SPEDOC NEWSLETTER 2011

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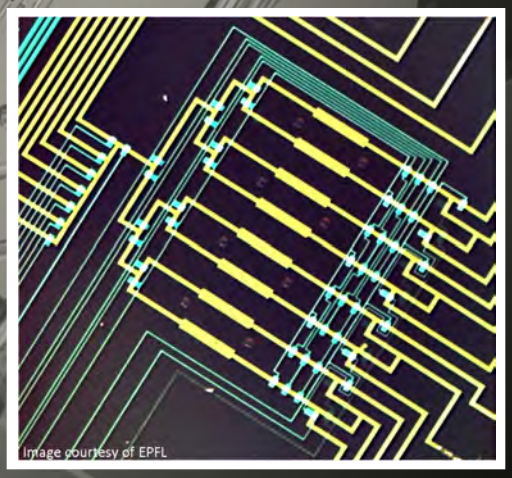
SURFACE PLASMON EARLY DETECTION OF CIRCULATING HEAT SHOCK PROTEINS & TUMOR CELLS

Started on January 2010, **SPEDOC** is a research initiative funded by the European Comission's Seventh Framework Programme for Research. **SPEDOC** aims at combining the latest advances of nano-optics, optical manipulation and microfluidics with recent discoveries in Heat shock Proteins (HSP) to develop the precursor of future individualized **cancer diagnosis and treatment follow-up devices**.



The developed platform, integrated in a microfluidic environment, will exploit the surface plasmon resonances supported by micro- and nano-gold nanostructures

(i) to track HSP70 proteins in the peripheral blood and
(ii) to monitor its over expression at the surface of cancer cells.

This innovative platform aims to be the precursor of a high sensitive point of care device **to be used in biological labs** by medical doctors. It should also permit providing treatment to cancer patients at an earlier stage and at lower doses with the consequent decrease of secondary effects.

In this first Project Newsletter we would like to present the basis of our investigation as well as the main results obtained during this first year of work.

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This project is funded by the European Commission's Seventh Framework Programme for Research (FP7)

CONTEXT AND OBJECTIVES

Cancer has become the leading cause of death in the world and costs more in productivity and lost life than any other illness, according to the American Cancer Society report presented at the 2010 World Cancer Congress. Although the risk of dying from cancer has been decreasing since the early 1990s, the rate of new cancers remains stable. The treatment of cancer is therefore progressively improving but the disease remains devastating. In this "war" against cancer, one main strategy, complementary with the improvement of treatments, aims at detecting the disease at an earlier stage.

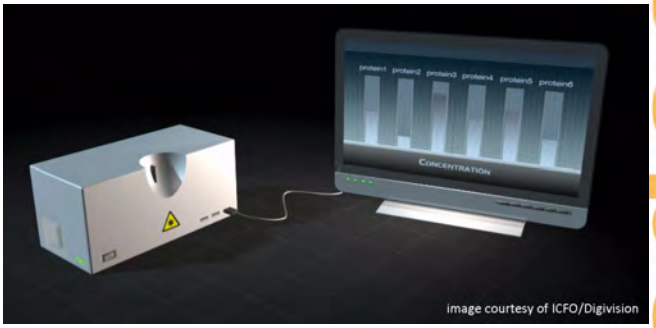
In practice, the higher the sensitivity of the detection is, the earlier the cancer can be identified and treated. This high sensitivity is currently not available, neither in clinical nor point-of-care environments, nor at an institutional oncology research level. Indeed, today diagnosis still relies mainly on microscopic (but not molecular) cues, when the tumor is already composed of several millions of cancer cells. Alternatively, tracking cancer at the molecular level by monitoring the presence of cancer markers in the patient's body would enable to better anticipate the development of the disease. In this context, the SPEDOC project is a multidisciplinary European initiative that joins forces of physicists and oncologists to develop a novel ultrasensitive cancer-marker sensing platform for early detection and accurate treatment monitoring.

Prior research studies have demonstrated that in cancer patients, HSP70 (Heat Shock Protein 70) is over-expressed at the surface of cancer cells and in the peripheral blood. Therefore, HSP70 is an interesting target to be tracked in the organism of a patient for at least 2 reasons: Firstly, HSP70 over- expression is associated with many cancers. Secondly, since HSP70 is expressed on the outer membrane of cancer cells but not normal cells, it may also be possible to detect circulating tumor cells in serum.

While conventional ELISA (Enzyme-Linked ImmunoSorbent Assay) tests have been recently used to detect circulating HSP70 proteins, the maximum sensitivity remains limited to a few ng/ml and requires labels that may denature the protein compromising the target affinity for the receptor and extend and complicate the assay. Beyond the sensitivity issues, there is not, to date, any technique, which enables detecting HSP70 directly at the cell membrane surface in serum, which would provide much higher reliability in diagnosis.

