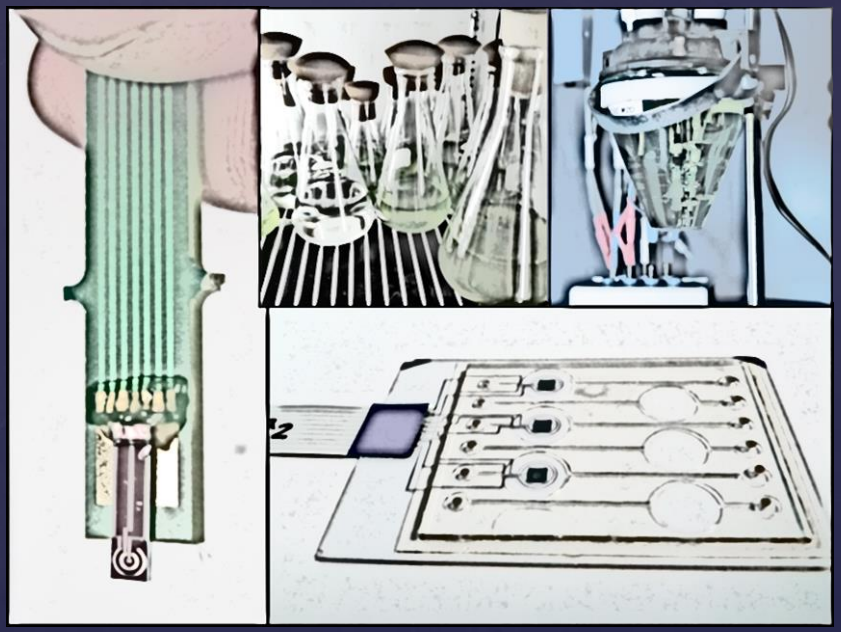
Development of a lab-on-chip platform integrating electrochemical and optical microsensors for the detection of water contaminants based on algal physiology monitoring



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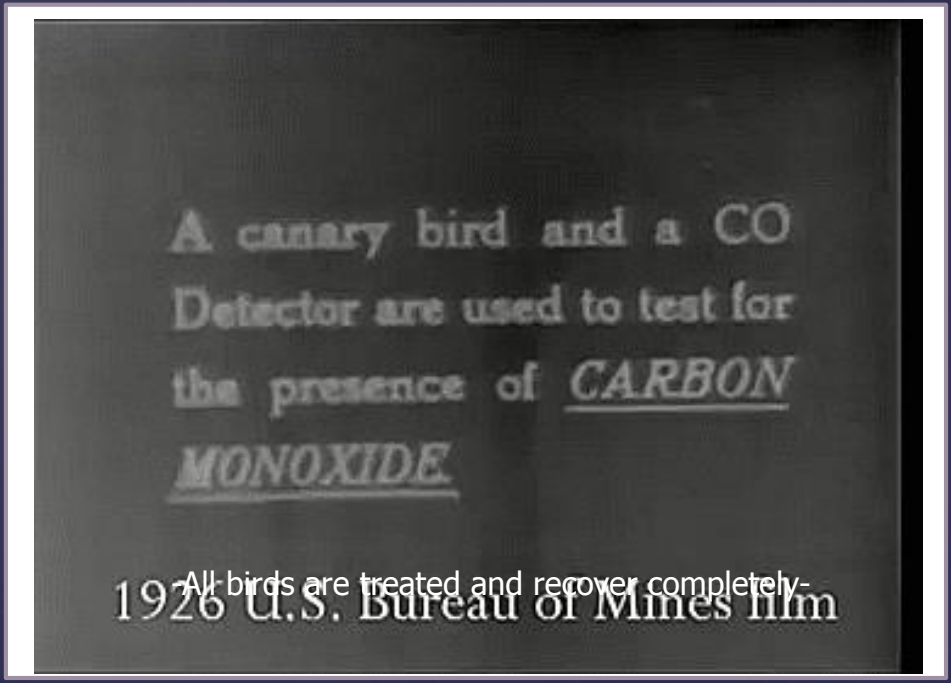
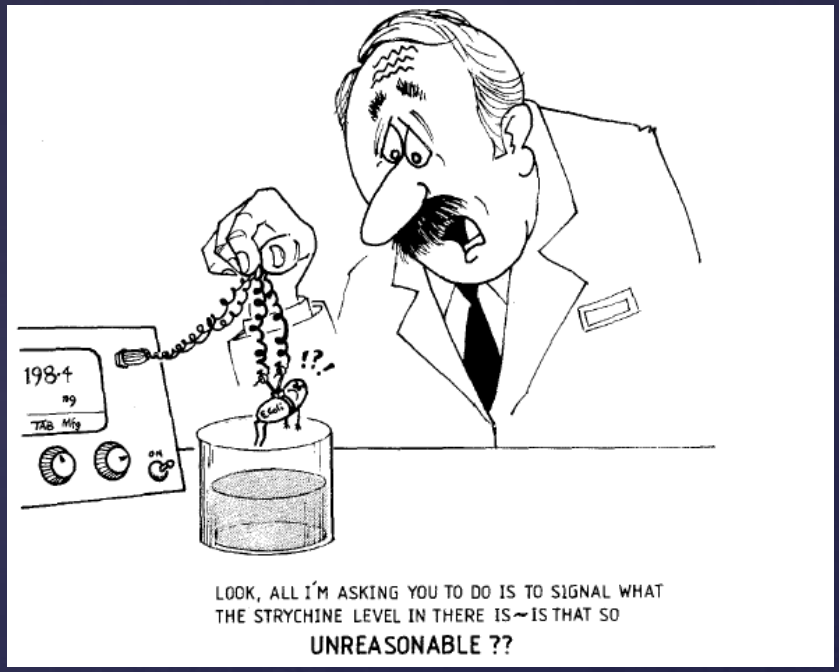
Toxicants detection methods – Alternative systems

Goal ⇒

- On-site, rapid measurements
- Threshold detection
- Early warning system





Biosensor

Biological sensing element

Transduction system

Biosensors – Axes of development

Herbicide detection → **optical** and **electrochemical** sensors

	optical	electrochemical
Low Limit of detection (LOD)	+	-
Easily miniaturized	+	+
Low cost	+	+
Stabilization time	-	+
Insensitive to contamination	+	-

Algae

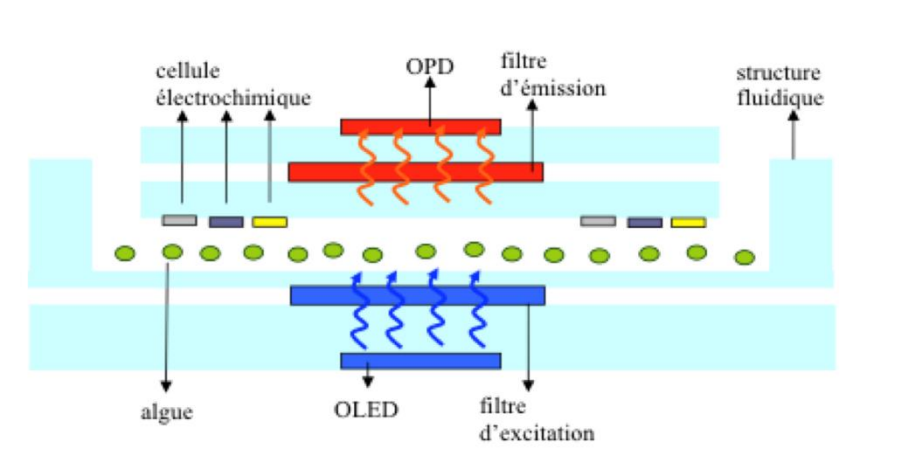
Electrochemical sensor | Optical sensor

Herbicide detection

Biosensors – Aim of the development => Lab on Chip (LOC)

Study, development and integration of building blocks :

- multi-tank fluidic structure
- optical components : OLED /OPD
- all integrated electrochemical microcells

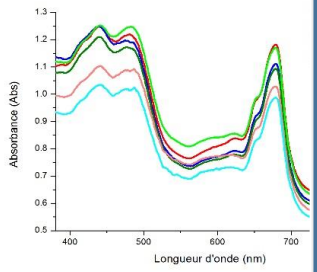


Scientific aims :

- Assessing the feasibility of combining optical and electrochemical sensors for the measurement of algae metabolism changes
- Evaluating the measurement reproducibility with biological material
- Integrating a multi-analysis system on a same LOC plateform
- Miniaturisation of the whole system (=> portable device)

