

A polyphenol biosensor realized by Laser printing technology



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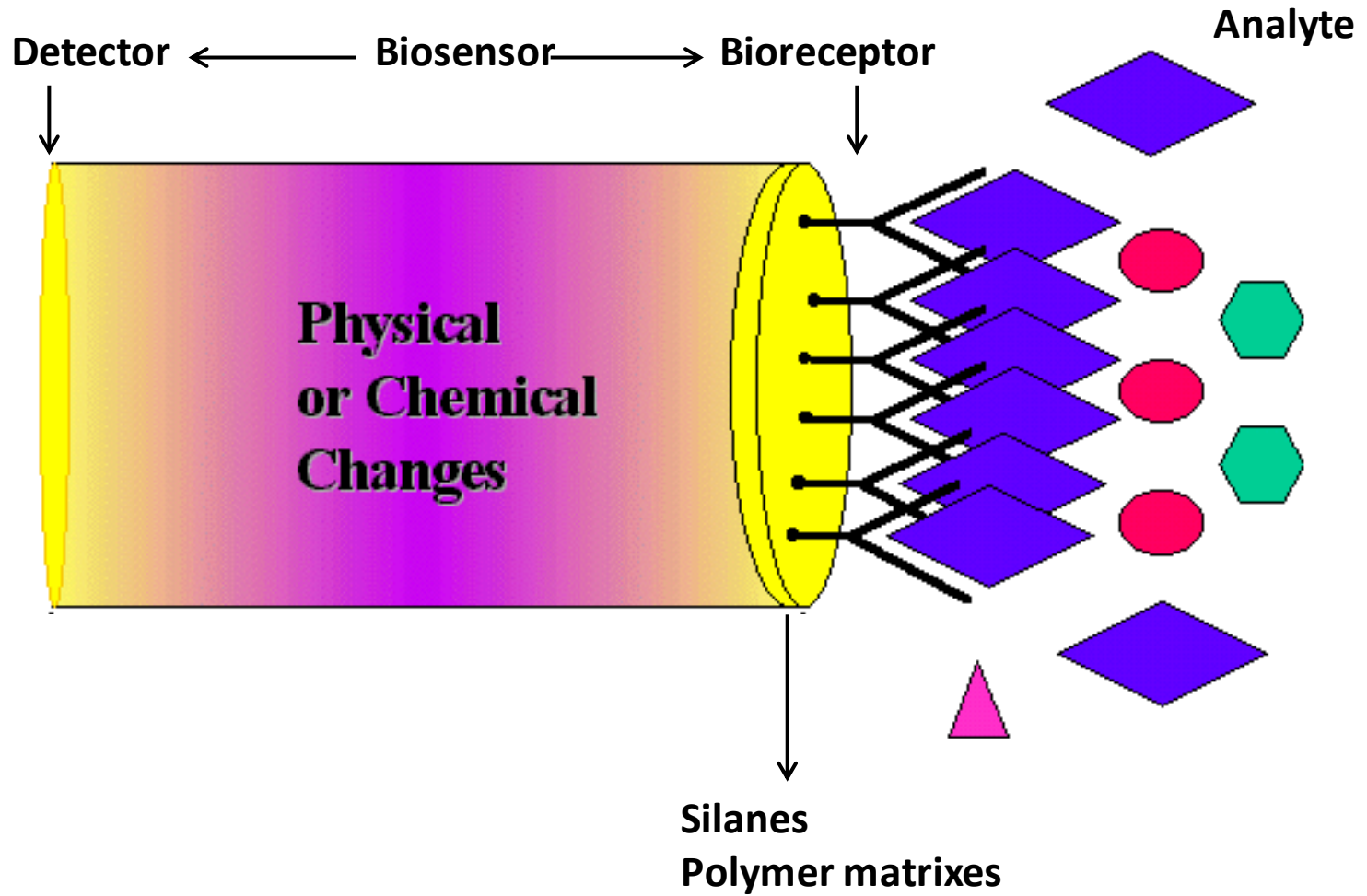


CNR- Institute of Ecosystem Study, Italy



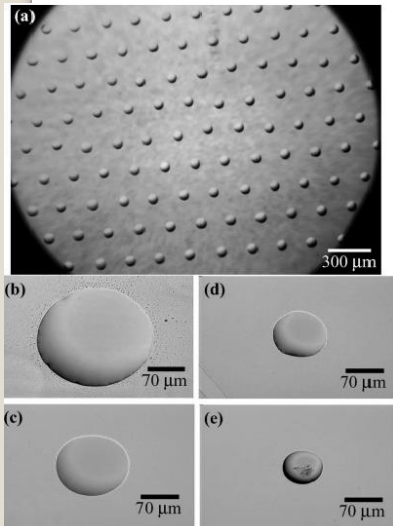
Introduction

Biosensor overview

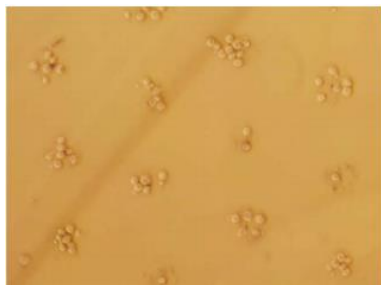


Laser Induced Forward Transfer (State of the Art)

