Ion sensing real-time quantitative monitorization of isothermal DNA amplification

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Jornadas do CENIMAT|I3N

OUTLINE

- Objectives
- State-of-the-art
- Application of Field effect based sensors
- Ta_2O_5 sensitive layer production and optimization
- EIS Label-free detection of DNA amplification
- Oxides based ISFET sensors
- Conclusions and Future perspectives

OBJECTIVES

Application of Field Effect based Sensors

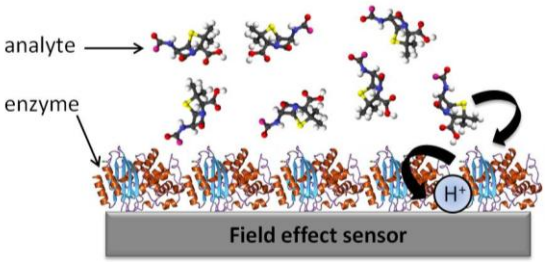
- Optimization of Ta_2O_5 thin films for quantitative real-time DNA amplification
- Development of the Loop mediated Isothermal DNA amplification method (LAMP) for cancer biomarkers
- Monitoring of DNA amplification
- Real-time monitoring of c-MYC LAMP amplification through field effect DNA detection.
- Gene expression analysis – mRNA quantification (application to cMYC gene)

Previous work

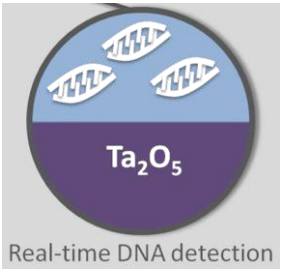
Production & Optimization
Ta_2O_5 thin films for enhanced pH sensitivity