Bio-sensor study

- Algal bio-sensor
- Pollutant effects (herbicide)

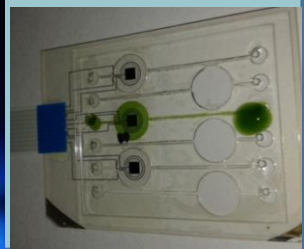


Electrochemical cell & microfluidic plateform

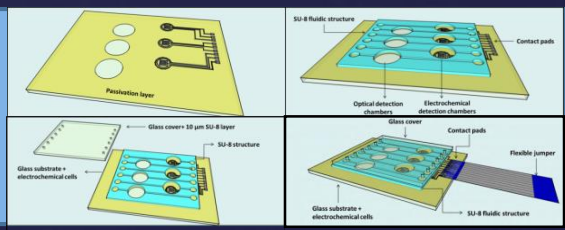
- Design
- Fabrication
- Calibration tests
  - O_2

Validation measurements

- Photosynthesis activity
- Fluorescence



Conclusion

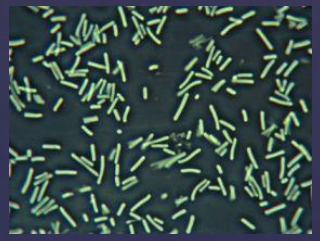


Which bio-sensor ?

Why micro-algaes ?

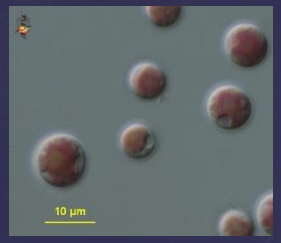
- Very sensitive to stress (pollutants, temperature…)
- Very sensitive to pesticides, herbicides, metals

Several physiological indicators : photosynthesis, fluorescence …



Cyanobacteria

Green algae



Red algae

[16] Shao N. et al. , Planta 2008, 228(6):1055-1

Chlamydomonas reinhardtii

Pollutants :

- Herbicides and pesticides : **Diuron**, Paraquat, …
- Drugs
- Heavy metals
- Toxins

Photochemistry

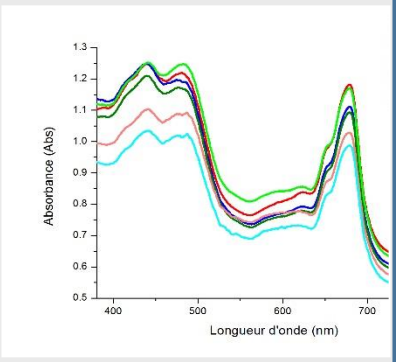
Fluorescence

Energy transfer

Heat

Bio-sensor study

- Algal bio-sensor
- Optical response
- Pollutant effects (herbicide)

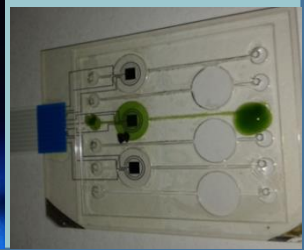


Electrochemical cell & microfluidic plateform

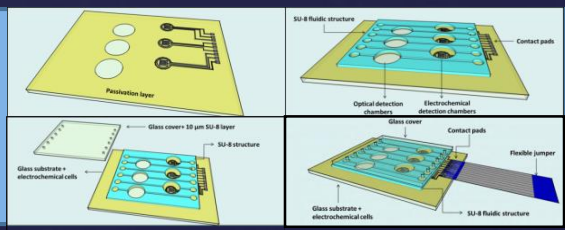
- Design
- Fabrication
- Calibration tests
  - ➢ O_2

Validation measurements

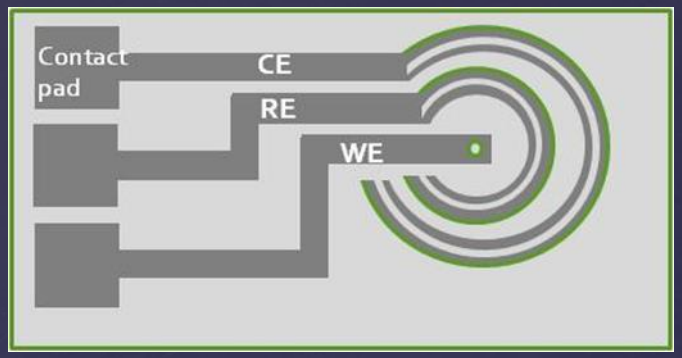
- Paraquat detection (pH, H_2O_2)
- Diuron detection (O_2)



Conclusion



Fully integrated electrochemical microcell

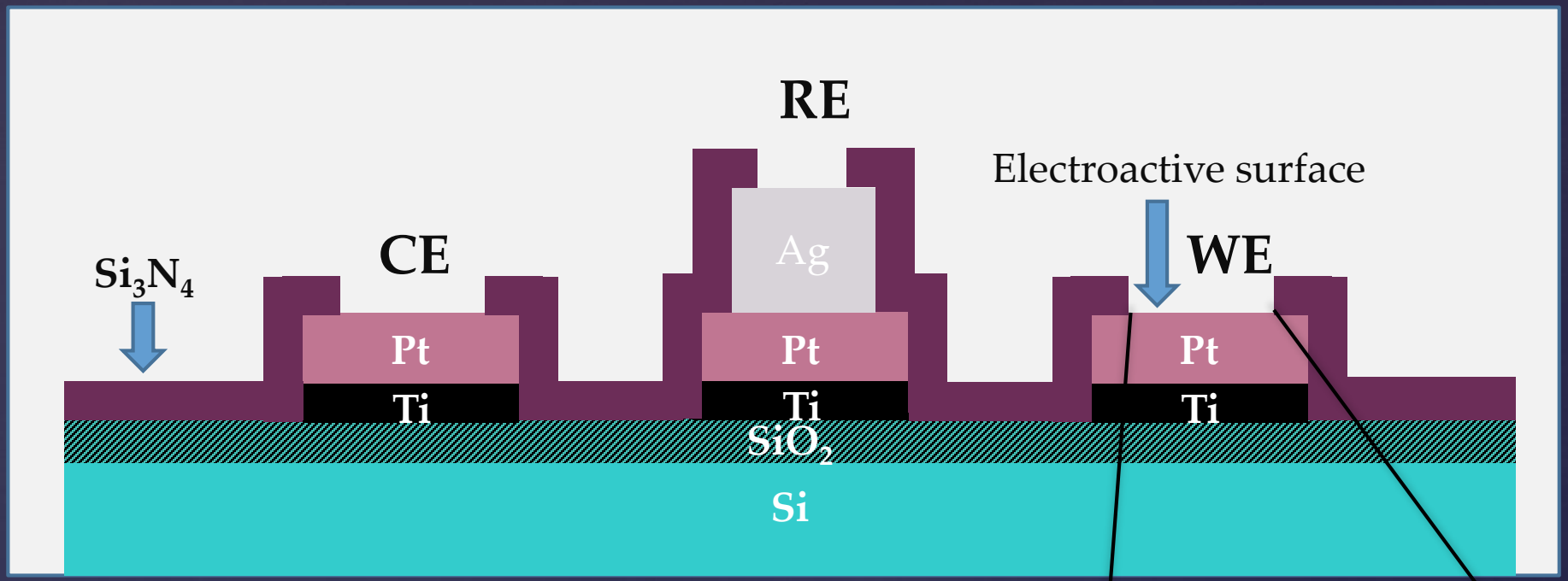


CE ⟶ Pt
RE ⟶ Ag/AgCl
WE ⟶ …

Working electrode materials

Species to be detected	Working electrode material	Functionalized electrode material
Dissolved O_2	Pt	Pt black