Living cells



Othon et al. *Biomed. Mater.* 3 (2008) 034101



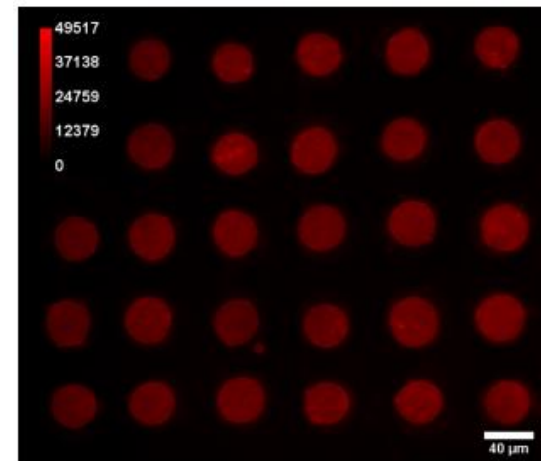
F. Guillemot et al. *Acta Biomaterialia* 6 (2010) 2494–2500

DNA microarrays

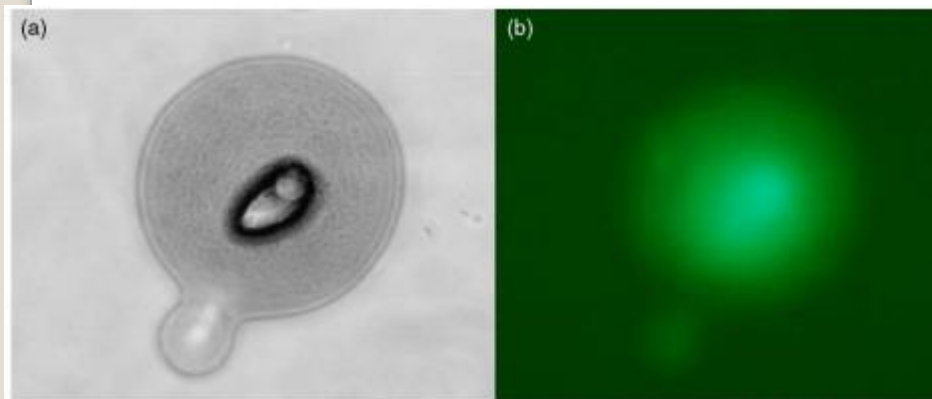


Serra et al. *Appl. Phys. Lett.*, Vol. 85, No. 9, 30 August 2004

Biotin microarrays



C. Boutopoulos et al., *phys. stat. sol. (a)* 205, No. 11 (2008)

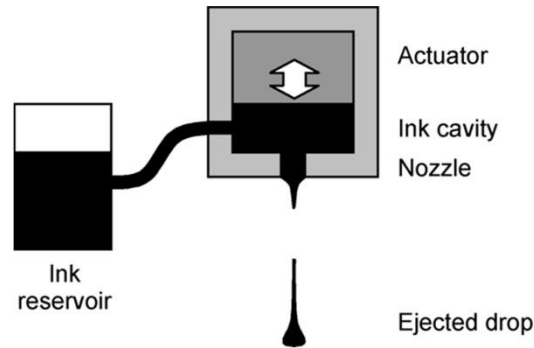


B.R. Ringeisen et al. *Biomaterials* 23 (2002) 161–166

Deposition of biomolecules on sensor devices

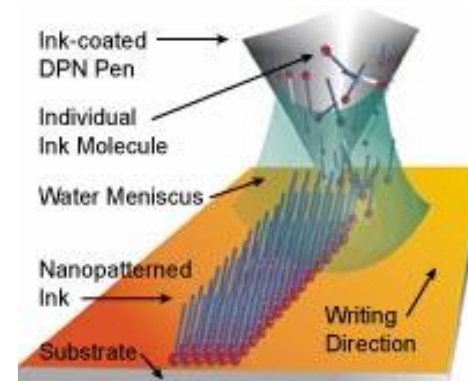
Ink-Jet Printing

Spatial resolution:
30 μ m.
Printing velocity:
100-500 spots/sec.
Non- Contact



Drop on demand ink-jet (DOD) (K.K.B.
Hon et al. *Annals - Manufacturing
Technology* 57 (2008) 601–620)

