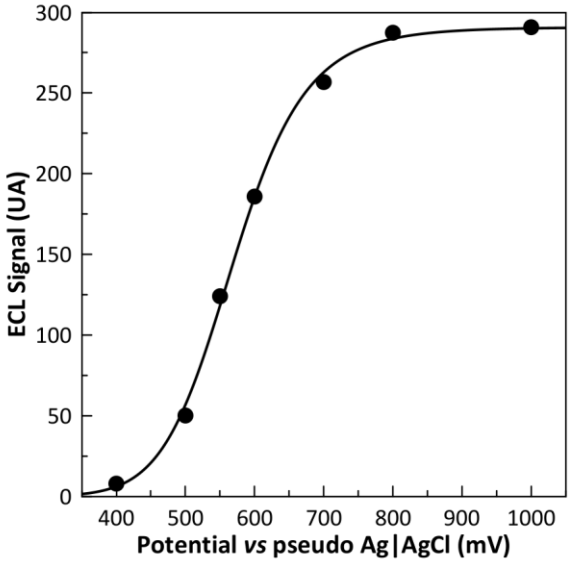
Experimental setup

[H_2O_2] = 50 nM

[Luminol] = 100 µM

Veronal buffer 50 mM, pH = 8.5

Maximum signal obtained for 800 mV vs Ag|AgCl pseudo ref. (non modified SPE)



Experimental setup

[H_2O_2] = 0.8 - 50 nM

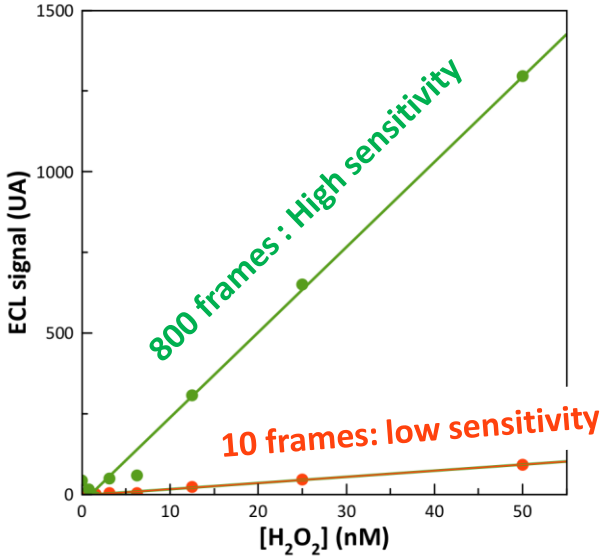
[Luminol] = 100 µM

Véronal buffer 50 mM, pH = 8.5

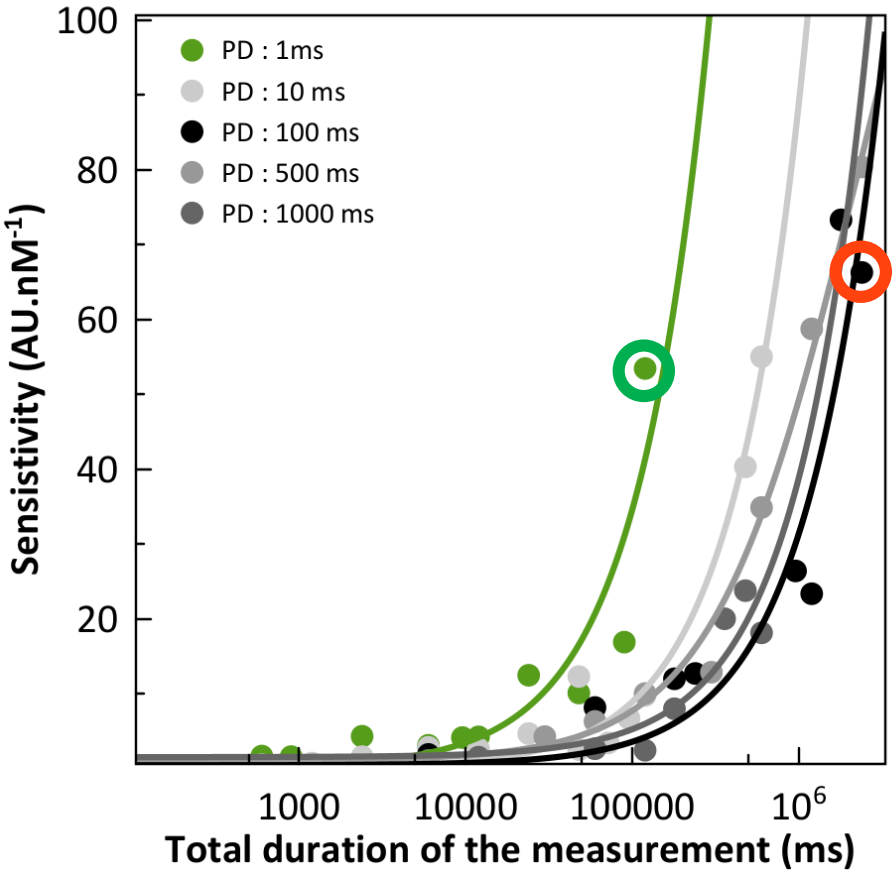
Applied potential : 800 mV vs Ag|AgCl pseudo ref.

Higher frame number higher sensitivity but…

long analysis time



Pulse width is the key parameter for short time analysis



Pulse width is varied from 1 ms to 1000 ms:

- **Number of frame is constant**
- **Analysis time increases drastically (expected)**
- **For short pulses width, higher sensitivity is achieved**

Examples: for 1 ms pulse width, potential is applied every 12 ms on a column

Close to continuous potential application on the 96 electrodes…
… but potential could be modified on each of them

1 ms pulse: 2 min for 96 electrodes and sensitivity of 60 nM^{-1} H_2O_2

vs

1000 ms pulse : 40 minutes for the same results

What's next ?

Technicals:

- **Improve Screen-Printing**
- **"Higher" volumes: Drop (50 µL, hemispherical) to wells (400 µL, cylinder)**
- **Replace PCB by plastics, paper… (true disposable electrodes)**

Fundamentals:

- **"True" equations for analysis > Physical parameters**
- **Study more deeply the ECL reaction using IPA**

Applications:

- **Screening of chemical libraries for drug discovery (enzyme inhibitors)**
- **ECL and electrochemical Biosensors optimization**
- **Others ?**

Thanks you for attention and to

La Région Auvergne-Rhône-Alpes