Fabrication procedure



A. Substrate → Si

D. Passivation layer deposition → Si_3N_4

B. Photolithography

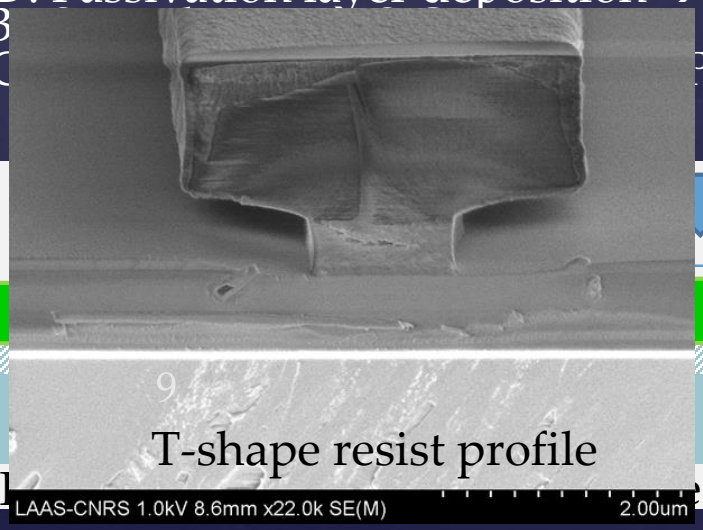
C. Pt, Ag

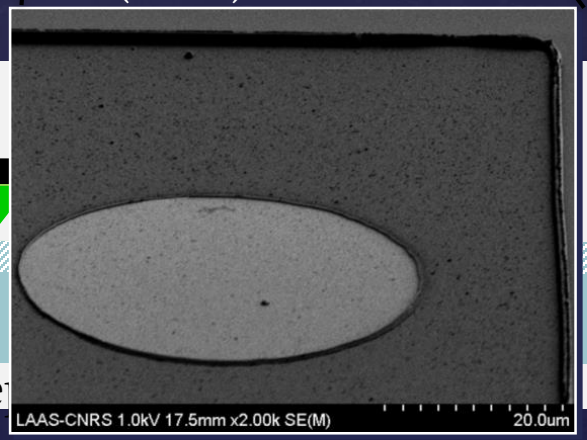
Photolithography

Si_3N_4 deposition (ICP CVD 100°C)

Metal evaporation (PVD)

Resist lift-off





1. I

3. Development

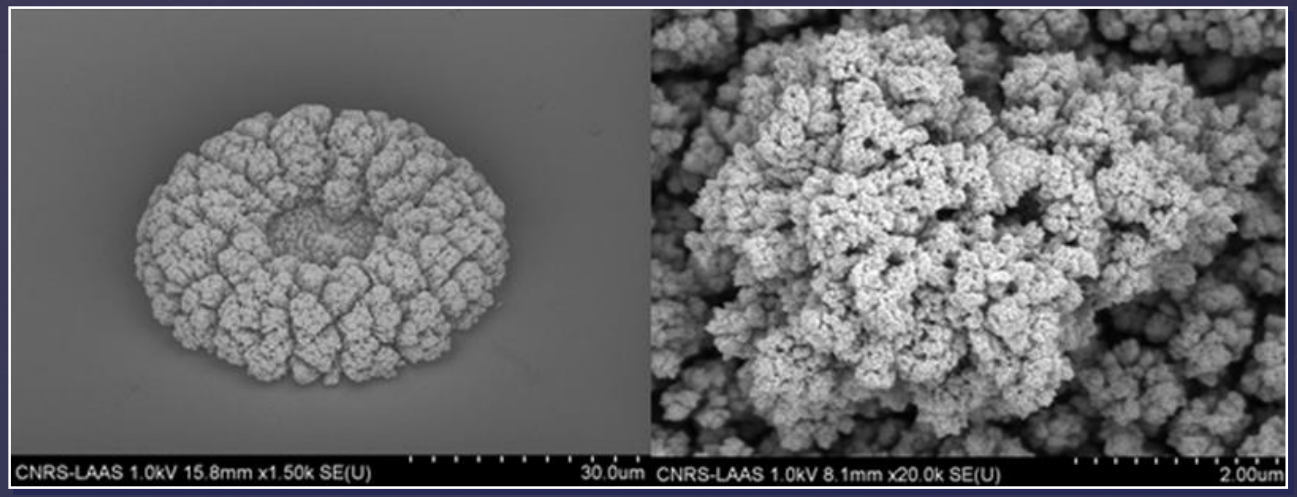
4. De

Functionalization

1. Ag oxidation [1] → Ag/AgCl for RE
2. Pt black electrodeposition[2] for WE

Linear sweep voltammetry
E_{range}: 0.1- 0.25 V/SCE
Scan rate: 1mV/s
KCl 0.01 M

ISM Bordeaux



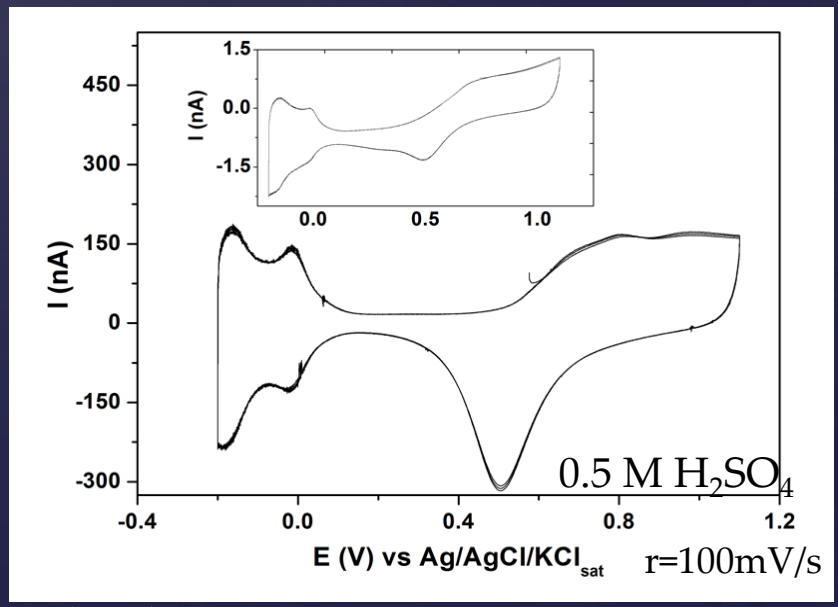
Potentiostatic
E = -60mV vs $Ag/AgCl/KCl_{sat}$
Q deposited : controlled

