PROJECT PERIODIC REPORT

Grant Agreement number: 610580

Project acronym: SYMPHONY

Project title: Integrated SYsteM based on PHOtonic Microresonators and Microfluidic Components for rapid detectioN of toxins in milk and dairY products

Funding Scheme: STREP, FP7-ICT-2013-10

Date of latest version of Annex I against which the assessment will be made:

Periodic report: 1st ☐ 2nd ☐ 3rd ■ 4th ☐

Period covered: from 1st November 2015 to 31st January 2017

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement .

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

SYMPHONY

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The SYMPHONY STREP project (FP7-ICT-2013-10) project aims to produce a platform enabling the rapid detection of toxins and contaminates, with an initial focus on the carcinogen Aflatoxin M1, in milk and milk products. The miniaturised smart system is aimed to perform label free detection of contaminants in milk and improve safety and quality of dairy products. The main goal is to produce an automated sampling and analysis system to be used at intake lab in dairies.

The project's broad impact[3]

The dairy industry actively contributes to the economies of communities, regions and countries across the globe. It is a vital part of the world's food system and currently the industry is globalising, increasing the scope and strength of trade.

The dairy sector has a steadily growing production trend (+2.2% annually on average since 2000)[4] that is expected to continue in the long-term. These trends are driven by an increasing population demanding animal proteins in growing economic countries. Consumption of dairy products is consequently expected to increase by 20% or more before 2021, according to the FAO and OECD.

From an economic standpoint, the dairy industry has a number of factors that makes it different to other sectors of the agricultural industry.

1. Milk as a raw material has several key factors making it special. As a liquid with around 90% water, it is bulky and heavy requiring high-cost transportation. Milk is also highly perishable and subject to adulteration, with the quality depending significantly on farm management. As a consequence, comprehensive quality regulations greater than other agricultural sectors are common and necessary.
2. The socio-economic factors of the dairy industry make it one of the most highly protected markets in the world. The majority of dairy farmers are small-scale producers so have a vulnerable position in the market with a high percentage of fixed costs. As a consequence, they are only able to adjust to market changes slowly. Therefore, many countries place a high degree of importance on the dairy industry with western countries implementing protection measures.
3. There are a high number of strong co-operatives involved in milk processing. In 2011, two of the world's five largest dairy companies were co-operatives, with combined sales of $29.1 billion. The rise to prominence of co-operatives can be summed up by the benefit to small family dairy farms of a guaranteed outlet and price for their product.
4. Milk is valuable, but at the same time is an expensive raw material. Milk can be converted into added value products and longer life products. However, processing the milk is crucial and so dairy processing is very important to the dairy farming sector, more so than other agriculture sectors. Therefore, dairy processing operations have high technical and quality standards to comply with.

The implementation of SYMPHONY as an automated analysis unit at Hazard Analysis and Critical Control Points (HACCP) will result in a more efficient management of quality control and an enhanced control of specific risk factors and increased public health safety. This will warrant strict control of milk batches entering the production chain, providing a better quality assurance and a timely feedback to the contaminated farm. In return, it will lead to a considerable reduction of the economic loss for farmers and dairies and in an improved quality of products.

[3] This is part of a survey produced by QCL.
[4] The Economic Importance of Dairying, International Dairy Federation, IDF Factsheet, 2013.

Project technical achievements

Development of integrated photonic sensors

For analyte detection, LioniX implemented high-resolution SiN asymmetric Mach-Zehnder interferometers biosensors. Chip included compact 8x array of sensing sites, with a LOD of $5x10^{-7}$ Refractive Index Units (RIU) and a sensitivity of 10600 rad/RIU (Measurements by UNITN). The sensor functionalisation, based on antibodies fragments (FABs) was developed at FBK, providing a final sensor detection limit for aflatoxin below 1µg/l. The sensor regeneration and reusability was demonstrated up to 5 regeneration cycles.

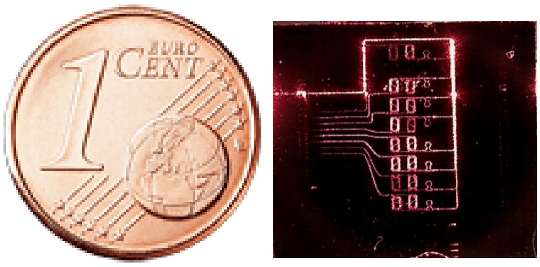

Figure 1: Picture of the aMZI chip

Sensor integration provided two complementary options: an on-chip integrated photodetector (FBK) and heterogeneous integration of VCSEL by grating coupling (LioniX and FBK), in collaboration with BioFOS EU Project. Monolithic integration can significantly reduce the system cost since the detector is realized together with passive components, in a single fabrication process. The 3D schematic representation of the proposed photodetector by FBK is shown in Figure 2. Previously, we implemented photoconductive detector and first batch of PIN photodiodes based on such design. The applicability of these devices for monitoring of the biosensor response has been proved. The best responsivity measured by UNITN and FBK was as high as 0.38A/W (at bias of 5V) has been estimated at 850 nm. We demonstrated that the performance of our diodes is comparable with that of commercial external photodetectors.