Application in Field effect based sensors

Enzyme-based sensors

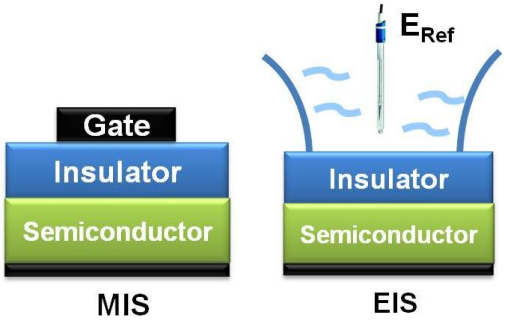


Label-free DNA detection

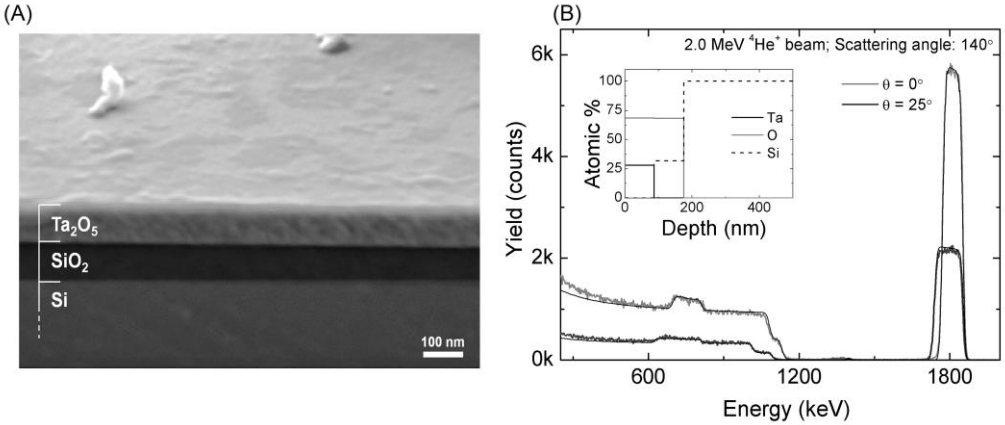


FIELD EFFECT BASED SENSORS PRODUCTION AND CHARACTERIZATION

Electrolyte-Insulator-Semiconductor



Ta_2O_5 rf Sputtering Deposition @ RT



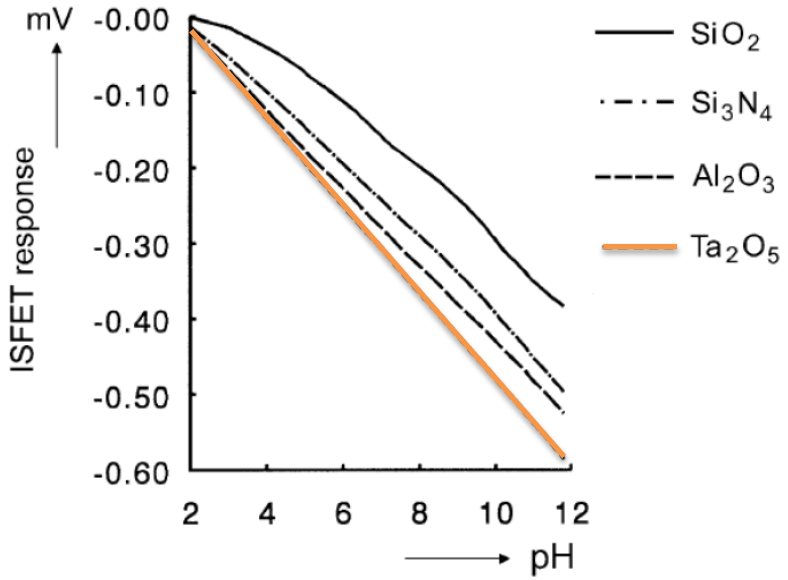
pH Sensitivity

58.2 mV/pH

(A) SEM

(B) RBS

Ta_2O_5 thin films for enhanced pH sensitivity



Linear relation to pH

High buffer capacity

Large number of active surface sites

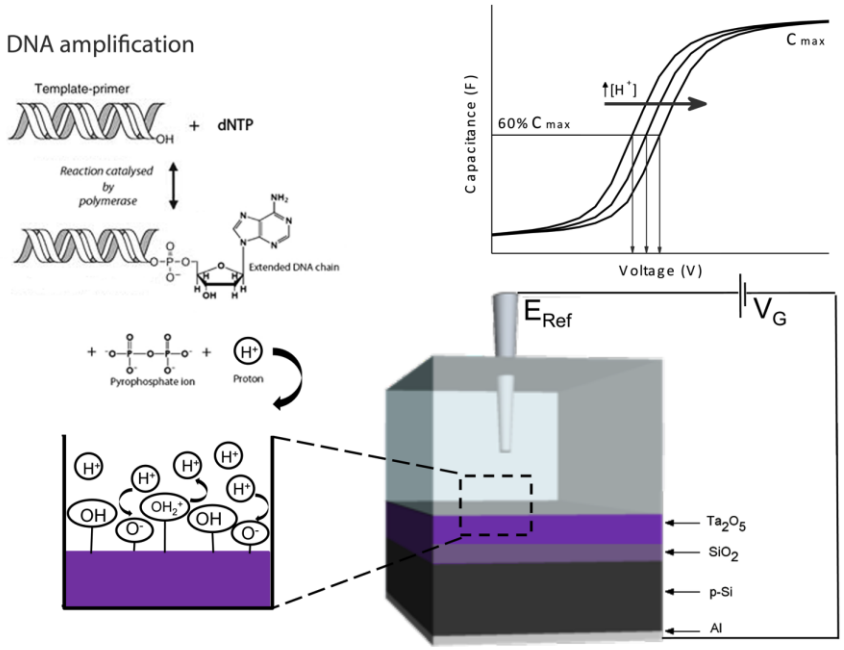
High pH sensitivity

rf magnetron sputtering production of Ta_2O_5

Low processing T

FIELD EFFECT BASED SENSORS

Electrolyte-Insulator-Semiconductor



Dielectric is amphoteric accepts and releases protons

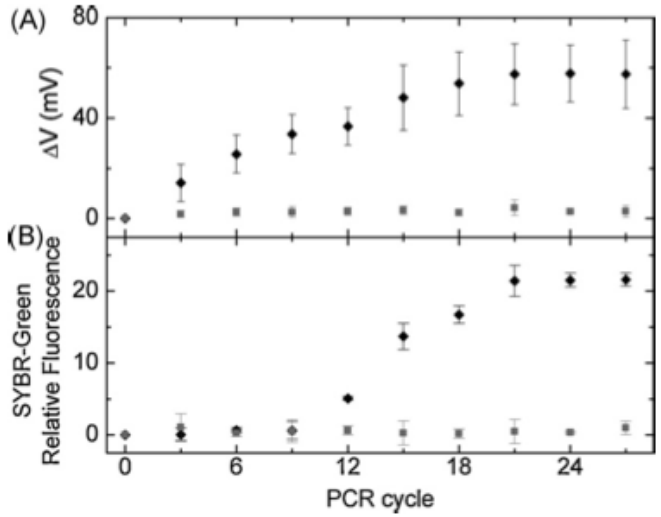
Surface potencial varies with $[H^+]$ **inducing a voltage shift**

pH variations modulate the devices characteristics

LABEL-FREE DETECTION OF BIOMOLECULES

FIELD EFFECT DETECTION OF DNA

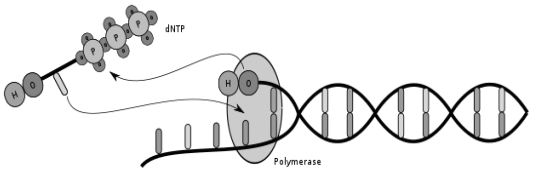
Monitoring PCR DNA amplification



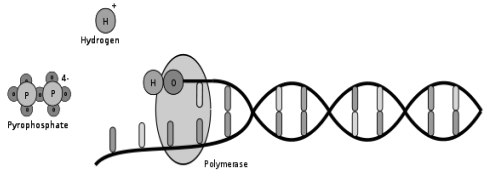
EIS sensor
DNA quantification through direct detection.

Real-time PCR
DNA quantification in real time through detection of a fluorescent label.

DNA polymerisation



Polymerase integrates a nucleotide.



Hydrogen and pyrophosphate are released.

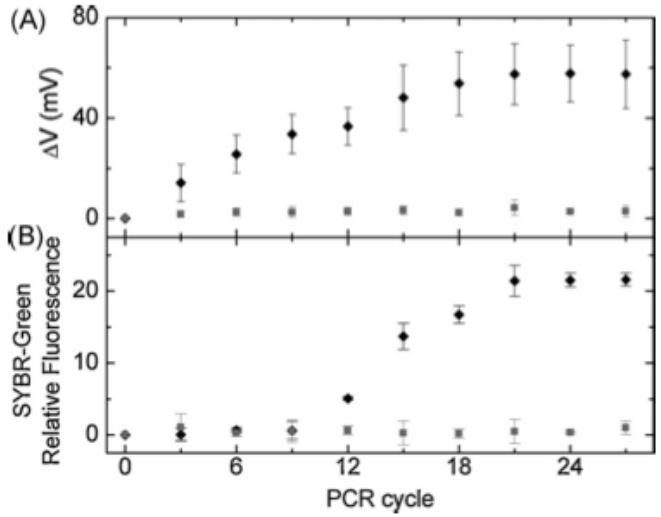
Monitoring LAMP DNA amplification

Real-time monitoring of c-MYC LAMP amplification through field effect DNA detection.