Electrochemical characterization

Cyclic voltammetry

Quality of the deposited material ⇨ Pt signature

Quality of passivation layer ⇨ No extra peaks



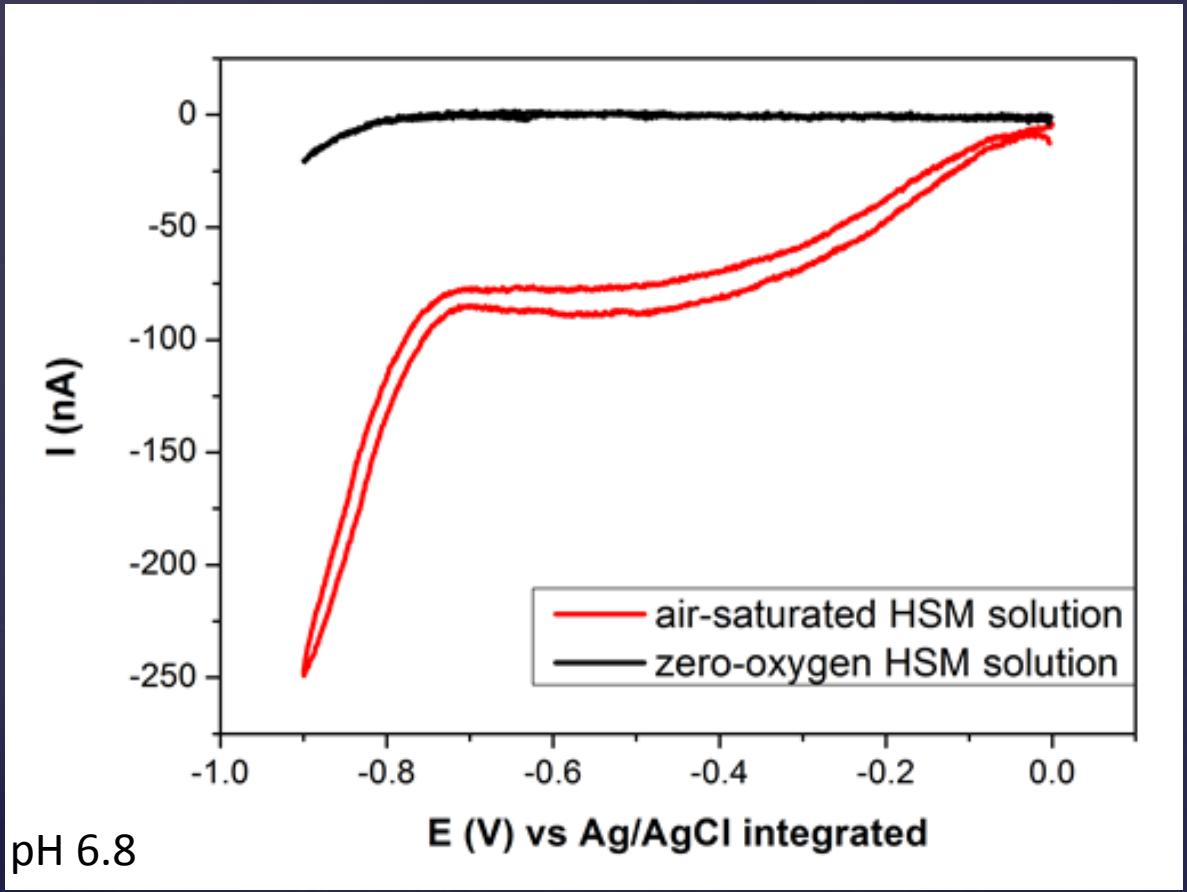
[1] Thesis Christophe C., 2010

[2] Ben-Amor et al., 2013, Electroanalysis, vol. 25, pp. 656–663

Sensor calibration

O_2 monitoring $\quad O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

Cyclic voltammetry

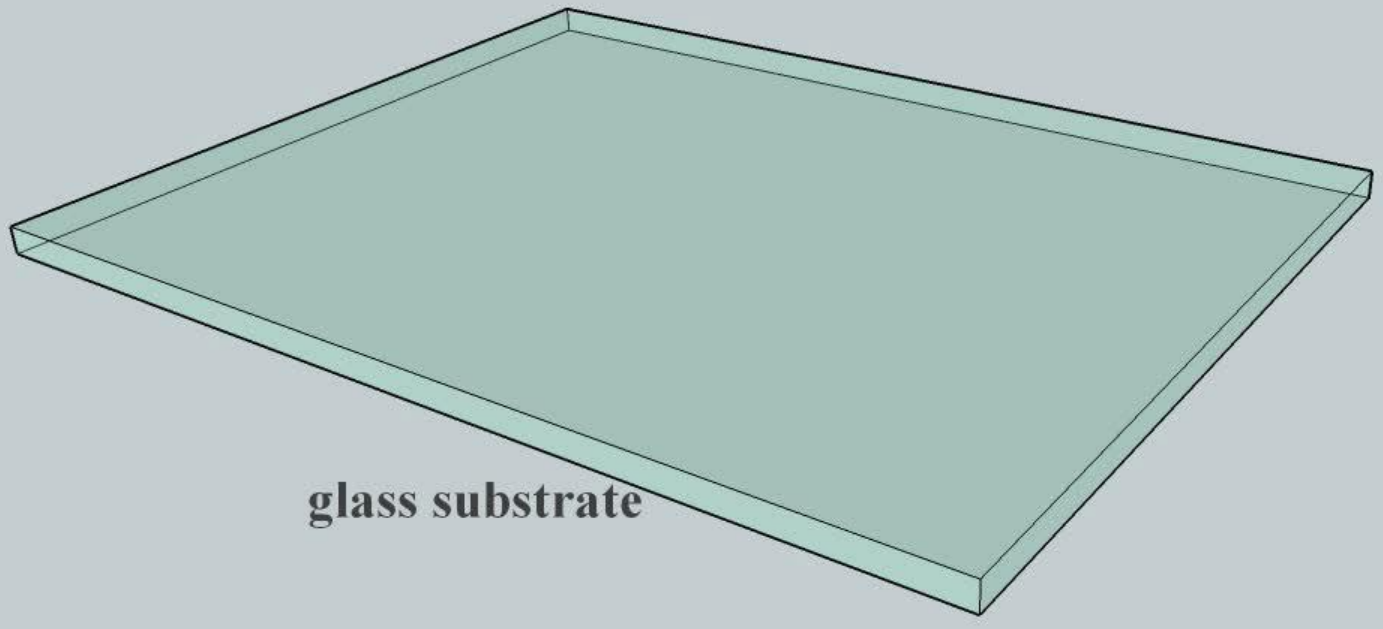


Temperature (°C)	Oxygen concentration (nmol/mL)
0	443
5	387
10	341
15	305
20	276
25	253
30	235
35	219

Dissolved oxygen concentrations for saturated water [5]

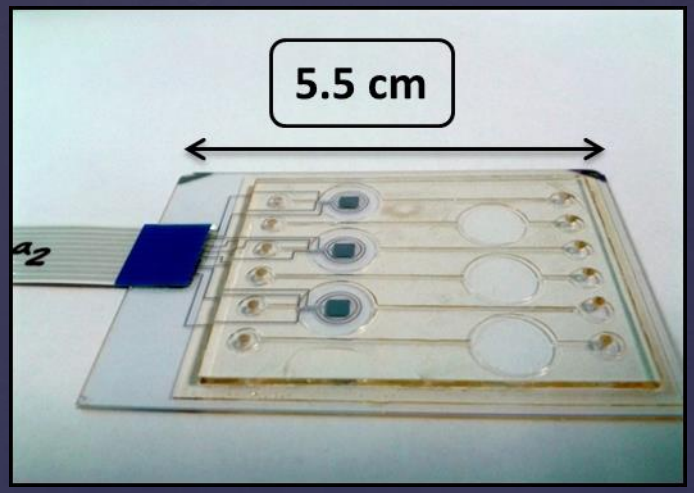
[5] Truesdale and Downing, Nature 173:1236, 1954

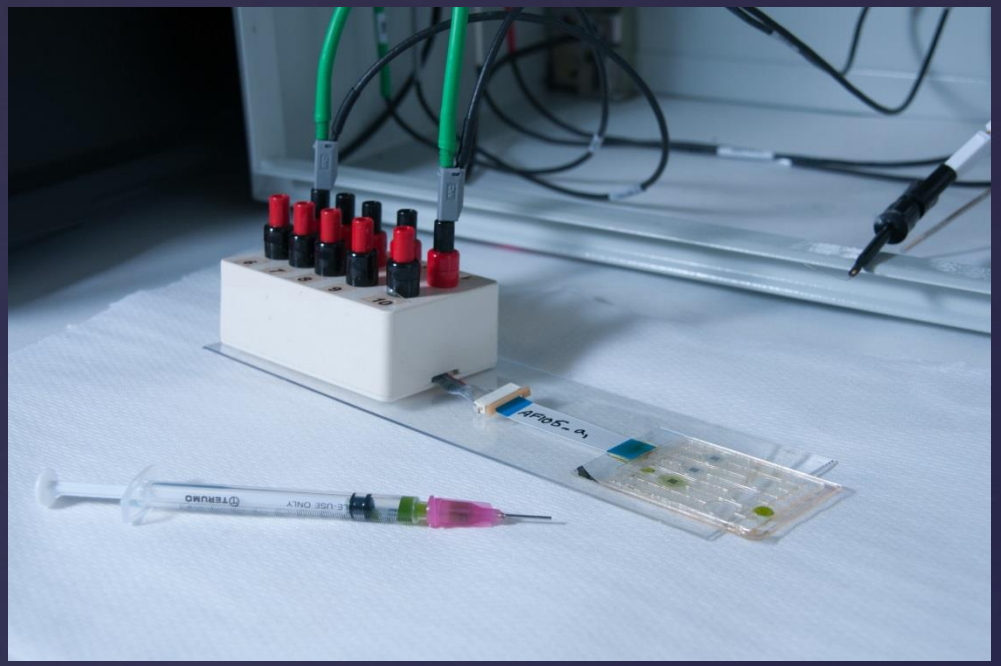
Fabrication procedure

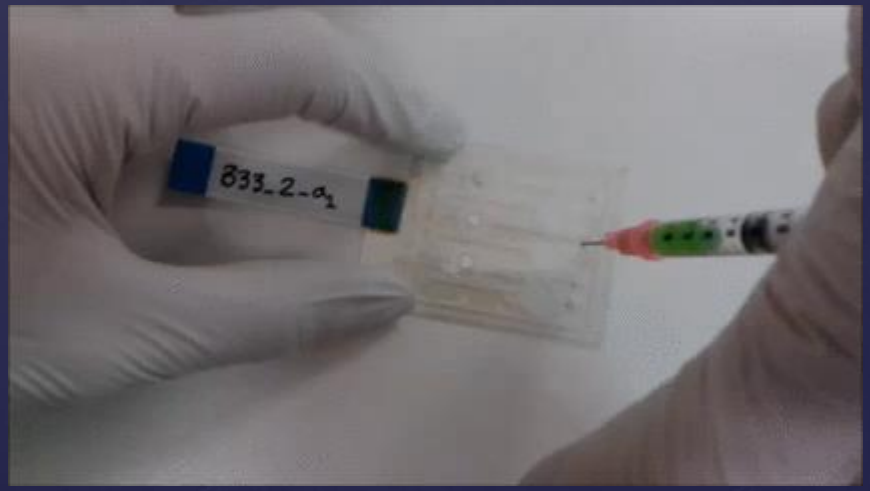


- Compatibility with optical technology
- Large detection chambers
- Large channels