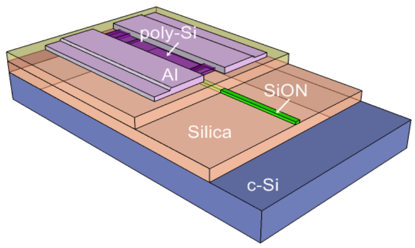

Figure 2 3D schematic of the photodetector integrated on top of the waveguide

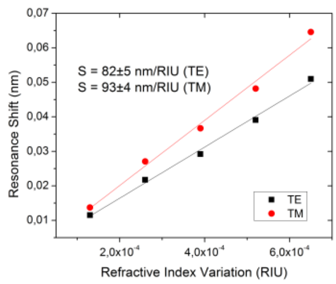

Figure 4 Bulk sensitivity measurement on the sensor chip, integrated with PIN photodetector.

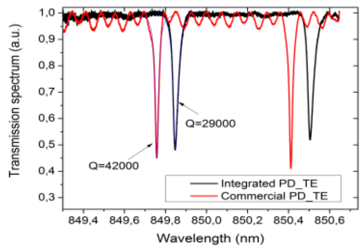

Figure 3 Transmission spectra of sensor mRR, measured with both commercial and integrated photodetectors.

Fab' based functionalization (FBK) on SiN aMZI show a good specificity of the detection. Figure 5 shows the measurements by UNITN for the three sensors when a 100 nM concentration of Aflatoxin M1 (black lines) or of Ochratoxin (red lines) is added to the buffer solution. The phase shifts in time following the kinetic of the binding of the toxins to the antibody on the surface of the exposed aMZI arms. The functionalization is shown to be specific. In fact, in the case of AFM1, after MES rinsing, the phase shift is 2 radians larger than the one before the toxin injection, while in the case of Ochratoxin it is only about 0.8 radians. Regeneration procedure demonstrated the reusability of the chip up to 5 measurements.

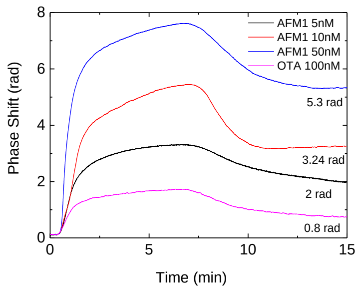

Figure 5 Sensing measurements of 5 nM, 10 nM and 50 nM pure AFM1 and 100 nM OTA diluted in MES

Sample preparation and system setup

Milk is a complex matrix, including multiple phases and a number of chemical species. Aflatoxin has low solubility in water, with good affinity with proteins. The small molecule size of aflatoxin (MW: 328.3 g/mol) is an additional challenge if detection is based on label-free direct methods monitoring the change of surface properties like measurement of refractive index (photonic sensors) or impedance (electrochemical capacitive sensors or impedance spectroscopy) at the surface. Clearly, phase and chemical separation are needed for an efficient detection of aflatoxin with a biosensor and procedure of separation needs to guarantee high aflatoxin recovery after the phase separation.

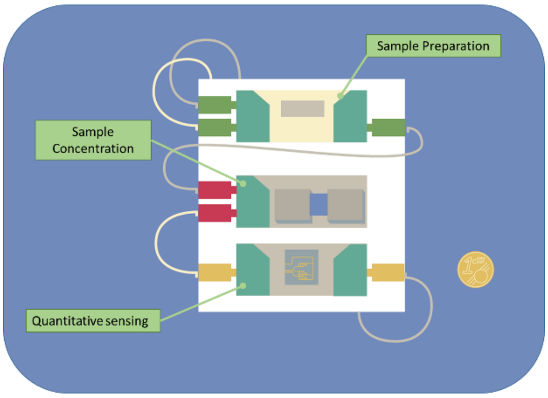

Figure 5: Symphony system concept.

In SYMPHONY, the sample preparation strategy is organized as follow:

- FBK developed a separation module based on TFF filtering, coupled with a thermal and chemical process, which showed to be able to reduce the fat content below 0.1% and proteins below 0.5% with a flow rate in the order of few ml/min. The AF recovered in the clean solution is about 75%, suitable for high performance concentration and measurement.
- A concentration module to increase the limit of detection of the system and to reduce the interference with the milk matrix. The concentrator was realized by Epigem and functionalised with mussel foot protein (mfp-1), a universal and simple method to have a sticky layer suitable for the immobilization of anti-aflatoxin antibodies by ACREO and could achieve up to 50x concentration by thermal release of bound AF.
- Sensor is consistently and quickly quantifying aflatoxin in few minutes.