Dip Pen Nanolithography



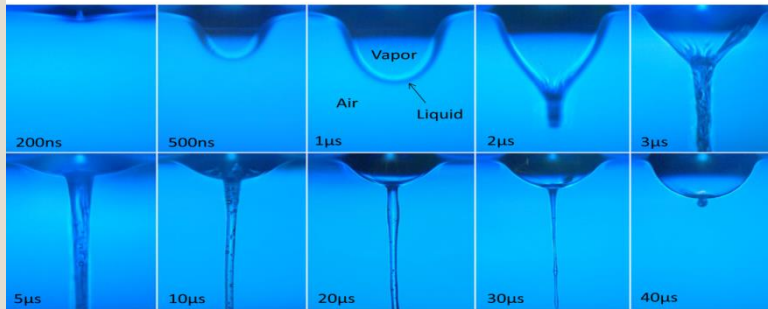
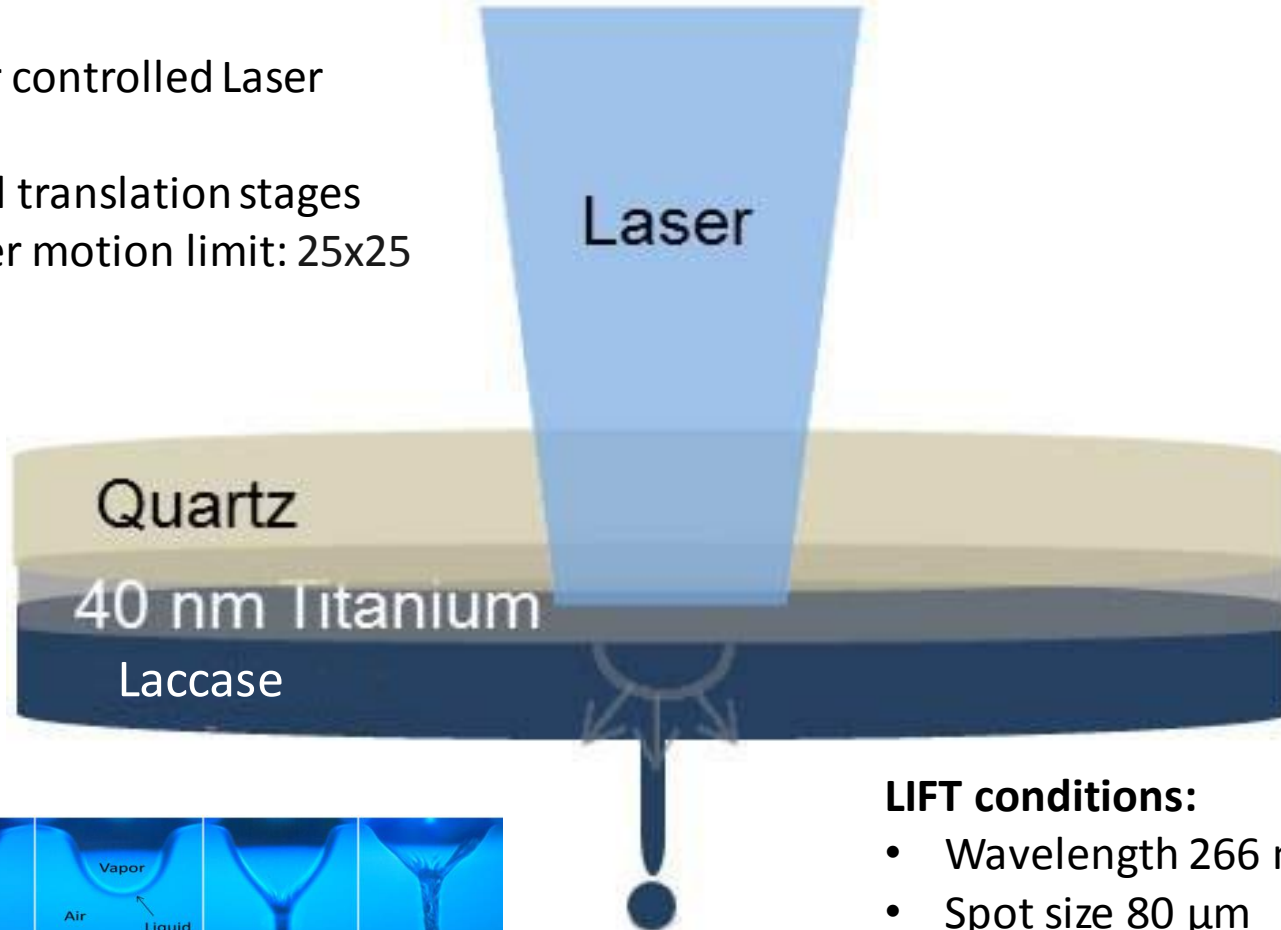
Spatial resolution: 40nm
Printing velocity : 64spots/ sec
Contact (1nN)

Laser Induced Forward Transfer Advantages:

- Direct write technique
- Non Contact technique
- High spatial resolution (5 μ m)
- High speed printing

Laser Induced Forward Transfer mechanism

- Computer controlled Laser trigger
- Motorized translation stages
 - Upper motion limit: 25x25 mm