- Biocompatible system
- No detrimental procedures for fragile electrode materials
- Compact and solid device

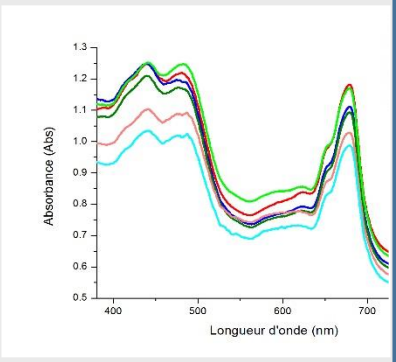

5.5 cm
a2


TERUMO


833_2_a1

Bio-sensor study

- Algal bio-sensor
- Pollutant effects (herbicide)

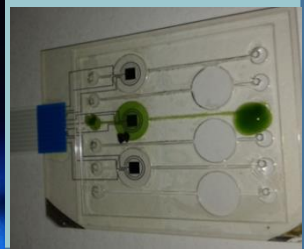


Electrochemical cell & microfluidic plateform

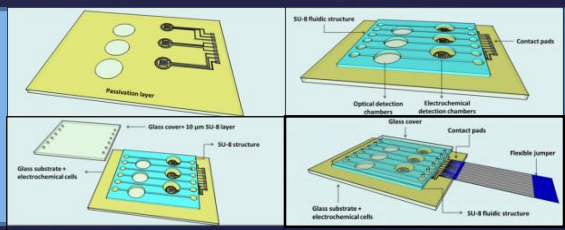
- Design
- Fabrication
- Calibration tests
  - ➢ O_2

Validation measurements

- Photosynthesis activity
- Fluorescence



Conclusion



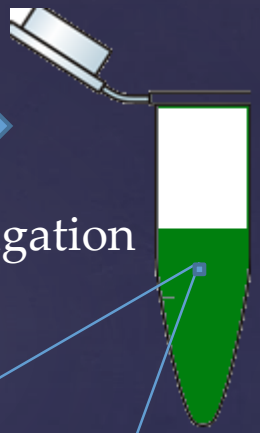
Bioassays

O_2 monitoring Protocol

Diuron

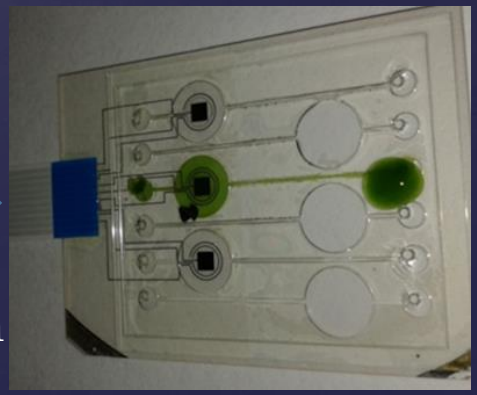
$H_2O \rightarrow 2\ H^+ + \frac{1}{2}\ O_2$