LABEL-FREE DETECTION OF BIOMOLECULES

FIELD EFFECT DETECTION OF DNA

Monitoring PCR DNA amplification



EIS sensor
DNA quantification through direct detection.

Direct

Label free

Low cost

Real-time PCR
DNA quantification in real time through detection of a fluorescent label.

Indirect

Chemical Label

High cost

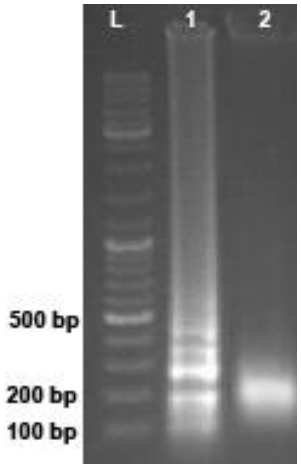
Monitoring LAMP DNA amplification

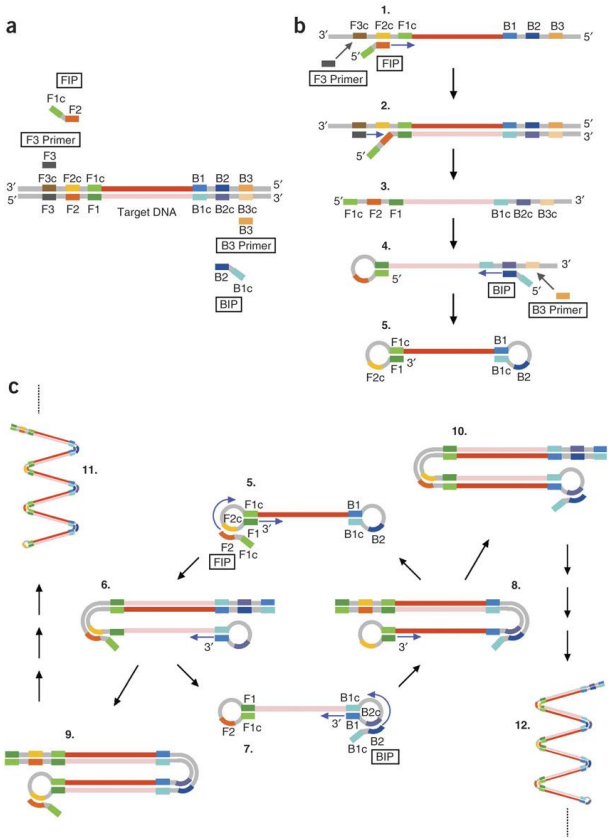
Real-time monitoring of c-MYC LAMP amplification through field effect DNA detection.

LAMP DNA AMPLIFICATION MONITORING

LAMP AMPLIFICATION

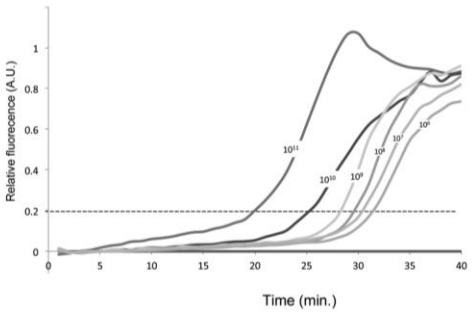
Electrophoretic analysis of the LAMP amplified *cMYC* product. Agarose gel electrophoresis of the *cMYC* amplified products (1% agarose gel with GelRed® staining). Lane L - GeneRuler™ DNA Ladder Mix; Lane 1- *cMYC* LAMP amplification product; Lane 2- *cMYC* LAMP amplification product digested with *Hinf* I.

