SYMPHONY

Coordinator contact:
Fondazione Bruno Kessler
Lorenzelli Leandro; email: lorenzel@fbk.eu
via Sommarive 18 38123, Trento, Italy

Project context

In the dairy industry, one of the most pressing needs for food quality and safety is the timely detection of aflatoxin M1 contaminations that originate from animal feed and are secreted into milk. This issue represents a hazard for human health and an economic loss for the dairy industry. The available technology for aflatoxin detection is laboratory-based, in most cases requiring sample preparation and does not provide timely identification of contaminants, thus fails to deliver cost-effective management of milk quality. The entire supply chain relies on the fact that the dairy products delivered to consumers are safe. Current best practices for Aflatoxin management in milk in the modern dairy industry recommend aflatoxin M1 analysis to be carried out routinely for quality assurance and quality control purposes as well as for feed-back mechanisms to farm and animal health management, as requested by European Commission regulation (EC) No. 1881/2006. The current procedure involves the collection of samples from collection tanks. The milk samples are analysed in a laboratory with screening tests such as Enzyme-Linked Immunosorbent Assay (ELISA). Contaminated milk that exceeds the 50 ng/Kg (ppt) aflatoxin limit is not allowed in the market and has to be disposed.
The SYMPHONY STREP project (FP7-ICT-2013-10) 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.

Objectives

The objective of the SYMPHONY project is the development of a novel system for the detection of aflatoxin M1 in milk for the dairy industry. The integration of cutting-edge technologies in SYMPHONY will empower the application of innovative heterogeneous systems by the dairy industry, driven by end-user needs.

The technical goal is to develop a continuous flow analytical system that can take samples at various stages during milk production when milk is transferred between storage sites. The SYMPHONY project approach is based on the integration of polymer-based technologies for microfluidics and Si-based photonic structures.

The system will be supplied in a form suitable for trials within the dairy production supply chains and it is meant for application at the incoming storage tank of a dairy producer, which was evaluated as the “most beneficial location” in our preliminary analysis, performed by means of questionnaires and meetings with dairy industry stakeholders.

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.
SYMPHONY is a demand-driven project pursuing:

1. Advancement of MNBS devices toward real-world applications by providing:

a. Integrated sample preparation for analytical tools in dairy industry,
b. Advancement in high sensitivity photonics biosensors, by using aptamers to provide selectivity to targets and improved sensor stability and hybrid integration of sources and detectors.

2. Improvement of milk quality assurance by making available new technologies and procedures for the detection of aflatoxin and other contamination
3. Improvement of dairy industry competitiveness by early detection and effective corrective action against aflatoxin outbreaks and contaminants:
   a. On field simultaneous detection of various contaminants, thanks to arrays of photonic sensors;
   b. Analysis of raw milk and other liquid dairy products, such as yogurt, butter milk, kefir, whey;
   c. Quality assurance at various HACCP
4. Improvement of food quality with positive long-term impact on human health

Description of the work performed and the main results achieved

The activities carried out during the reporting period of the project have been developed mostly for the implementation and evaluation of modules and general architecture of the system as described below.

Stakeholders needs, system specifications & architecture

Starting from user requirements and guidelines surveyed and elaborated by QCL, the general architecture of the system has been defined, with still open options to be discussed during the second year. Since the system is heterogeneous and consists of several modules realized with different technologies, an integration plan has been defined with the aim of having a first prototype of the whole system by the end of the second year of the project. The system developed for aflatoxin M1 will be the starting point to extend the functionality to other analytes in order to exploit the potential of sensor technology and microfluidics for the realisation of a multi-analyte system for milk quality. The modular approach and the sensor architecture are very suitable for the implementation of sensor array for the detection of multiple targets. An evaluation of the system extension to multianalyte approach has been completed. Antibiotics were selected as best target of multianalyte extension, considering end-users expectations, technical feasibility (in particular the availability of both antibody and aptamer for the same analyte), compatibility with the EU regulation, time and costs of the technical work to adapt the system to the target analytes.