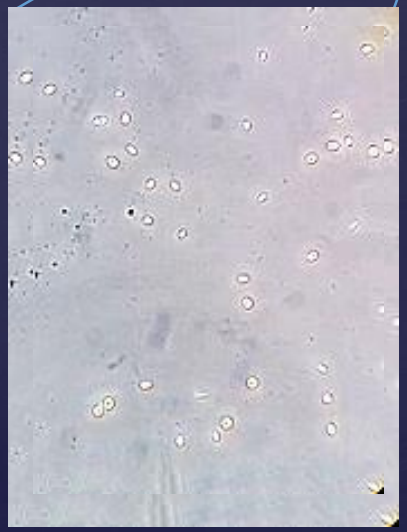
Centrifugation



Herbicide addition

Injection in LOC





O_2 monitoring through CA

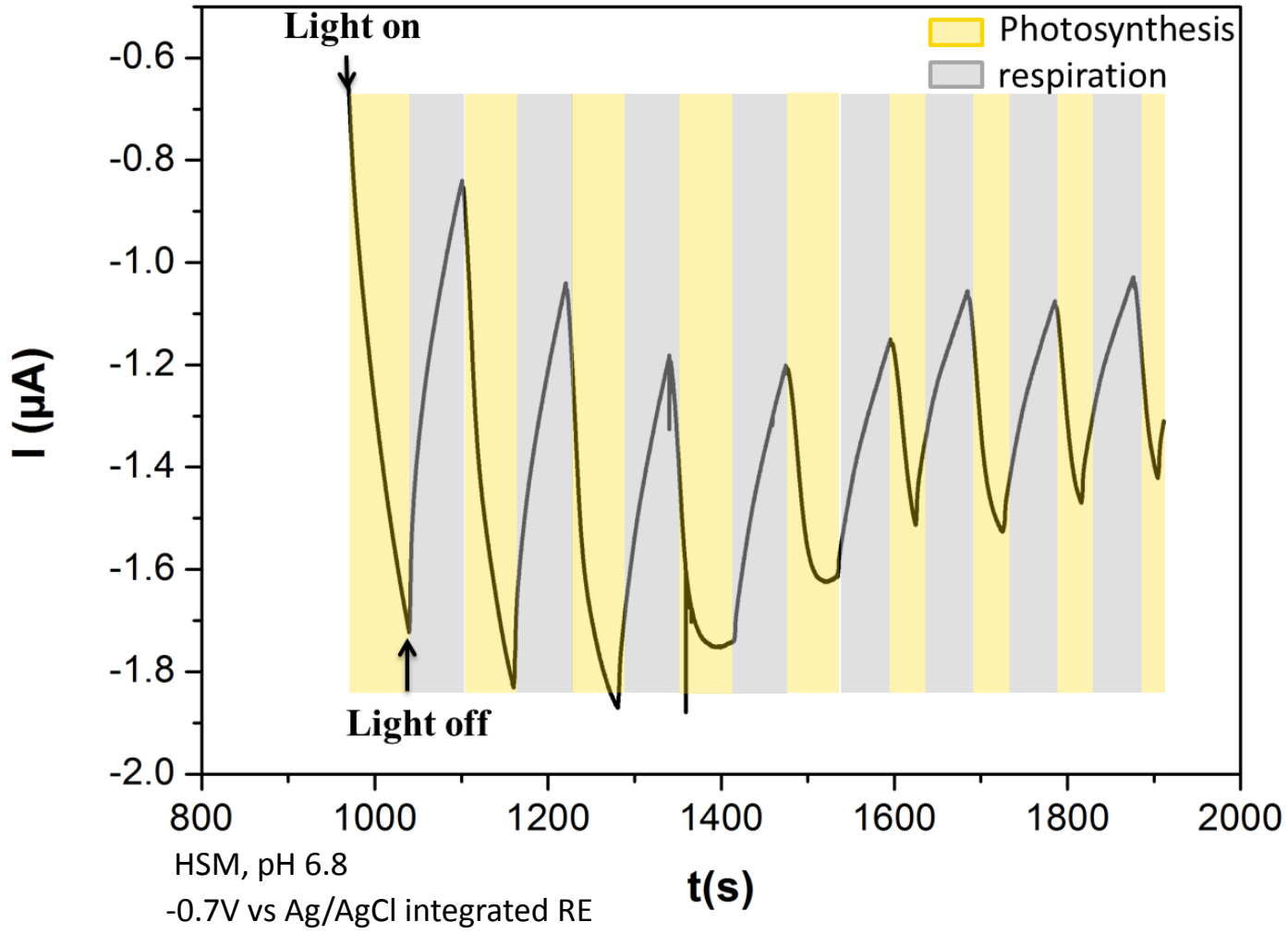
O_2 measurement without herbicide

Halogen white light

Current linearly depends on O_2 concentration

$$I_{lim} = 4\,nFDC^{sol}r_d\,z$$

Chronoamperometry

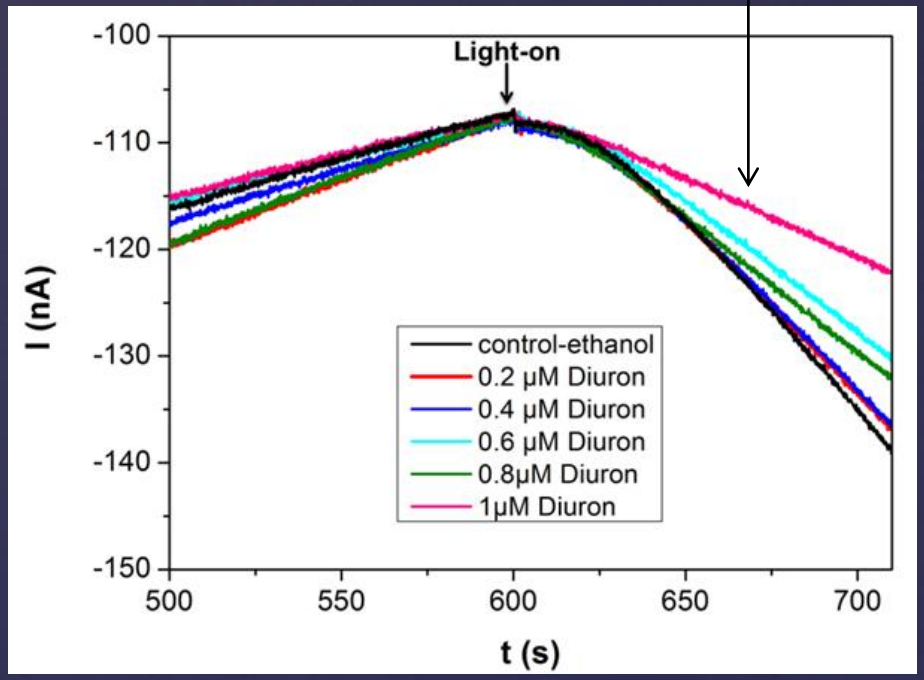


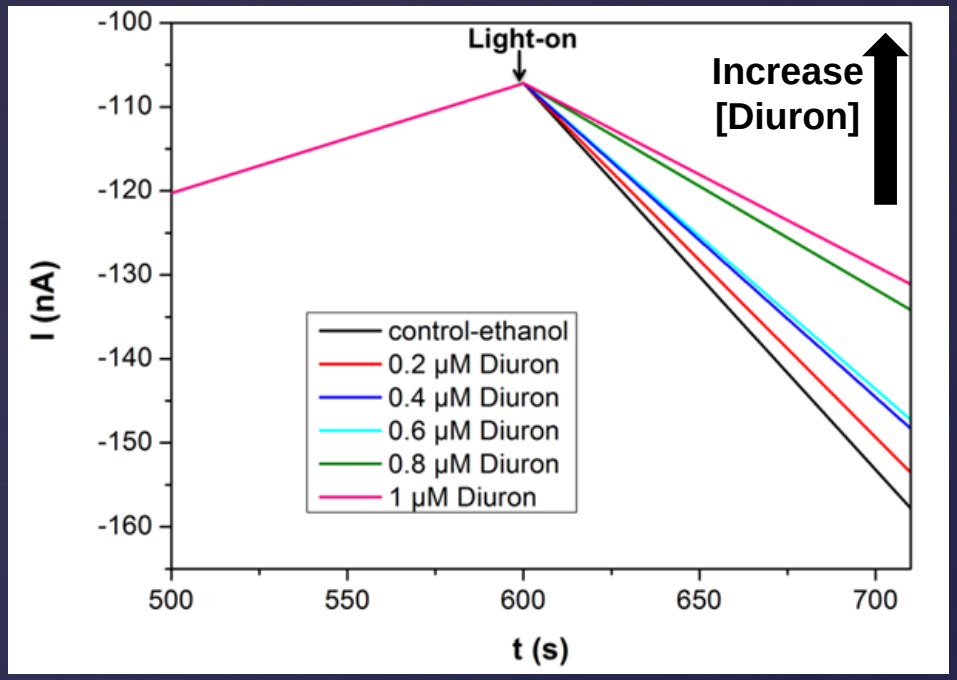
HSM, pH 6.8

-0.7V vs Ag/AgCl integrated RE

O_2 measurement with herbicide

Photosynthesis slope = O_2 production rate





Diuron has no impact on respiration

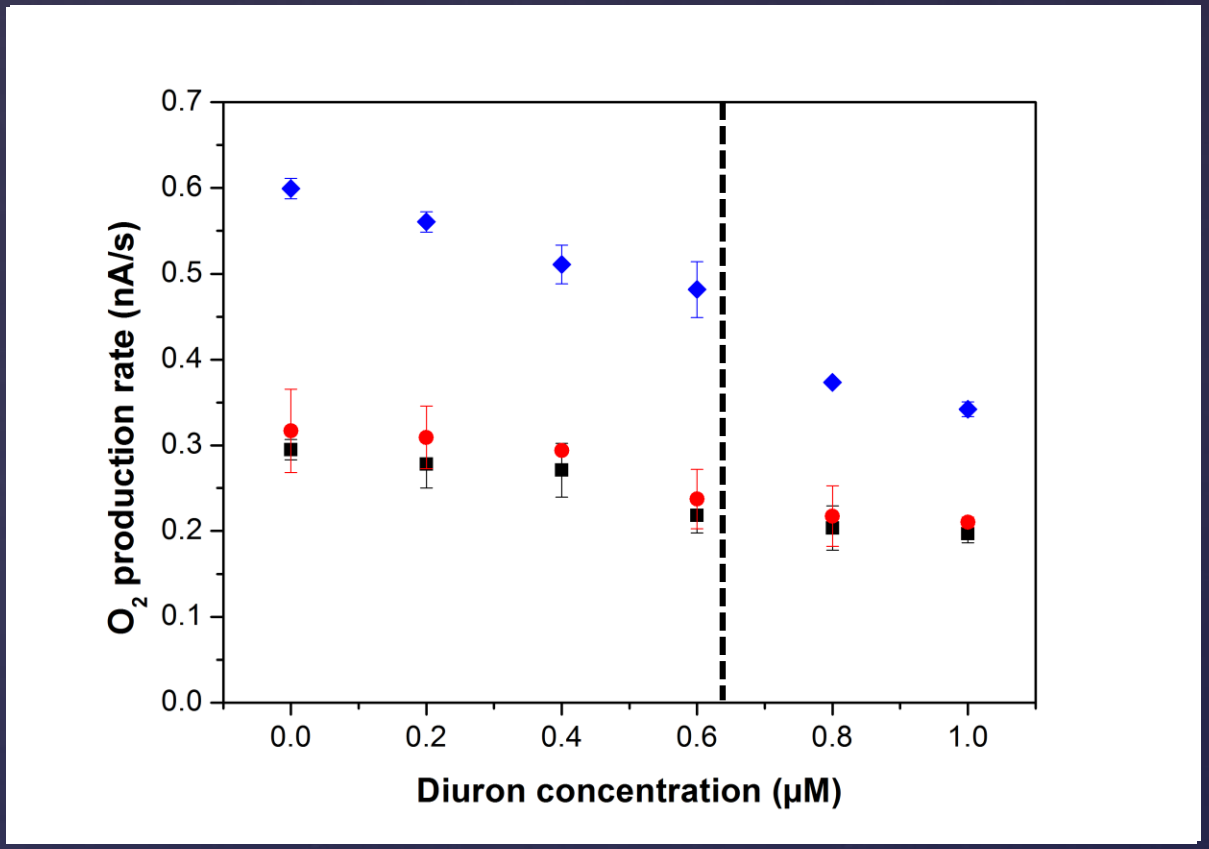
Respiration slope constant for different Diuron concentration

