



## 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 contaminants, 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.

### *Global dairy market<sup>1</sup>*

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)<sup>2</sup> 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.

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<sup>1</sup> This is part of a survey produced by QCL.

<sup>2</sup> The Economic Importance of Dairying, International Dairy Federation, IDF Factsheet, 2013.



### ***System requirements***

SYMPHONY addresses the needs of the dairy industry with novel microfluidic technologies and biochemistry for sample preparation, photonic integrated sensors and compact hardware for integration in the production chain of the dairy industry, leading toward precision process management.

After a consultation with stakeholders, main specifications for the SYMPHONY system were elicited and reported in the following table.

<b>Specification</b>	<b>SYMPHONY Target USP</b>
LOD (ppt)	<10
Test Accuracy	±10ppt @25 or 50ppt
Robustness	Very High
Test Time	20 min
User-friendliness	Very Easy to Use by non-skilled operator.
Multianalyte Testing	Important
Cost per test	1-5 euro

Aside these functional requirements, to be successful, SYMPHONY solution should meet non-technical needs and provide benefits for users. These include the fact that SYMPHONY system should match and improve existing processes, meet the most updated legislation, bring cost savings, improve quality and add value to operations.

### ***Development of photonic sensors***

High-resolution biosensors, such as SiN asymmetric Mach-Zehnder interferometers (aMZI developed by LioniX) and SiON microring resonators (MRR developed by UniTN and FBK), were studied for analyte detection. Both types of sensors gave good results in terms of sensitivity, Limit Of Detection (LOD) and reproducibility. The aMZI solution was selected for final implementation because it was demonstrated to be more suitable for the integration steps.

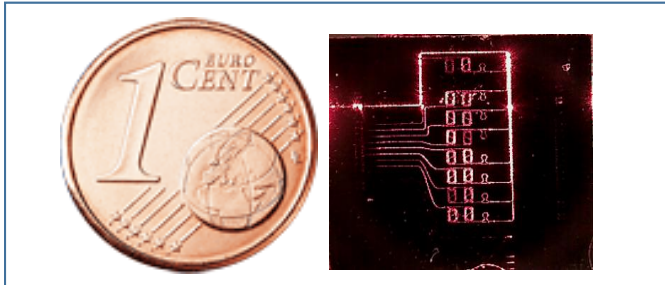


Figure 1: Picture of the aMZI chip

The initial aMZI design presented in the first year annual report has seen many improvements during the second year, manufacturing technology and design wise, resulting in higher yield and better and stable performances of the aMZI sensor (see Figure 1). While retaining a similar sensitivity, the path length of the sensor is greatly diminished down to 6.2 mm, resulting in a more efficient use of chip surface, stable performance, less discrepancy between each sensor characteristics and lower optical losses, allowing designs with more sensors. UNITN measured for these sensors a LOD of  $5 \times 10^{-7}$  Refractive Index Units (RIU) and a sensitivity of 10600 rad/RIU. The sensor regeneration and reusability was demonstrated up to 5 regeneration cycles. Next steps will be the hybrid integration of VCSELs and photodiodes on the sensor and the surface modification with proprietary alkene-modified SiN surfaces for robust aptamer immobilization, in collaboration with LioniX sister company Surfix.

### ***Development of a monolithically integrated on-chip photodetector***

An on-chip integrated photodetector represents an interesting alternative with respect to the butt-coupled photodiodes. 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 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 0.33A/W (at bias of 9V) at the wavelength of 850 nm.

In the last run, we improved the fabrication process in order to enhance the performance of the integrated sensor-detector system. UNITN measured the transmission spectra at the output of the of sensor rings with both a fiber-coupled Si detector and the integrated PIN photodetector (Figure 3 and Figure 4). The average responsivity of 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.

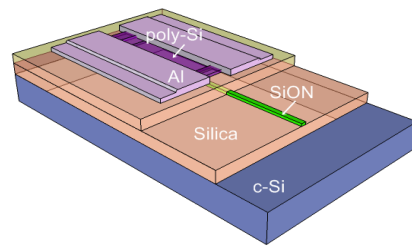


Figure 2 3D schematic of the photodetector integrated on top of the waveguide

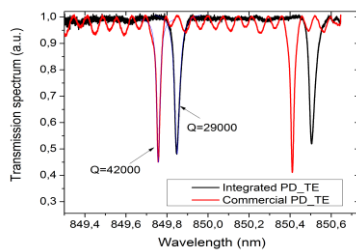


Figure 4 Transmission spectra of sensor mRR, measured with both commercial and integrated photodetectors.

