# 3D structured biochip for label-free multi-analyte determinations at the Point-of-Need

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## Introduction:

### Motivation:

- Optical biosensors, especially those offering label-free detection, are the most promising platforms for fast, accurate and quantitative operation at the Point-of-Need due to their proved high detection sensitivity and specificity.

- However, market has set several challenging requirements for the commercialization of such platforms into products - which rarely met - such as:

  - simplification of the measurement procedure
  - cheap and disposable consumables
  - compact and semi-automatic devices

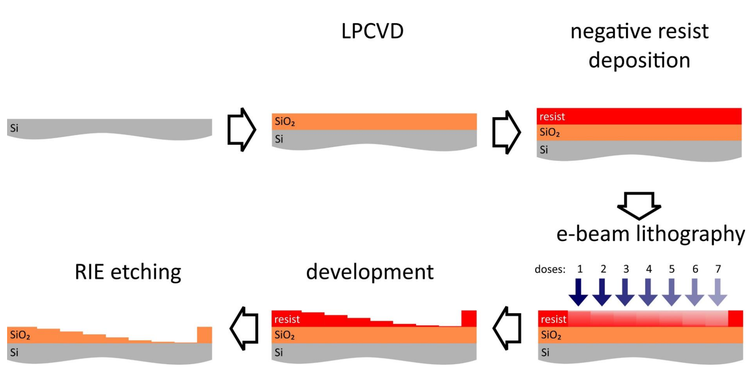
### Conceptualization:

- The proposed optical immunosensor implements 3D structured biochips with a $SiO_2$ layer of spatially varying thickness to monitor biomolecular interactions that are taking place on top.
- This detection principle provides an oversimplified measuring setup, since:
  - illumination is based on LEDs and spectral filters
  - the signal is recorded by a CCD/CMOS camera
  - The biochip is cost-effective in HVM
- The proposed principle of operation is demonstrated through the immunochemical determination of Aflatoxin M1 ($AFM_1$) which is the most carcinogenic and toxic aflatoxin produced by fungi.

## Experimental Description:

### Process flow-chart:

The fabrication procedure of the 3D structured biosensing surface is illustrated below. A $SiO_2$ layer of 3-μm thickness on top of silicon substrate was engineered by carefully tuned grayscale e-beam lithography, followed by dry etching of the $SiO_2$ layer.




Multilevel resist profiles with defined steps of various heights were fabricated using "tailor-made" exposure doses. This exposure dose modulation results in a locally non-uniform etch rate of the resist leading to removal of different resist quantities during the development.

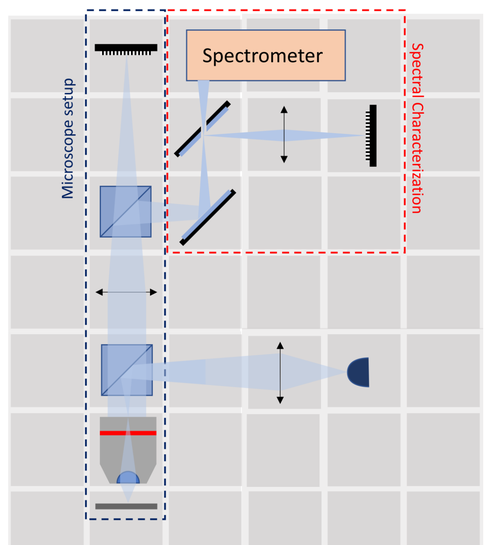
### Optical Characterization Setup:

The illumination of the 3D structured biochip by a standard and low-cost LED causes a spatial modulation in the reflectance signal that is recorded by an optical set-up built around a CCD/ CMOS camera.

Biomolecular interactions monitoring using a simple *microscope setup*:

- CMOS sensor (2048×2048 px)
- Light source: LED (460nm ± 20 nm)
- Objective lens: 5X

- Simultaneous thickness monitoring using *spectral characterization* of the biochip during the validation of the setup.