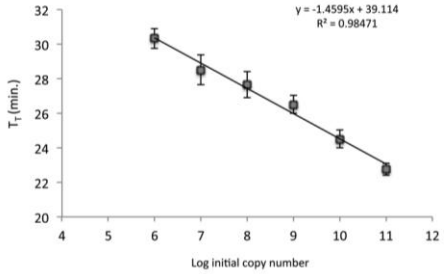




Real-time LAMP

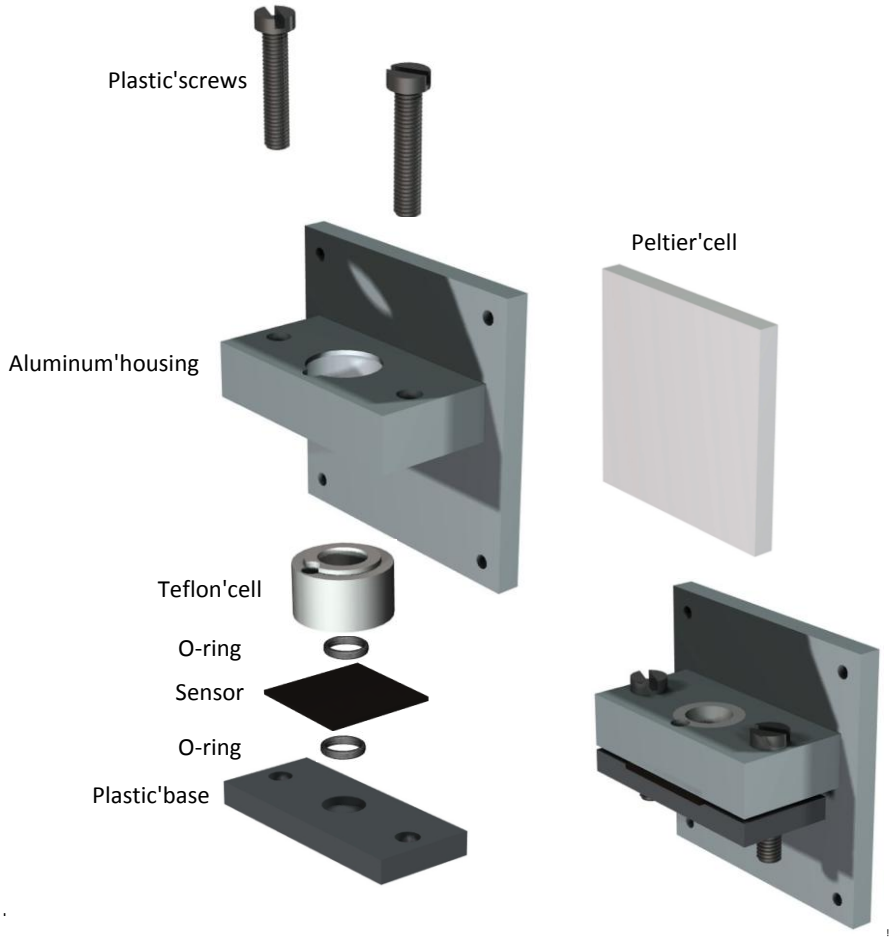
DNA quantification in real time through detection of a fluorescent label.