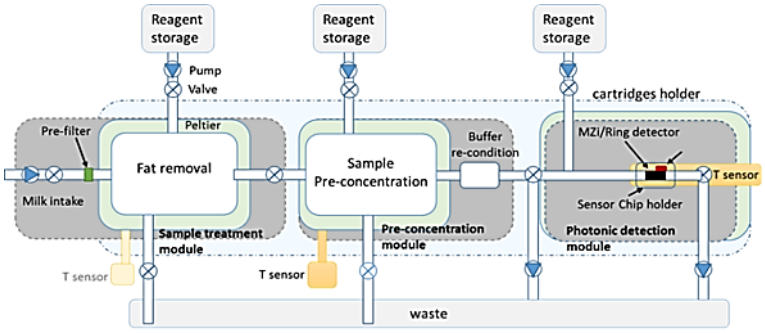

Figure 1: Sketch of Symphony architecture

Development of photonic sensors

The photonic device proposed in this project is based on a low cost Si-based fully integrated optical sensor. The development of the photonic sensor, that will be able to efficiently detect Aflatoxin M1 in milk, is an essential step towards the realization of the SYMPHONY project. During the first year of this project we designed, developed and tested two types of photonic sensors in order to define their performance in term of an Aflatoxin M1 sensor. The first design is based on SiN

asymmetric Mach-Zehnder interferometers (aMZI) developed by LioniX, while the second sensor is based on SiON microring resonators (mRR) and developed jointly by FBK and UniTN.

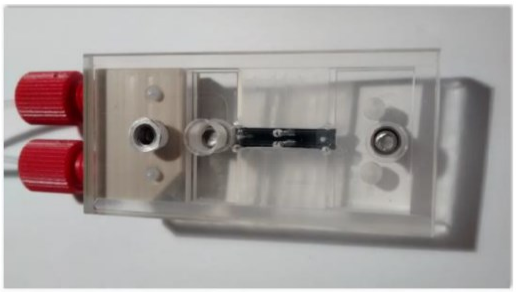

Figure 2: Picture of photonic sensor

Innovative designs were simulated and tested, and close collaborations with WP3 concerning the functionalization procedure as well as WP4 concerning the microfluidic platform were established. We obtained a Limit Of Detection (LOD) of $0.8*10^{-6}$ Refractive Index Units (RIU) and $1.6*10^{-6}$ RIU in the case of aMZI and mRR, respectively. Preliminary Aflatoxin sensing measurements are also available. Integration of VCSEL sources and photodiodes by means of heterogeneous assembly will provide a module which is simple to use and without the need for complex alignment procedures, where only electrical contacts are needed to connect the sensor to the system.

Functional surfaces and analytical methods

During the reporting period the scope of this WP was twofold: to investigate the suitable procedures for sample treatment and pre-concentration, and the sensor functionalization and regeneration procedure. 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. Anti-aflatoxin aptamers were instead immobilized by FBK on the detector surface for the capture and detection of aflatoxin previously purified on the antibody-functional surfaces. The aptameric layer captures around 10^{13} aflatoxin molecules per cm^2, matching the requirements to achieve the lowest limit of detection in EU regulation. The reusability of the aptameric layer has also been tested, showing a significant reduction of the binding efficiency after 5 regeneration cycles (SiON surfaces) or 10 regeneration cycles (Si_3N_4 surfaces), and therefore opening the promising perspective of using the sensor more than once, reducing the overall analysis cost.

Development of microfluidic components

Sample preparation, (i.e. sample clean-up, extraction and pre-concentration), has been the main limiting factor of automatic and on-line analysis systems integration in the dairy industry. Clean-up kits available on the market are labour intensive and require a skilled operator to perform the procedure and therefore an automated procedure greatly contributes to the overall value of the system. In the project, this will be achieved with a joint activity of ACREO, EPIGEM and FBK using a micro/milli fluidic system, able to manage milk sample quantities of millilitres that will be moved, concentrated and interconnected with the sensor with a range of commercially proven interconnection and packaging technologies available to the consortium. The main purpose of the sample preparation stage is to clean the milk sample from unwanted components like fats, which may interfere with the following stages and cause clogging of the system, and to concentrate and make the toxin available for detection. Then, sample preparation can be schematically divided in the following steps: fat removal, aflatoxin extraction, pre-concentration and elution.