Normalization protocol

O_2 measurement with herbicide

Halogen white light

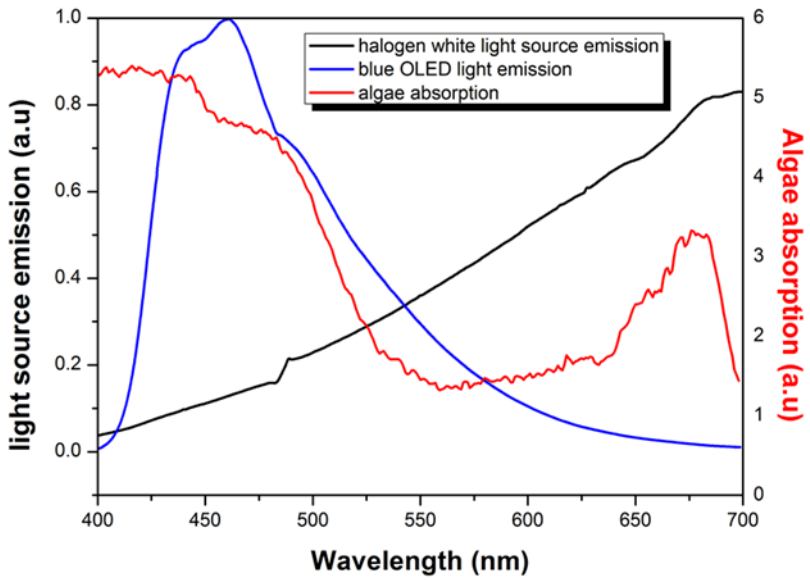
Light intensity effect (light stress)

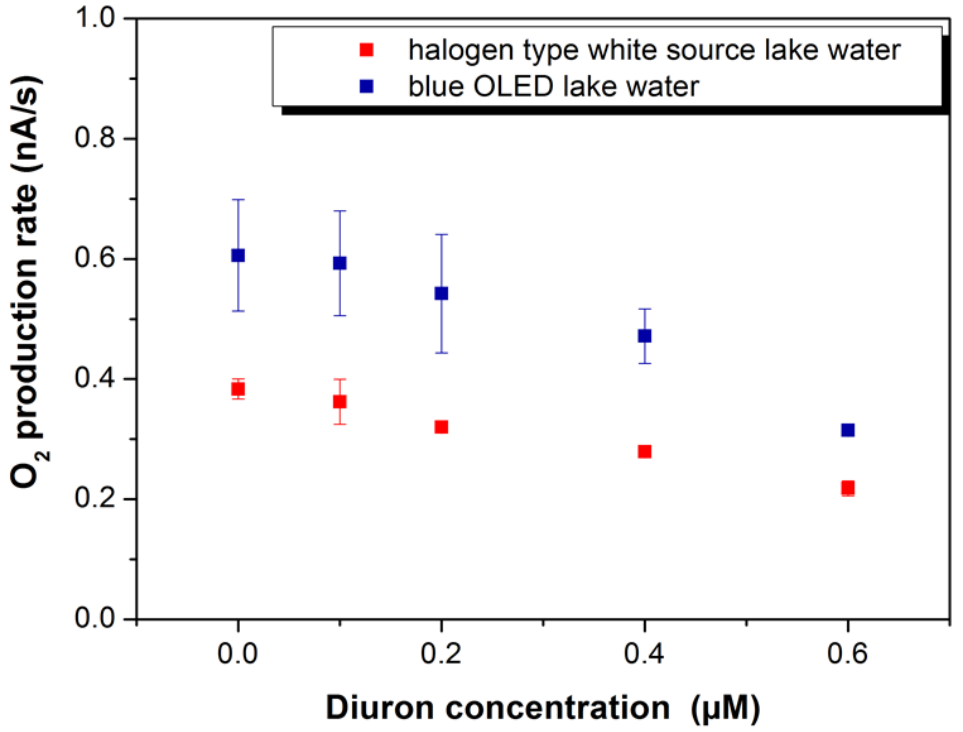


	Sensor	Light intensity (µmol photons.m^{-2}. s^{-1})	Sensitivity (blank-0.6µM)
●	Sensor 1	**1800**	**0.12**
■	Sensor 2	**1800**	**0.11**
◆	Sensor 2	**600**	**0.20**

O_2 measurement with herbicide

OLED light source
Sample : lake water



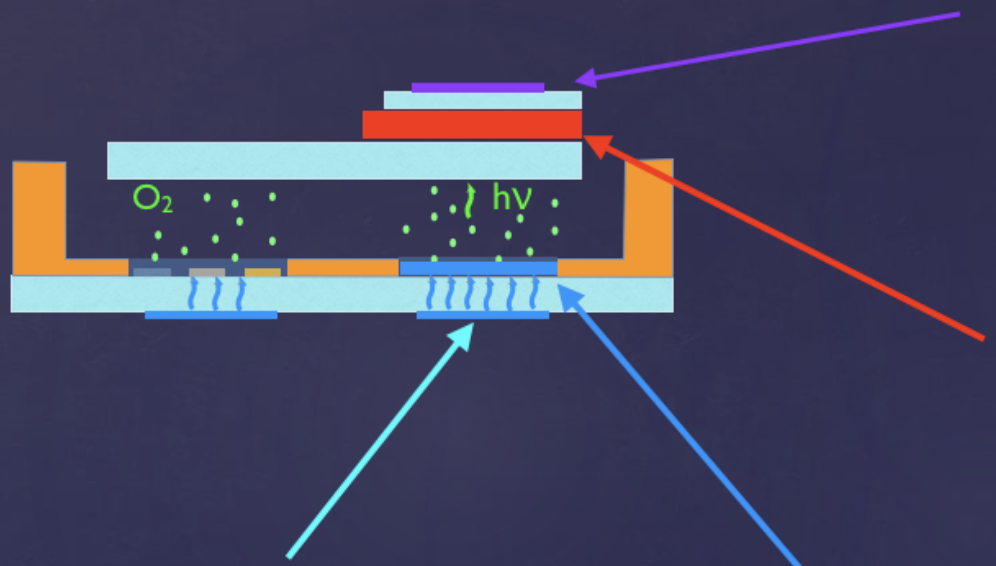


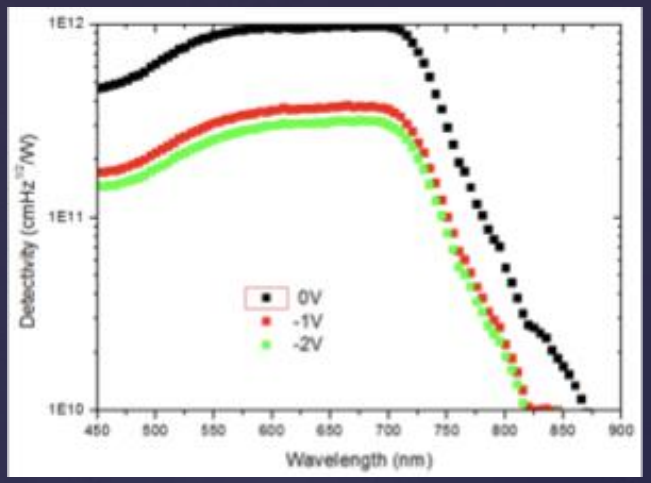
	Sensitivity nA. s^{-1}. μM^{-1}
OLED	0.48
Halogen white lamp	0.26

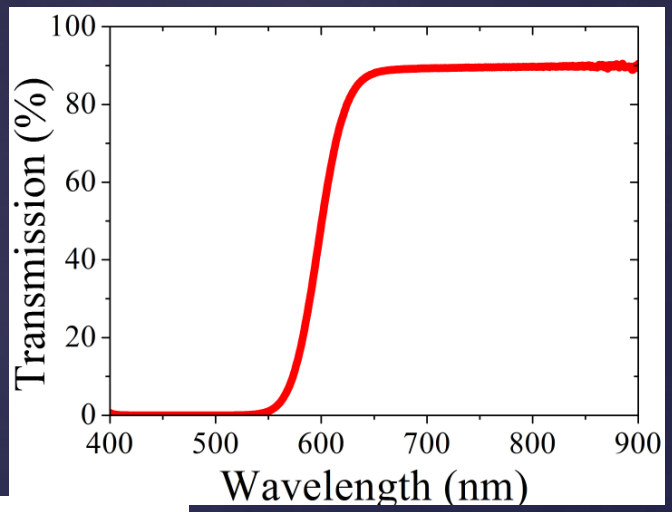
Improved sensitivity:

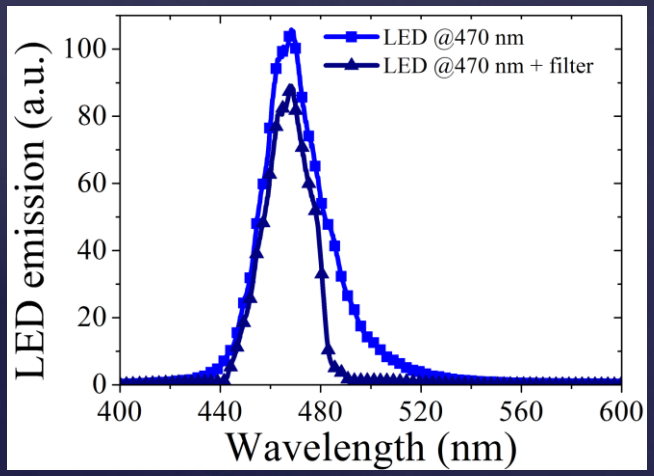
1. Temperature increase → enhanced enzyme activity
2. Wavelength more adapted to algal absorption spectrum

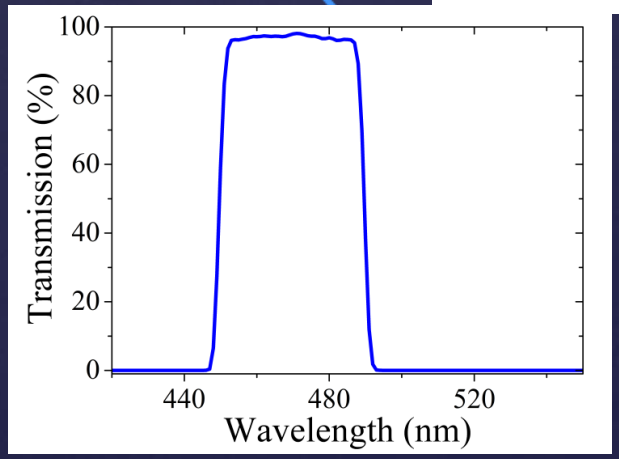
Optical measurement principle



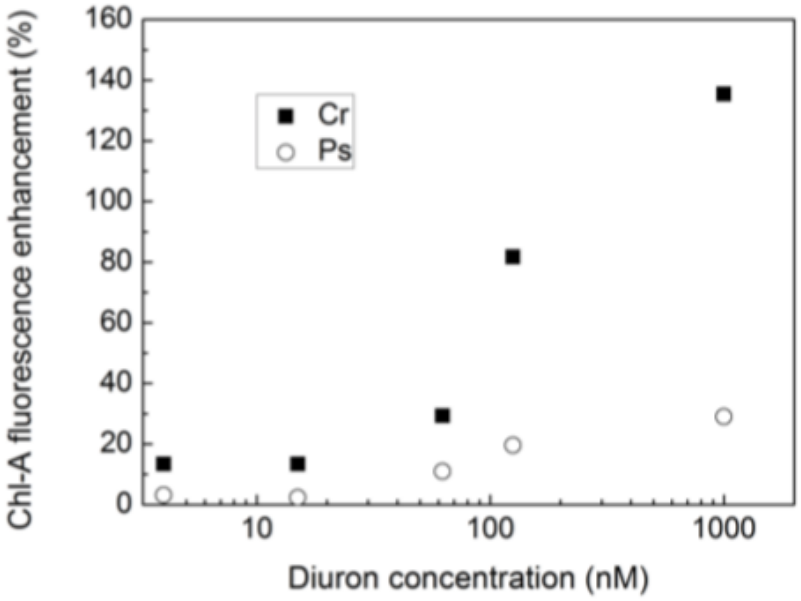


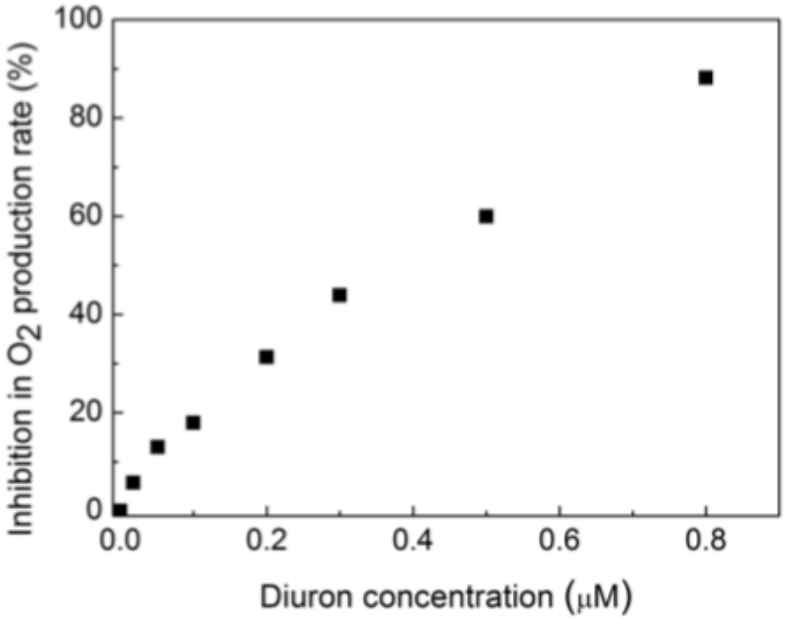






Herbicide detection using dual microsensors

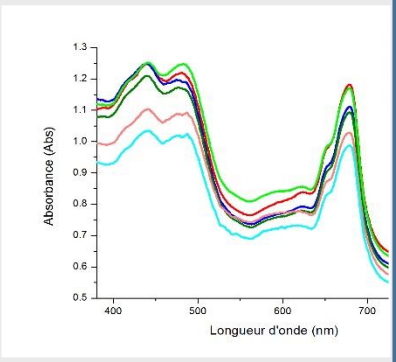




- Large range of herbicide concentration (0.1 to 1 µM) => pollutant traces
- Early Diuron detection of 20 nM with optical detection
- Early Diuron detection of 1 nM with electrochemichal detection

Bio-sensor study

- Algal bio-sensor
- Pollutant effects (herbicide)



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- Fabrication
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  - ➢ O_2

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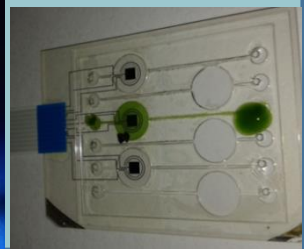
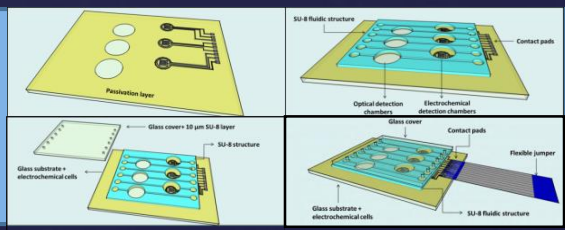
- Photosynthesis activity
- Fluorescence

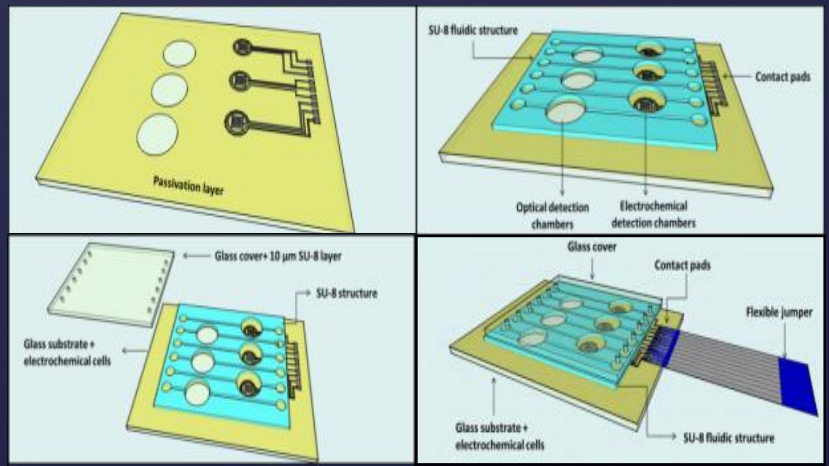


Conclusion



Conclusion

- Identification of electroactive species
- Validation of detection properties for O_2
- Transfer of multianalysis system on lab-on-chip platform
- Microfabrication process on glass substrate
- Light source optimized through OLED use
- Fluorescence detection measurement thanks to OPD
- Optimization of fabrication using low cost technologies
- Decrease of needed sample volumes
- Herbicide multi-detection validated through bioassays



Thanks for your attention

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