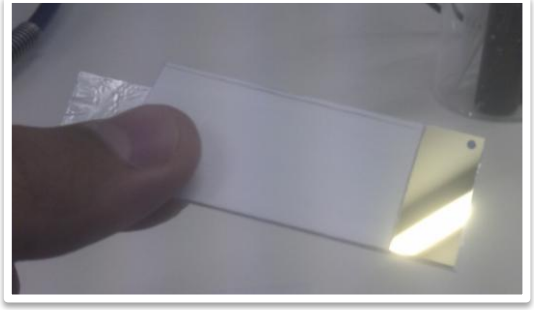


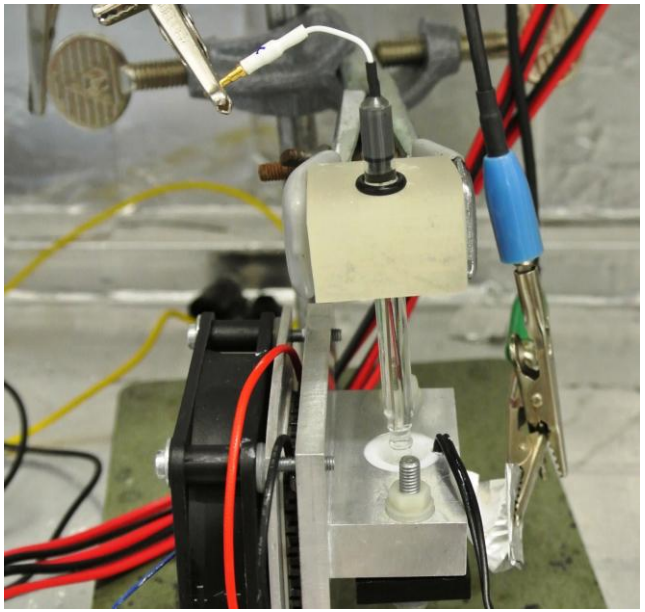


LAMP DNA AMPLIFICATION MONITORING

REACTION CELL

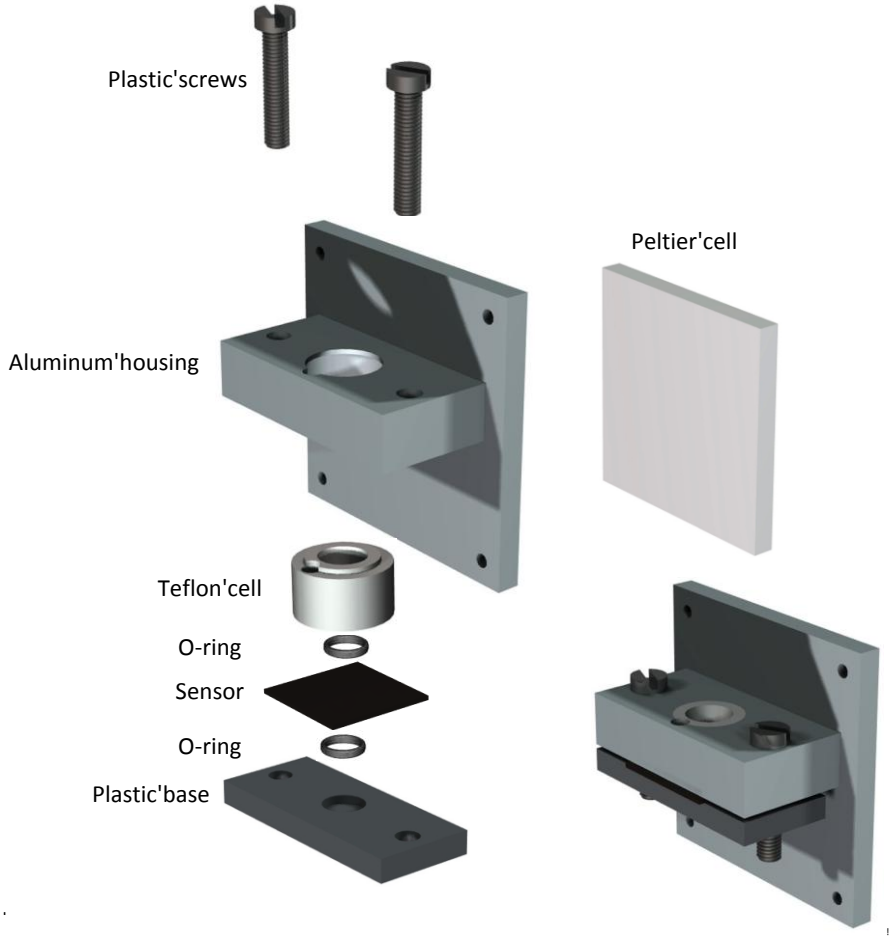




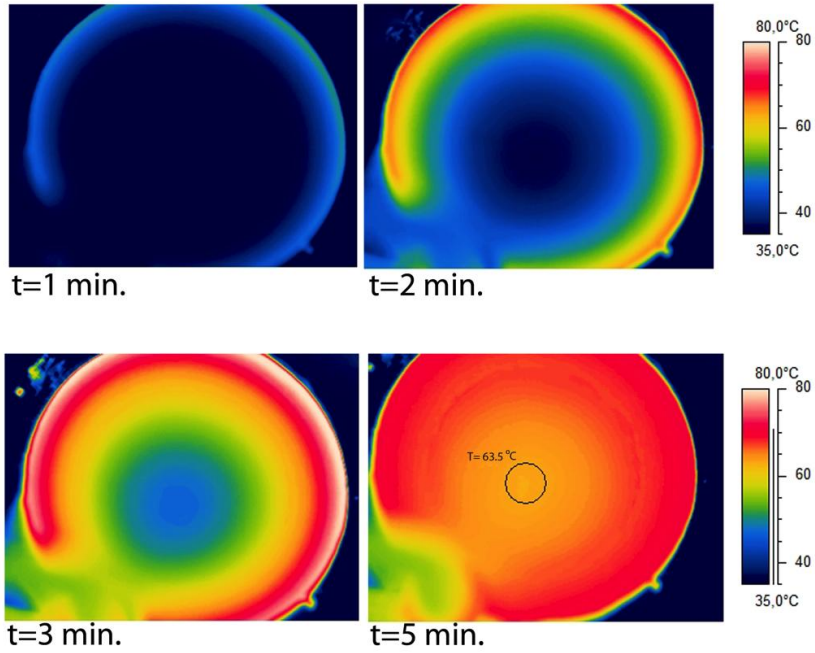


LAMP DNA AMPLIFICATION MONITORING

Reaction Cell

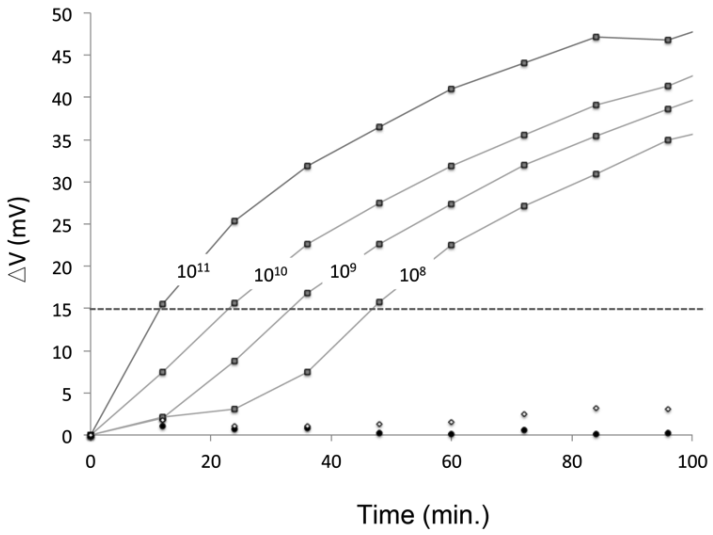


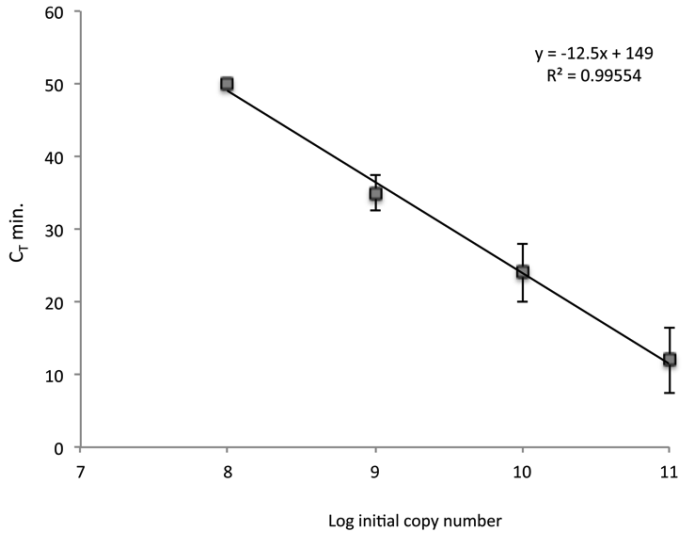
Thermal heating profile characterization



LAMP DNA AMPLIFICATION MONITORING

EIS REAL-TIME AMPLIFICATION DETECTION





Clear distinction between **Positive** and **Negative** LAMP reactions.
Very low sensor drift as demonstrated by **Buffer** solution measurements.