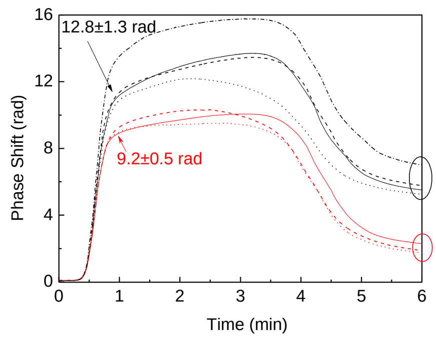

Figure 7: Sensor response at 1.2µg/l (black, 4 replicas; from 77ng/l milk processed with the system) and blank samples (red, 3 replicas).

The outcome of the project is a PC-controlled system able to demonstrate the procedure autonomously.

An additional project outcome was the development at FBK of a cleaning module designed to separate residual proteins before the detector (optional) based on electrophoresis. Vertical cross-flow electrophoresis of charged species was implemented by FBK as electrodes on glass for the realisation of a “SPLITT-like” structure (Figure 6), which could achieve up to 40% separation of BSA at flow rates ranging from 100 to 1000 µl/min. In perspective, the module can be used in addition to previously described devices for improved functionality.

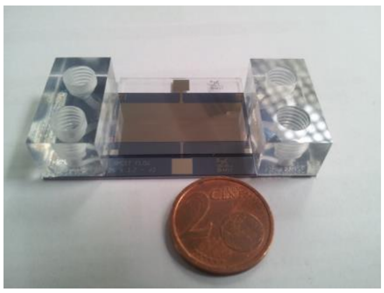

Figure 8: Electrophoretic separation device

Dissemination

For dissemination purpose, the Symphony project prepared a set of public material available on the website (www.symphony-project.eu):

- A newsletter.
- FBK prepared a dissemination video on Symphony concepts, which is available through the website homepage.
- Innovation brochures on major results.

SYMPHONY participated in the collaborative optofluidics demonstrations stand and several dedicated workshops and special sessions, e.g.:

- SELECTBIO at exhibition of Lab-on-a-Chip & Microfluidics Madrid March 2016,
- Microfluidics Matchmaking & Networking Event 2016, 14 September 2016, Basel, Switzerland,
- Special Session at RAFA2015,
- Exhibition at "Festival della scienza" (Science Festival), Genova (IT), on Symphony concepts,
- Booth at the World Congress on Biosensors 2016 in Gothenburg,
- MNBS2016
- Invited presentation at kick-off meeting of the EU Mycokey project.

The list of latest publications by Symphony project:

- T. Chalyan, L. Pasquardini, F. H. Falke, M. Zanetti, R. Guider, D. Gandolfi, E. Schreuder, C. Pederzolli, R. G. Heideman, and L. Pavesi "Biosensors based on Si3N4 Asymmetric Mach-Zehnder Interferometers" Proc. SPIE 9899, Optical Sensing and Detection IV, 98991S (April 29, 2016).
- T. Chalyan, L.Pasaquardini, D. Gandolfi, R. Guider, A. Samusenko, M. Zanetti, G. Pucker, C. Pederzolli, and L. Pavesi. "Aptamer- and Fab'- functionalized microring resonators for Aflatoxin M1 detection". IEEE JSTQE, PP(99), September 2016, doi: 10.1109/JSTQE.2016.2609100.
- A. Adami, A. Mortari, E. Morganti, L. Lorenzelli - Microfluidic sample preparation methods for the analysis of milk contaminants – Journal of Sensors, Volume 2016 (2016), Article ID 2385267, 9 pages, doi: 10.1155/2016/2385267.
- T. Chalyan, R. Guider, L. Pasquardini, M. Zanetti, F. Falke, E. Schreuder, R. G. Heideman, C. Pederzolli, L. Pavesi - Asymmetric Mach–Zehnder Interferometer Based Biosensors for Aflatoxin M1 Detection - Biosensors 2016, 6, 1.
- A. Samusenko, D. Gandolfi, G. Pucker, T. Chalyan, R. Guider, M. Ghulinyan, and L. Pavesi - A SiON microring resonator-based platform for biosensing at 850 nm - IEEE Journal of Lightwave Technology 34, 969-977 (2016).
- G. Pucker, A. Samusenko, M. Ghulinyan, L. Pasquardini, T. Chalyan, R. Guider, D. Gandolfi, A. Adami, L. Lorenzelli and L. Pavesi - An integrated optical biosensor platform - SPIE Newsroom, March 21 (2016).

More information: http://www.symphony-project.eu/