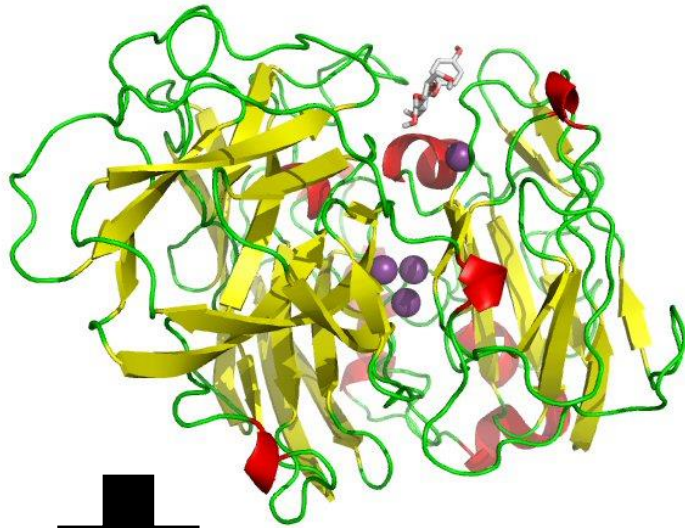
LIFT conditions:

- Wavelength 266 nm to 1064 nm
- Spot size 80 μm
- Energy fluence: 450 mJ / cm^2
- Distance between the donor and the substrate: 200 μm
- 10 μL on target
- ns to ps pulse duration

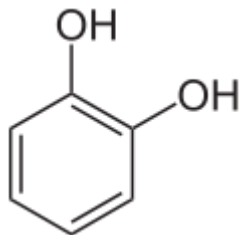
Catechol biosensor

Applications

Laccase



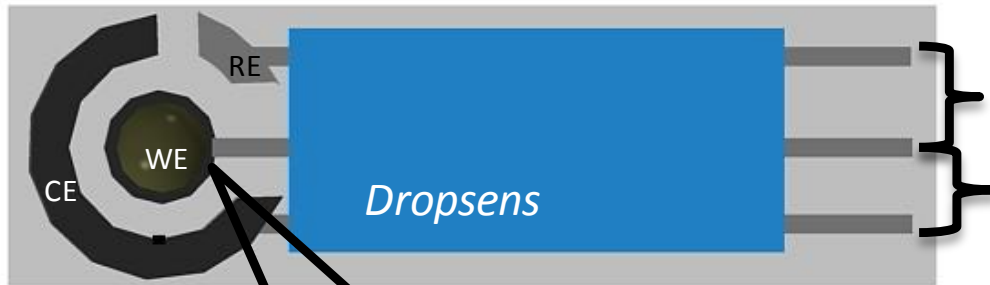
Catechol



Food Analysis



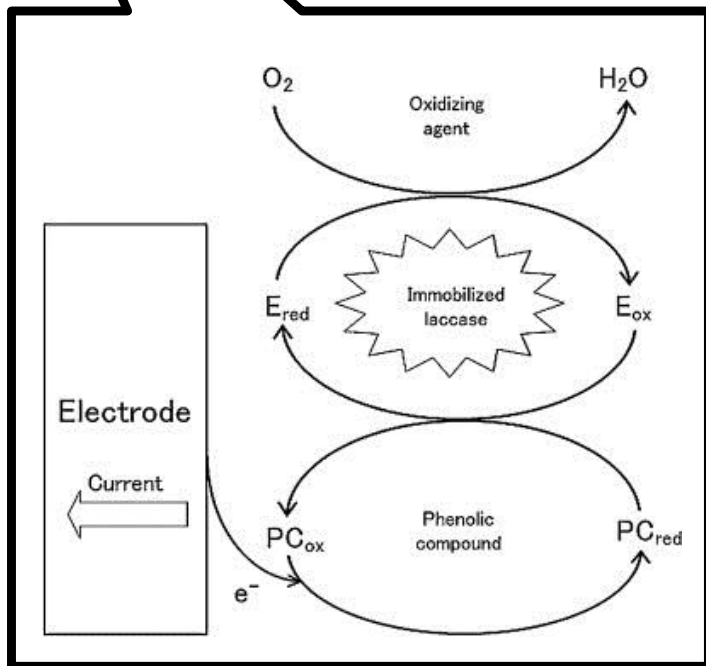
Principle of detection for enzymatic based biosensors



Applied potential
(- 30 mV)

Measuring current

Oxidation of phenols by Laccase enzyme results in Quinone compounds

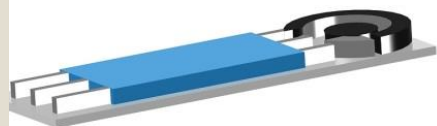
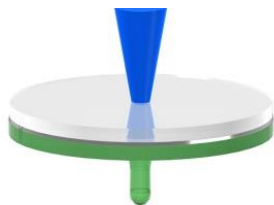


Quinone can be electrochemically reduced and detected in a concentration-dependent manner.