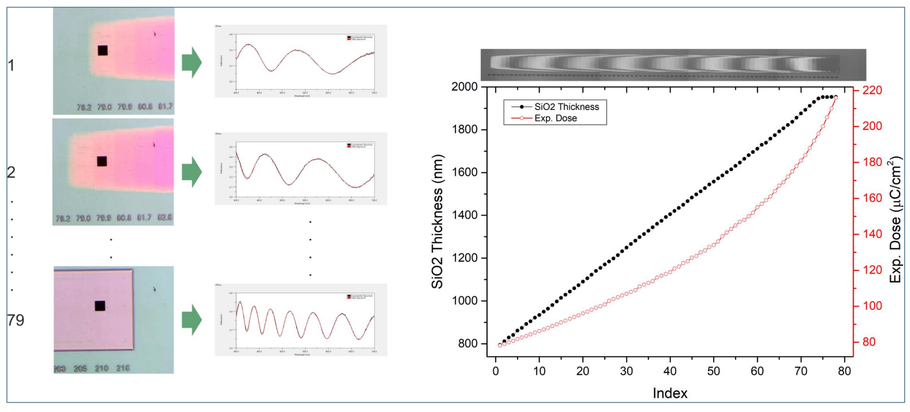



## Results:

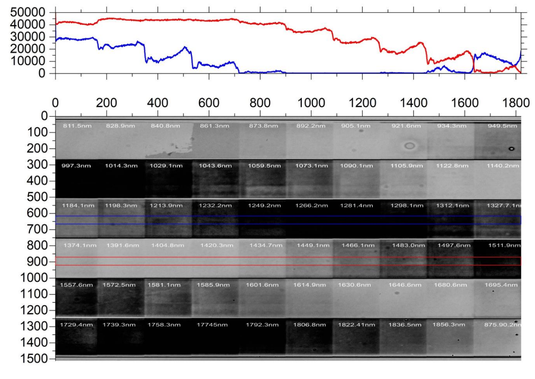
### Characterization of the 3D structure:

Method: Microscope-based reflectance spectroscopy (FR-uProbe-ThetaMetrisis) with a measurement spot size of 25μm.

- Thickness measurements at each area exposed to different e-beam dose.
- The black square area corresponds to the area of measurement.
- From the reflection spectrum the thickness was calculated by analyzing the interference fringes.
- Thickness values obtained vs exposure dose are shown in the graph.
- Linear trend of the thickness slope thanks to the "tailor-made" exposure conditions.

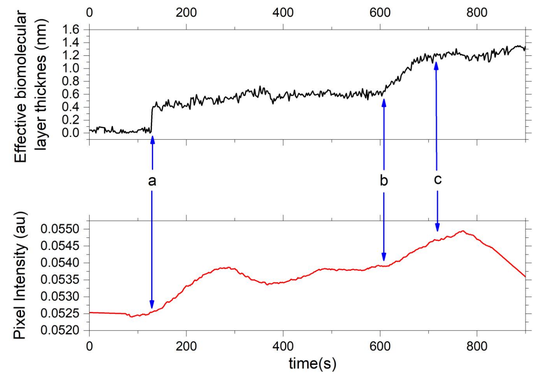



### $AFM_1$ immunochemical detection:



- Final 3D structure consisted of 10×6 sensing areas of different thickness values.
- Intensity profiles of images were taken during the biomolecular interactions and
- Simultaneous determination of real-time biomolecular adlayer thickness for validation of the results.

- Real-time signal recordings corresponding to $AFM_1$ calibrators (0–2.0 ng/mL) prepared in assay buffer.
- The arrows show the sequence of solutions run over the biochip:
  - start – a: mixture of calibrators with a rabbit anti-AFM1 antibody
  - a – b: biotinylated anti-rabbit IgG antibody
  - b – c: streptavidin




## Conclusions:

- A new radical biosensing concept in which reflectance from a $SiO_2$ layer with spatially varying thickness is recorded.
- Promising preliminary results on the qualitative determination of Aflatoxin M1.
- Future plans: further optimization and evaluation of the technique's performance

## Acknowledgments:

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