PUBLICATIONS

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Ion sensing (EIS) real-time quantitative monitorization of isothermal DNA amplification

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Real time
c-Myc
Cancer
Label free

ABSTRACT

Field-effect-based devices are becoming a basic structural element in a new generation of microbiosensors. Reliable molecular characterization of DNA and/or RNA is of paramount importance for disease diagnostics and to follow up alterations in gene expression profiles. The use of such devices for point-of-need diagnostics has been hindered by the need of standard or real-time PCR amplification procedures. The present work focuses on the development of a tantalum pentoxide (Ta_2O_5) based sensor for the real-time label free detection of DNA amplification via loop mediated isothermal amplification (LAMP) allowing for quantitative analysis of the cMYC proto-oncogene. The strategy based on the field effect sensor was tested within a range of 1×10^8–10^{11} copies of target DNA, and a linear relationship between the log copy number of the initial template DNA and threshold time was observed allowing for a semi-quantitative analysis of DNA template. The concept offers many of the advantages of isothermal quantitative real-time DNA amplification in a label free approach and may pave the way to point-of-care quantitative molecular analysis focused on ease of use and low cost.

1. Introduction

Molecular diagnostics based on DNA detection has increased tremendously over the past few years, in particular towards pathogen identification, drug screening and diagnosis of genetic diseases (Sadik et al., 2009). Most standard technologies showing high sensitivity and low detection limits are usually performed via optical methods, with emphasis on fluorescence intensity measurement from a reporter molecule (Espy et al., 2006). Still, these methods can be expensive and complex to implement, and the majority of these methodologies rely on the enzymatic amplification of DNA via polymerase chain reaction (PCR), generally regarded as an essential method in molecular genetics (Aoi et al., 2006). Real-time monitoring of enzymatic DNA polymerization reaction is of paramount relevance in molecular diagnostics, in particular quantitative DNA amplification real-time PCR (qRT-PCR) is highly effective for monitoring gene expression. Following reverse transcription (conversion of mRNA to cDNA), the amplification reaction can then occur allowing for comparison between samples and/or comparative quantitation (Ginzinger, 2002; Sakurai and Husimi, 1992; VanGuilder et al., 2008; Wong and Medrano, 2005).

Loop-mediated isothermal DNA amplification (LAMP) has emerged as a powerful amplification technique to be used as simple and rapid diagnostics tool (Notomi et al., 2000; Parida et al., 2008). LAMP relies on auto-cycling DNA synthesis performed by a DNA polymerase with strand displacement activity and a set of two specially designed inner and two outer primers. Because of the strand displacement capability, the reaction can be performed at the same temperature without the need for temperature cycling. The final products are a mixture of stem-loop DNAs with various stem lengths and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target sequence. Because LAMP is performed under isothermal conditions and at a relatively low temperature, reverse transcription can simultaneously

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Ion sensing (EIS) real-time quantitative monitorization of isothermal DNA amplification.

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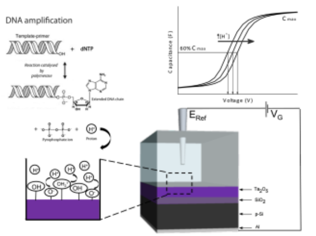
Abstract

Field-effect-based devices are becoming a basic structural element in a new generation of biosensors that are a non-optical alternative for real-time amplification and detection of nucleic acids, at lower costs and easier to integrate in a miniaturized platform. Molecular diagnostics based on DNA detection has increased tremendously over the past few years. Amongst these, Real-time quantitative DNA amplification is highly effective for monitoring gene expression. The present work focuses on the development of a tantalum pentoxide (Ta_2O_5) based sensor for the real- time label free detection of DNA amplification via loop mediated isothermal amplification (LAMP). Accumulation of the reaction by-product of polymerization (protons) is detected by the Ta_2O_5 sensor. We demonstrate the potential to quantify in real-time c-MYC, a proto-oncogene amplified and overexpressed in most human cancers. The strategy based on the field effect sensor was tested within a range of 10^8–10^{11} copies of target DNA, and a linear relationship between the log copy number of the initial template DNA and threshold time was observed allowing for a semi-quantitative analysis of DNA template [1]. The integration of this sensing technology with reverse transcription (conversion of mRNA to cDNA), could allow gene expression analysis. This concept offers many of the advantages of isothermal quantitative real-time DNA amplification in a label free approach and could significantly lower the costs associated with gene expression analysis and consequently reducing the costs for the molecular diagnostics of cancer.

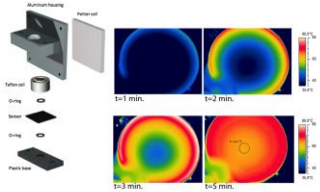
Method & Platform Fabrication

Representation of the DNA amplification detection scheme with typically obtained C–V curves.



The elongation reaction results in accumulation of protons, thus producing a pH shift of the surrounding solution proportional to the number of nucleotides incorporated. Hydrogen protons accumulation measured by impedance spectroscopy.