Figure 3: Picture of microfluidic concentrator

Up to now, microfluidic modules for fat separation and milk fractionation have been produced and tested showing a fat reduction comparable with laboratory techniques. The procedures for aflatoxin extraction and concentration are currently under evaluation. At the other end of the system, sensor

packaging with integral flow cells, has been designed and fabricated. This packaging can be reversibly sealed to the main microfluidics processor board using Epigem's microseal technology.

Exploitations

Significant observations and requirements for exploitation were:

- Primary exploitation application route for AFM1 detection in milk is as a tanker acceptance test at dairy processing milk intake laboratories.
- End-user requirements and competitive analysis indicate that the SYMPHONY system should provide comparable or better test time, cost per test and better uncertainty of measurement than commercially available lateral flow immunoassay test kits as well as be simple to use by non-skilled operators.
- Ability to measure multiple analytes in parallel would be a unique selling point and the ability to analyse antibiotic residues in milk could have significant exploitation potential as well as socio-economic benefits.

The value chain for SYMPHONY has been defined by QCL both for innovation and research and for production and sales

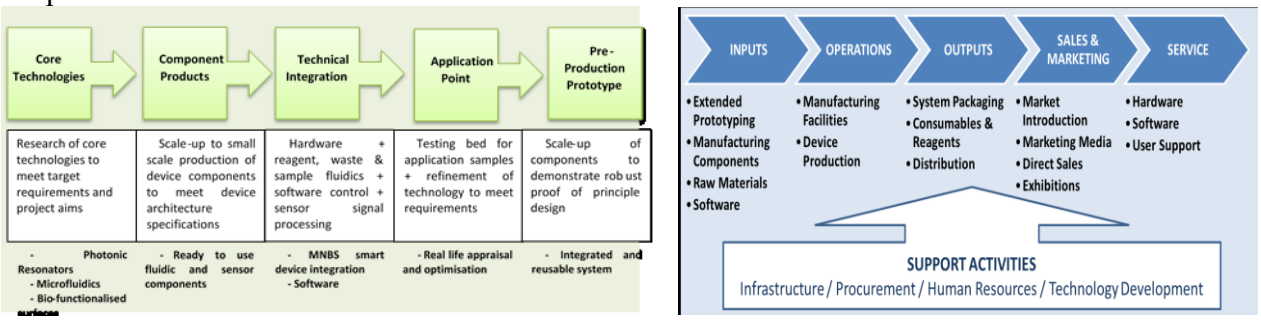

Figure 4: SYMPHONY Research and innovation (left) and production and sale (right) value chain

Dissemination

For dissemination purpose, a website was created and periodically updated. It can be reached at http://symphony-project.eu/. Moreover, a technical workshop was organized at the kick-off meeting and others are planned for the next year.

Scientific papers

- D. Gandolfi, G. Pucker, M. Guarisco, L. Pasquardini, M. Ghulinyan, L. Pavesi "Enhanced sensitivity in label free sensing using integrated wedge resonators" - oral presentation at EUROPT(R)ODE XII, April 13-16, 2014Athens, Greece.
- D. Gandolfi, M. Guarisco, L. Pasquardini, C. Pederzolli, M. Ghulinyan, G. Pucker, L. Pavesi " Enhanced integrated optical resonators for label-free biosensing"- Oral presentation at SPIE Photonics Europe, 14 - 17 April 2014 in Brussels, Belgium.
- G. Pucker "Development of microresonator based biosensors operating at ~0.8-0.9 µm", - Invited oral presentation in the Special session on Multifunctional Photonic Sensors IMEKO TCA 2014 Symposium, 15th to 17th September 2014 in Benevento, Italy.
- D. Gandolfi, F. Ramiro-Manzano, F. A. Rebollo, M. Ghulinyan, G. Pucker, L. Pavesi "Role of edge inclination in optical microdisk resonator for label-free sensing", Optic Express Journal, ArXiv library (ArXiv ID: 1408.3954).
- D.Ilver, K. Fogel, A. Krozer "MAP-assisted self-assembly of particles for spatially defined multi layered structures" - oral presentation at 2nd International Conference – NICE 2014 Nature Inspired Chemistry Engineers, Nice, France (15-17 October, 2014).

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