Amperometric Sensors

Outline of Experimental Process

AMPBIO-SPE

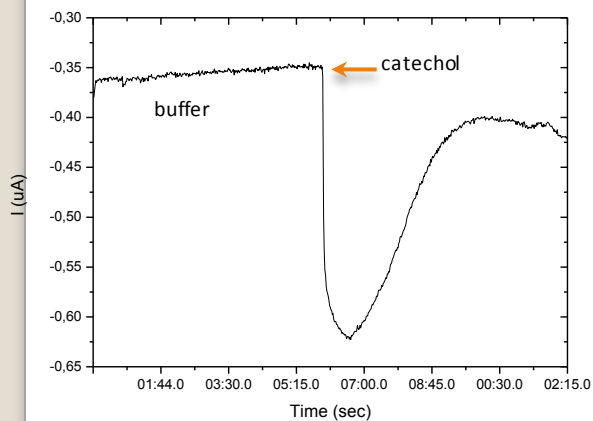


LIFT of Laccase in
graphite electrodes

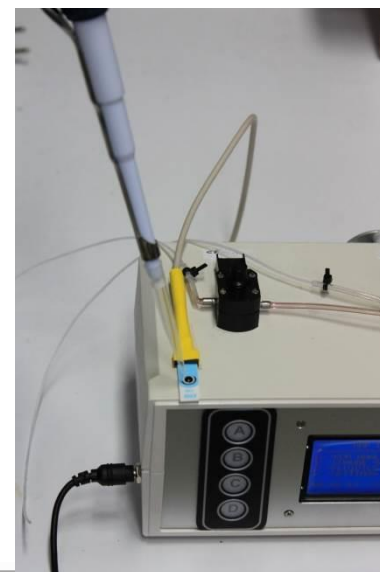
After washing step



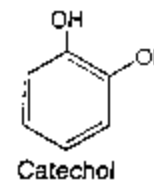
$\Delta V = -30 \text{ mV}$



Amperometric
signal response



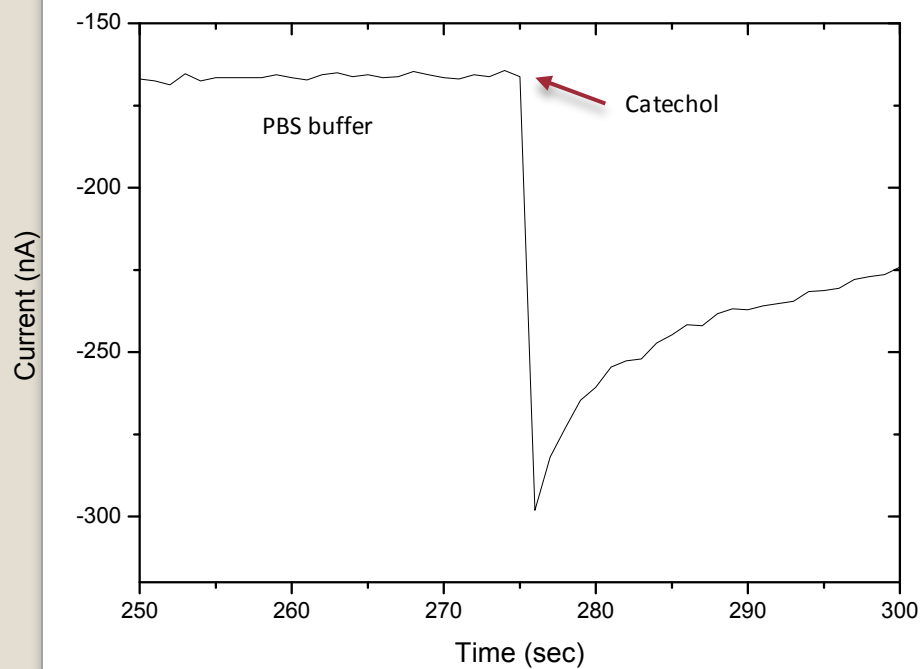
+ catechol



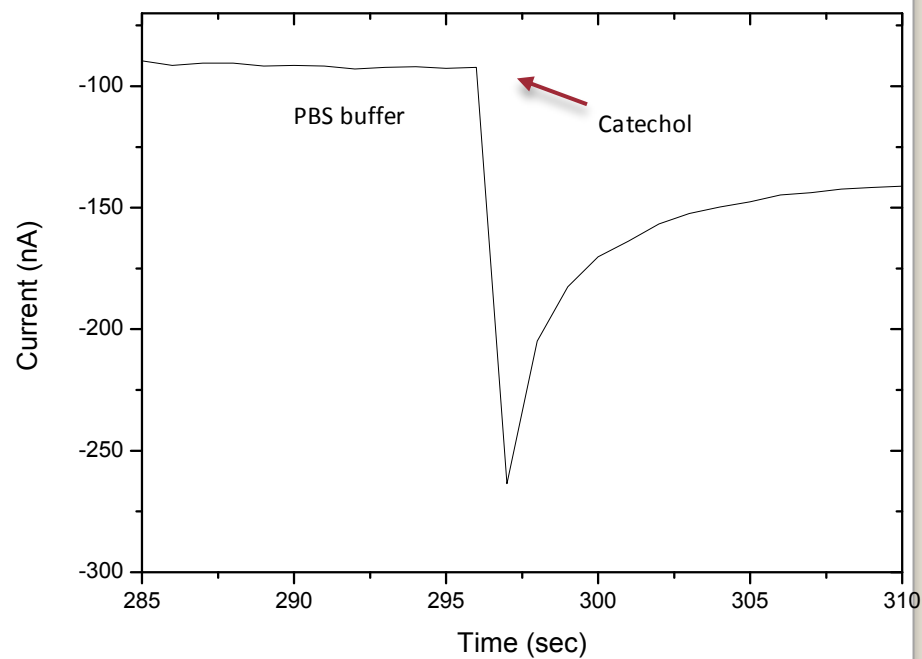
Successful functionalisation of laser printed SPEs with laccase

in presence of 300 nM catechol, after several washes.