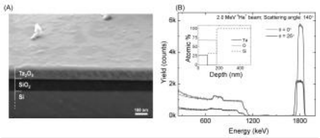
LAMP DNA amplification measurement cell



Prototype and schematic assembly representation (Left) and thermal heating profile characterization of the designed prototype. Thermal analysis of the final prototype was performed in real-time during the heating step. The observed circular shape corresponds to the entire heating cell with a diameter of 4 mm.

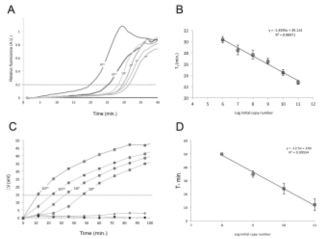
Results

Characterization of the Ta_2O_5 sensor



(A) SEM micrograph of cross-section view of the sensor; and (B) RBS spectra with respective fit curves, inset shows the calculated depth profiles. Surface morphology was studied by SEM and AFM and results show that the Ta_2O_5 films have a very homogeneous and smooth surface, with a root mean square roughness of less than 0.5 nm; RBS analysis showed that the Ta_2O_5 films present a quasi-stoichiometric oxygen proportion (2:4.8) – $Ta_2O_{4.8}$

Development of the real-time EIS quantitative LAMP Amplification of cMYC gene.



(A) Typical RT-LAMP amplification curves for cMYC DNA (A); (B) Relationship between log copies of initial template DNA and threshold time, T_T (RT-LAMP). (C) Typical EIS RT-LAMP amplification curves for cMYC DNA, with 10 fold dilutions of initial DNA template, buffer solution and LAMP negative control. (D) Relationship between log copies of initial template DNA and threshold time, T_T (EIS RT-LAMP). The observed potential shift occur towards more positive potentials, consistent with an increase of positive charge near the sensor's surface. Comparison of the electrochemical T_T values with those attained from the intercalating fluorescence dye show that, despite a slightly lower efficiency, the sensor response seems to be higher than that of the standard fluorescence method.

Conclusions and Future Work

Successful LAMP amplification of cMYC was achieved in the specifically developed isothermal amplification cell. The amplification reaction was monitored in real-time with the optimized Ta_2O_5-based sensor and a clear discrimination of template DNA initial concentration was observed. It was possible to develop a quantitative method for following DNA amplification in real-time comparable to the performance of available standard methodologies.

Because LAMP has been applied to the quantification of RNA in a single closed tube by adding reverse transcriptase, the strategy here proposed can be easily extended to the monitorization of gene expression levels. Significant progress can be expected from the development of an entirely automated device with an improved cell design for multiple sample analysis and lower volumes.

References

1 Veigas B, Branquinho R, Pinto JV, Wojcik PJ, Martins R, Fortunato E, Baptista PV., (2014). Ion sensing (EIS) real-time quantitative monitorization of isothermal DNA amplification, Biosens Bioelectron., 52:50-55.

2 Branquinho, R., Veigas, B., Pinto, J.V., Martins, R., Fortunato, E., Baptista, P.V., (2011). Real-time monitoring of PCR amplification of proto-oncogene c-MYC using a Ta₂O₅ electrolyte-insulator-semiconductor sensor, Biosens Bioelectron., 28 (1), 44–49.

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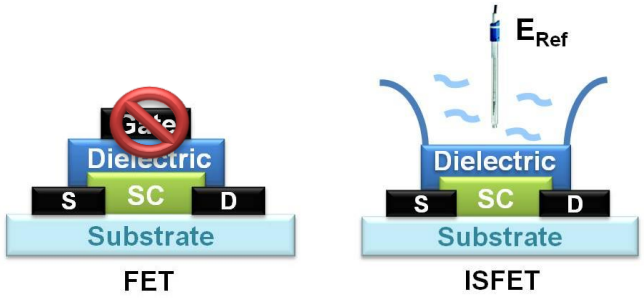
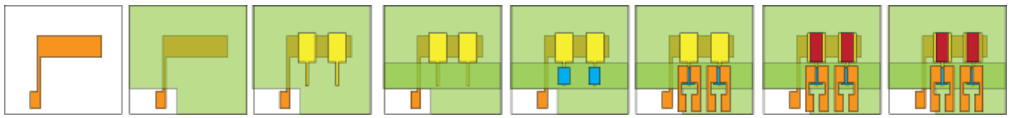
Conclusions & Future Perspectives