Independently, latest advances in nanotechnologies have led to new ultrasensitive sensing schemes able to detect low concentration of specific target molecules. Among the most promising approaches, metallic nanostructures, supporting the so-called surface plasmons resonances, combine (i) a high sensitivity to tiny changes of their surrounding refractive index as induced by the binding of molecules with (ii) intense optical fields suitable for enhanced Raman scattering and enhanced optical trapping.

The SPEDOC project aims at developing a novel integrated optical sensing platform based on surface plasmons (SP) for early diagnosis, treatment monitoring and follow-up of cancer at the level of oncology research institutes. By using the latest advances of surface plasmon nano-photonics, we investigate different configurations of compact and ultra sensitive sensors able to detect HSP70 proteins both in the peripheral blood and at the surface of cells of a mice model, using resonance perturbation, scattering imaging and Surface Enhanced Raman Scattering (SERS), respectively. The developed sensors will be implemented in an advanced microfluidics chip to increase reproducibility, reduce the volume of analyte involved and enable parallel detection experiments on a single chip.



Towards an integrated Optical Platform for the high sensitivity detection of cancer markers.

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The three main objectives of the project read

- Objective1: **Increasing the reliability and sensitivity of HSP70 tracking by using ultra-specific detection schemes (coupling highly specific receptors with state of the art plasmonic platforms) able to monitor the concentration of over-expressed biomarkers both circulating in the blood and accumulated at tumour cells membrane.** This would facilitate cancer detection in an earlier developmental stage for a more efficient population pre-screening and follow-up.

- Objective2: **Increasing the detection throughput**: The combination of microfluidics and plasmonic nanosensors will facilitate the implementation into a clinical setting due to faster and parallel assays with fewer steps and lesser sample consumption over existing methods.

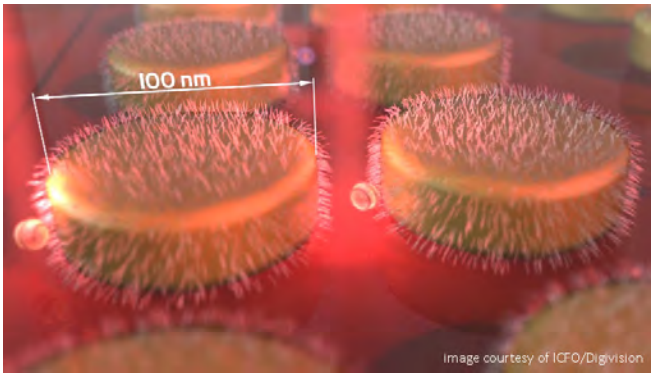
- Objective3: **Integration of optical transduction and plasmonic tweezers into a compact platform able to operate in a biology or oncology laboratory setting**. Such device should be understood as a precursor of a future portable device enabling point of care (POC) diagnostics in a medical environment.

Description of the work performed and Main research achieved so far

Over the first year of the project, the collaborative efforts of the SPEDOC partners have enabled to implement skills and knowhow from the different fields of expertise and some first main achievements towards the detection of circulating HSP70 have already been accomplished.

Design and fabrication of plasmonic architectures for the detection of circulating HSP70 Based on extensive numerical simulations, we have identified and optimized geometries of coupled gold nanoparticles that feature high sensitivity to a tiny change of their shallow refractive index, as induced by the binding of HSP70. Based on the optimized designs, samples were fabricated using e-beam lithography.

Surface chemistry and preparation of gold particles We have successfully elaborated a surface chemistry protocol that enables to bind to the gold sensors receptors with high binding specificity to HSP70. This accomplishment is essential since it determines the specificity and therefore the reliability of the different HSP70 sensing schemes that will be investigated.



Detection of HSP70 proteins in serum by plasmonic nano-sensors.

Design and production of microfluidic design compatible with LSP sensing. We have designed and fabricated a first microfluidic platform that is compatible with LSP sensing and could enable parallel sensing assays under a wide set of experimental conditions.

Successful demonstration of sensing of protein binding in microfluidic environment After integrating the fabricated sensors into the microfluidic platform, we have lately successfully achieved the detection of protein binding and study of the binding kinetics.

The expected final results and their potential impact and use

The expected final outcome of SPEDOC is the integration of the most successful plasmon-based sensing schemes into a compact microfluidic platform. We foresee the platform first to achieve label-free detection of free HSP70 proteins circulating in serum with sensitivity in the 10-100 pg/ml range i.e. at least one order of magnitude beyond state of the art sensitivity of prior label-based sensing schemes. Beyond, we aim at quantifying for the first time the actual over expression of HSP70 proteins at the membrane of tumour cells circulating in serum. By the end of the project, we expect the platform to be operated by a tabletop turnkey device that could be easily operated in a biology or oncology laboratory environment for carrying out parallel assays.