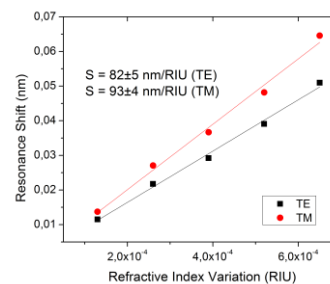


Figure 3 Bulk sensitivity measurement on the sensor chip, integrated with PIN photodetector.

Finally, UNITN performed the volumetric sensing experiments with water-glucose solutions at different concentrations, using the integrated photodetectors to collect the sensor response. The results evidence that also the resonance shift of the mRR can be efficiently monitored with our PIN photodiodes.

### Functional surfaces and analytical methods

During the reporting period, the scope of this WP was threefold: to investigate the suitable procedures for sample treatment, for the pre-concentration, and for the sensor functionalization and regeneration. The strategy implemented by ACREO for aflatoxin purification is based on the surface coating with mussel foot protein (mfp-1), a universal and simple method to have a sticky layer suitable for the immobilization of anti-aflatoxin antibodies. When comparing monoclonal antibodies versus polyclonal antibodies, we found polyclonal to be as good as the monoclonal in capturing AFM-1 from milk. However, we also found that the reversible heat inactivation of monoclonal antibodies to be more complete, as compared to polyclonal antibodies, indicating that it will be possible to elute AFM-1 in a smaller volume (=higher concentration) from monoclonals then from polyclonals. Both monoclonal antibodies coated substrate and polyclonal antibodies coated substrate could be stored in MES and re-used after several months.

Functionalization of detector surface on the detector surface for the capture and detection of aflatoxin previously purified is studied by FBK. Two different approaches were selected based on aptamers and antibodies fragments (Fab') respectively.

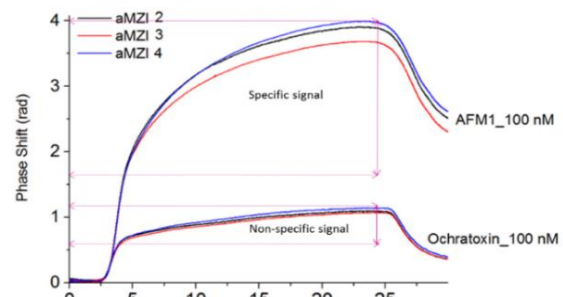


Figure 5 Sensorgram recorded on aMZI sensors by flowing AFM1 (black) and Ochratoxin (red) through the microfluidic chamber.



The aptameric layer captures around  $10^{13}$  aflatoxin molecules per  $\text{cm}^2$ , matching the requirements to achieve the lowest limit of detection in EU regulation.

In the case of *Fab'* based functionalization preliminary tests on 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 0.25 radians. Regeneration procedure is still under evaluation.

### **Sample preparation**

Milk is a complex matrix, including multiple phases and a number of chemical species.

- Fat globules, also including proteins, phospholipids, glycoproteins, neutral lipids, enzymes and other minor components. Typical size is about 0.2-8 $\mu\text{m}$ , concentration about 4%. The globule is stabilised by a membrane.
- Casein micelles, typical size is about 50-300nm (about 2.5% concentration),
- Water-based fraction includes whey proteins, salts, sugars, vitamins.

Aflatoxin is partially soluble 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 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. With this background, it is clear that phase and chemical separation are needed for an efficient detection of aflatoxin.

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

- A fat separation module based on continuous flow fat removal, which showed to be able to reduce the fat content down to 1% (comparable with content obtained by centrifugation) with a flow rate in the order of  $\mu\text{l/s}$ . A solution to increase the flow rate to  $\text{ml/s}$  is under evaluation.
- 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 currently under testing at ACREO and FBK.
- A cleaning module 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).

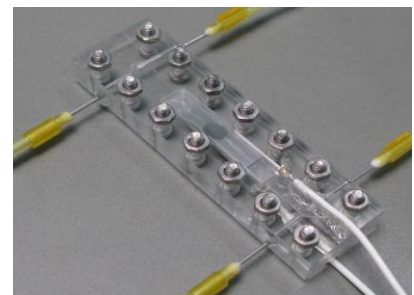


Figure 6: picture of the electrophoresis module.

### **Dissemination**

For dissemination purpose, a website was created and periodically updated. A first newsletter was issued in April and it is available at <http://symphony-project.eu/node/58>. In 2015, SYMPHONY participated in the collaborative optofluidics demonstrations stand at the World of Photonics Exhibition and several dedicated workshops and special sessions were organized (The Milk Day, Special Session at RAFA2015 and MinaB\_ICT), also in collaboration with other clustering projects. SYMPHONY results and approach were presented at 10 international scientific conferences and published in peer review journals. A complete list of publications can be found in the website.

**More information:** <http://www.symphony-project.eu/>