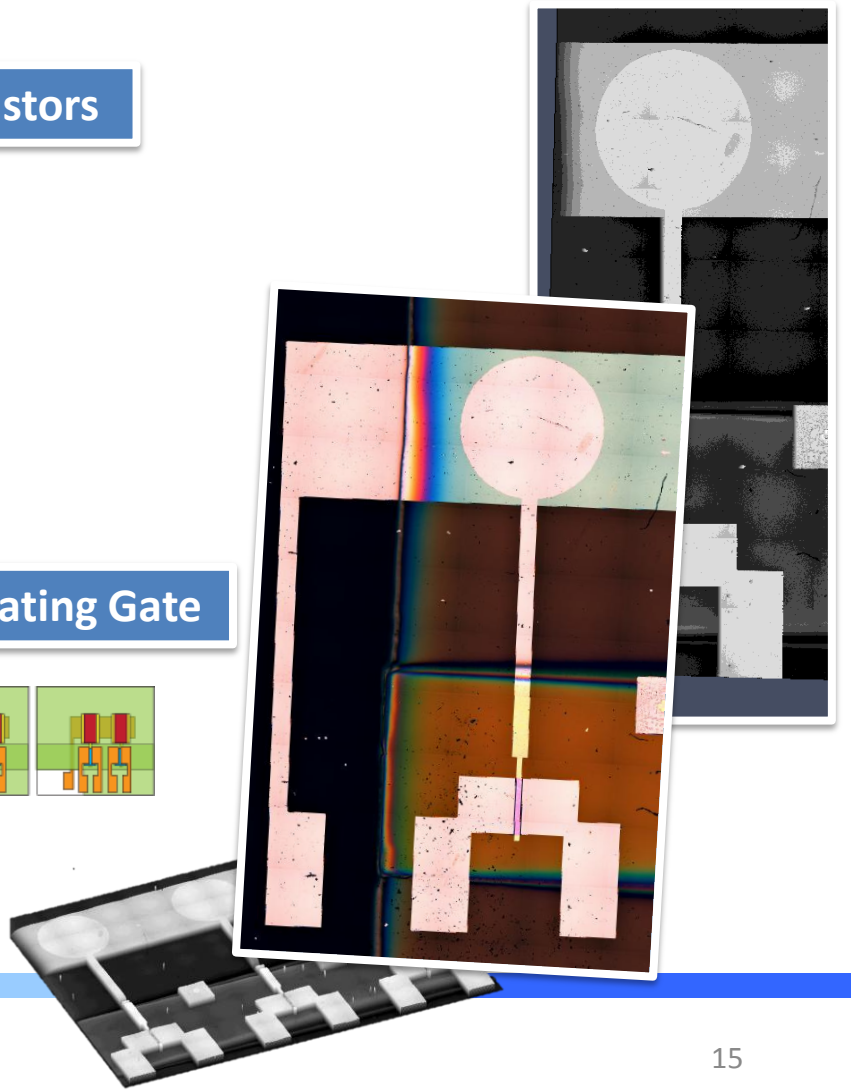
Label-free detection (30 mV/mM) and monitoring of DNA amplification were achieved with results comparable to fluorescence-based methods.

Oxide based Ion Sensitive Field Effect Transistors



Ion Sensitive Field Effect Transistor with Floating Gate

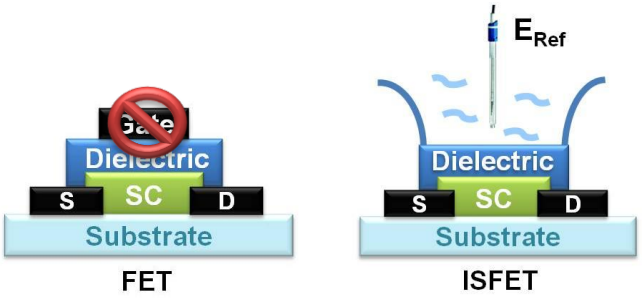




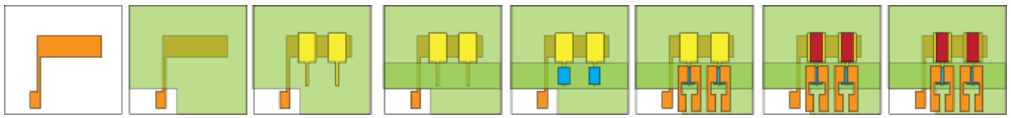
Conclusions & Future Perspectives

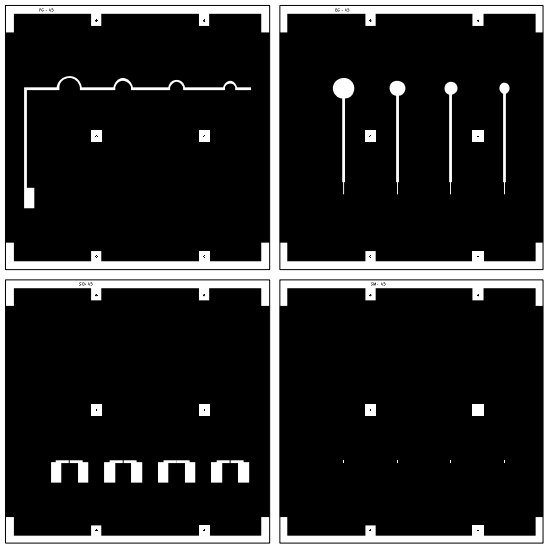
Label-free detection (30 mV/mM) and monitoring of DNA amplification were achieved with results comparable to fluorescence-based methods.

Oxide based Ion Sensitive Field Effect Transistors



Ion Sensitive Field Effect Transistor with Floating Gate





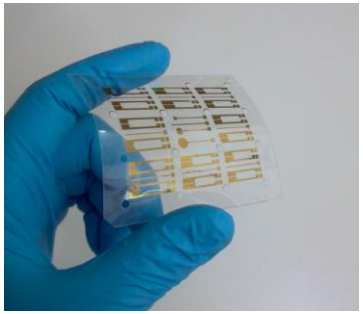
Future Perspectives

Reference Electrode free Devices

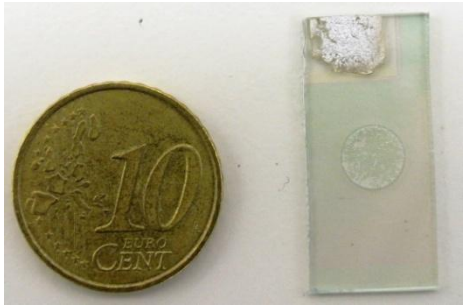
Device miniaturization

Flexible sensors

Alternative processing techniques: solution based synthesis; inkjet printing.



Transparent sensors



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