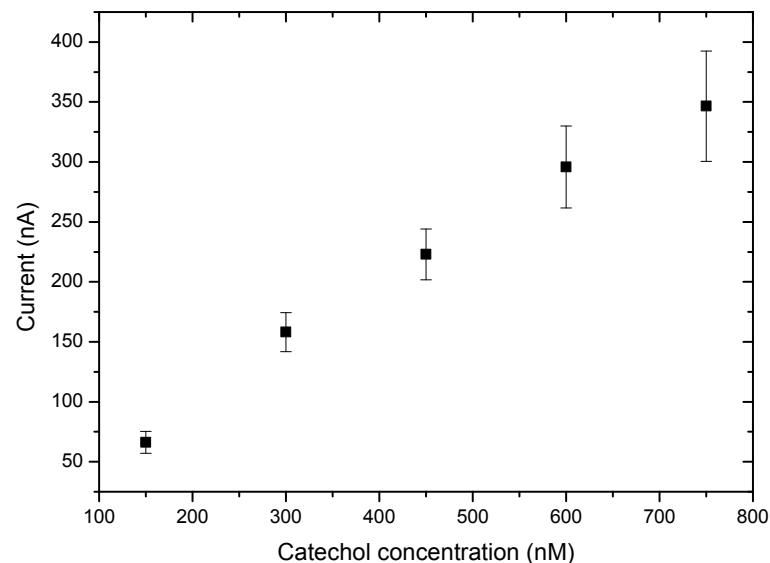
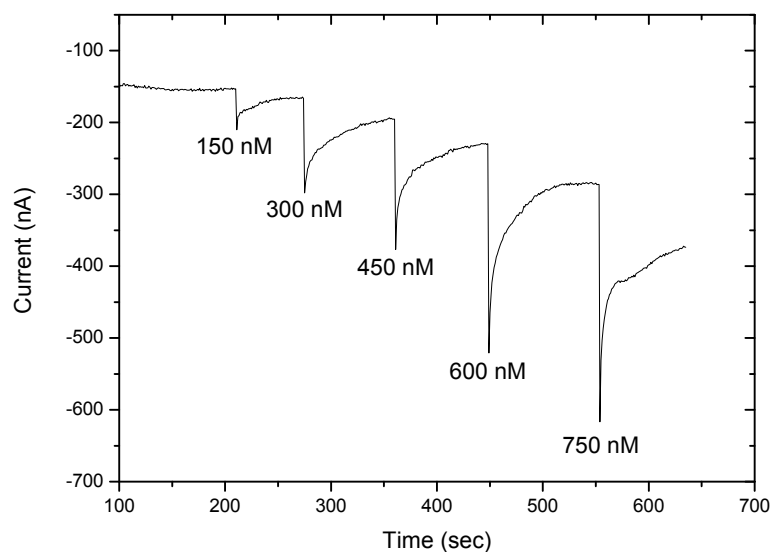
2 times washed



4 times washed



Response of laccase – based biosensor under various concentrations of catechol- calibration curve



Laccase concentration : $7 \cdot 10^{-2}$ U

Measuring conditions:

room temp

$\Delta V = -30$ mV

3 SPEs measured for each

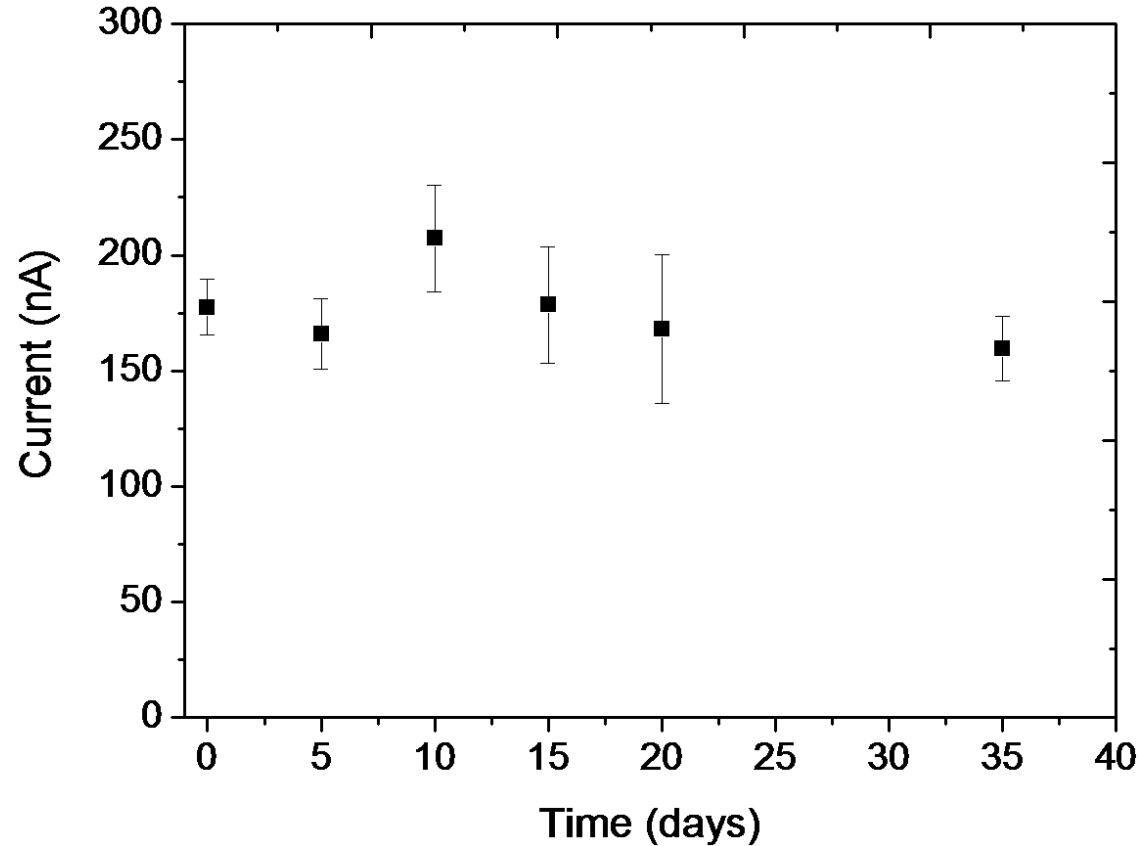
concentration

Volume of Laccase on WE: 1.61 nL

Immobilized enzyme: 0.086 ± 0.008 U.

Low LOD: 150 nM!!

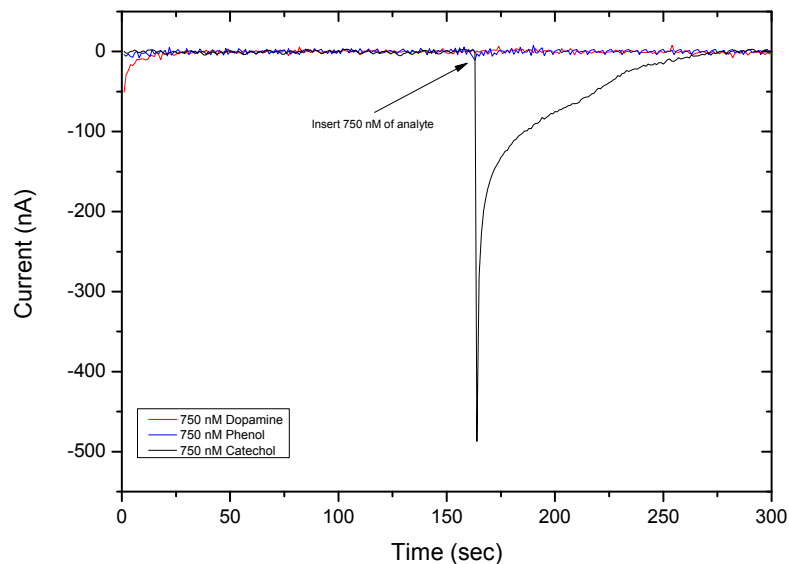
Stability laccase – based biosensor stored at 4°C in presence of 300nM catechol



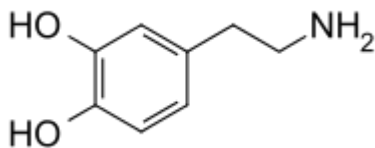
- Stored electrodes at 4°C.
- 3 SPEs measured for each day.
- Each electrode washed 2 times before the measurement.
- Each electrode was measured for 4 times.

Selectivity of Biosensor

Tested at 750 nM of Dopamine, Phenol, Catechol



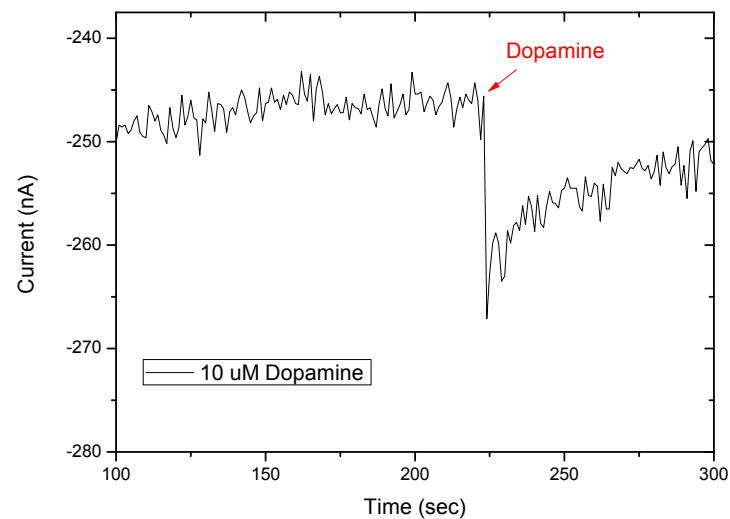
Detection of dopamine



LOD of Dopamine: 10 μM

Measuring Conditions:

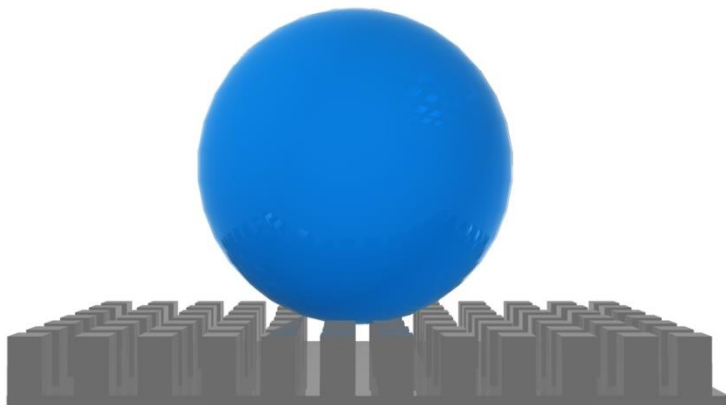
- Dopamine and Phenol soluted on PBS buffer pH: 4.5
- ΔV (between WE and RE): -30 mV



**Laser Direct
Immobilization of
Laccase enzyme**

Mechanism of direct immobilization via LIFT

Cassie wetting state:



Wenzel wetting state:

Laser:

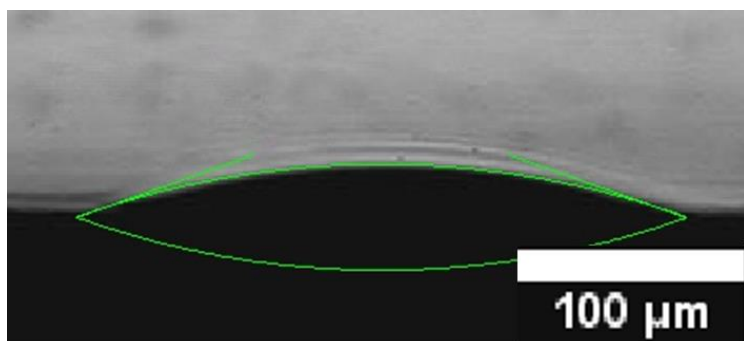
- High travel velocity of the liquid ~ 70 m/s
- High Impact pressure ~ 3 MPa



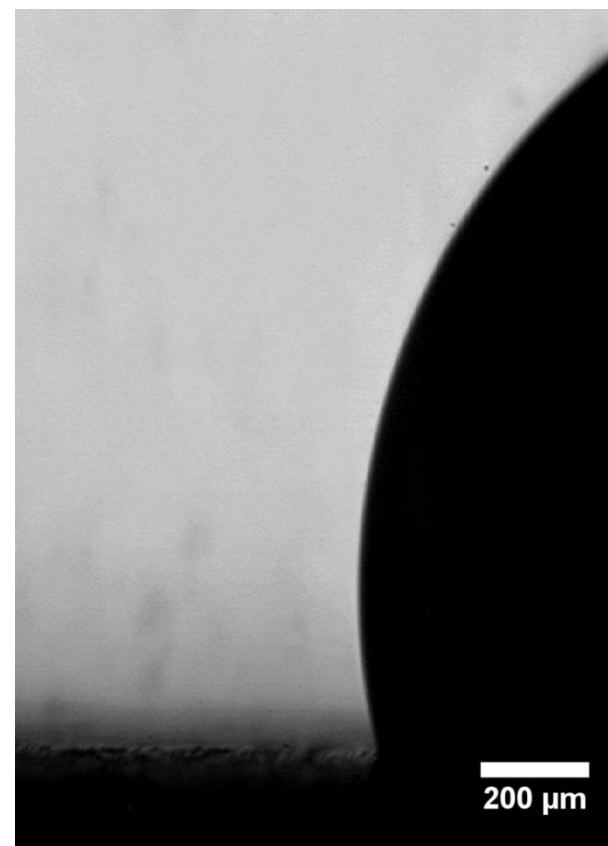
Contact Angle Measurements of Laccase on Graphite SPEs

Laser printed Laccase:

- ✓ *Volume of Laccase on the WE ~ 2 nL*
- ✓ *Calculated concentration of Laccase on the electrode ~ $7 * 10^{-2} U$*



Pipette drop casted Laccase



LIFT CA (°)

24,4 ± 2,4

Pipette CA (°)

95,2 ± 1,7

Conclusions

- Successful Laser printing of Laccase enzyme at a graphite SPE
- Direct immobilization of Laccase without any need of chemical functionalization layer
- Highest proportion of the printed enzyme is immobilized avoiding the waste of the biomaterials and the multiple functionalization steps.
- As a result of the above a Laccase based biosensor has been fabricated with high sensitivity and long term lifetime at storage conditions of 4⁰C.

Thank you

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Thermal Diffusion study