The current project focuses on the detection of a danger signal (HSP70 protein in serum) combined to the direct identification of circulating cancer cells (while many studies monitor cancer indirectly through byproducts). It is therefore highly suited for detecting cancer progression and relapse.

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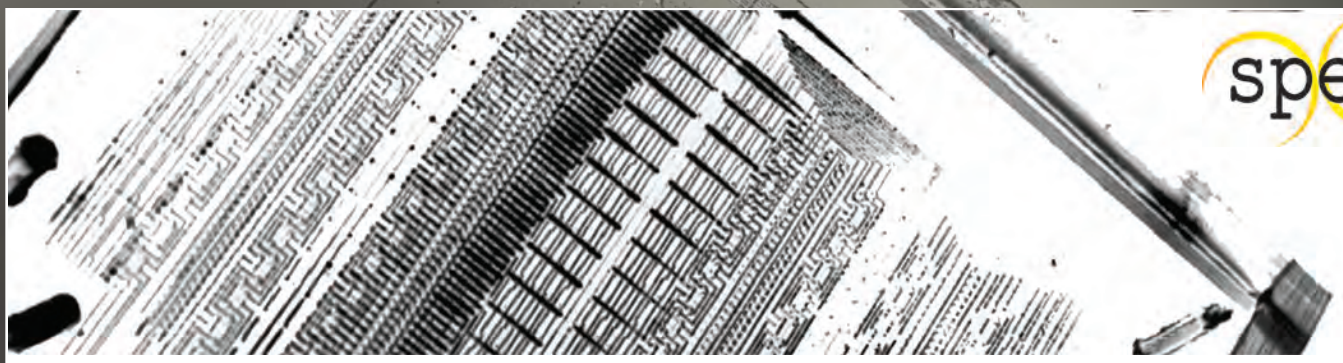

The project is based on blood samples, which are minimally invasive to collect and routinely used in clinical practice with well-established protocols. The integrated optical device within a microfluidics platform would allow a rapid and reproductive detection from small blood samples volumes without labour intensive and potentially error-prone laboratory manipulations. According to the "EMMA" 2007: Emerging Markets for Microfluidic Applications (from Market Research, http://www.marketresearch.com/), microfluidics components for diagnostic are expected to reach a market of a billion euros in 2011 (with a Total Accessible Market of about 5 billion euros), with major contributions due to point-of-care and clinical diagnostics.

Beyond the applications to diagnosis and treatment monitoring, the knowhow generated in SPEDOC is expected to contribute to the development of new treatments (e.g.: by the discovery of new molecular entities). In recent years new technologies (e.g.: monoclonal antibodies) and novel targeted approaches (e.g.: targeting EGF receptor) have provided significant new weapons in cancer therapy. HSP70 levels are high in tumour cells and are enhanced in response to certain anti-cancer drugs. For example, STA4783, a drug in Phase 3 clinical trials and which is the subject of a recent $1 billion deal between GlaxoSmithKline and the biotechnology firm Synta Pharmaceuticals, leads to stimulation of oxidative stress in tumour cells and a concomitant increase in HSP70 levels. HSP70 provides a major escape mechanism for tumours in response to therapies and it has been shown recently that specific targeting of HSP70 can increase tumour cell death. SPEDOC thus also provides a means to evaluate treatment efficacy or resistance. HSP70 antagonists have a very interesting potential for combination with drugs like STA4783, HSP90 inhibitors and others that induce HSP70 expression in tumours. INSERM is developing a candidate cancer treatment targeting HSP70 that fits in this innovative trend. SPEDOC is therefore at the cutting edge of efforts to exploit new scientific information for the discovery and validation of novel therapeutic approaches such as the one targeted by the end user INSERM.

A multidisciplinary Consortium

ICFO (coordinator) brings its expertise in the fields of advanced optical microscopy and spectroscopy on nanostructures, nanofabrication and optical trapping. **INSERM** is the expert in Heat Shock Proteins and will bring all the know how to enable biochemistry, providing serum of model mice and the necessary background to guide cells manipulation with optical tweezers towards pertinent results. Located in the same city as **INSERM**, **UB** will ensure the optimal interface between partners featuring expertises in nanotechnologies and the biologists of **INSERM**. Moreover, **UB** brings its full support to the consortium in the design and nanofabrication plasmonic devices, near-field optical measurements, surface functionalization and biostatistics. **EPFL** provides to the consortium strong expertise in both numerical simulations (Martin's group) and advanced microfluidics (Maerkl's group). Finally, **COSINGO** is specialized in the integration of optical devices. **COSINGO**'s skills and experience in optical engineering will enable the development of a compact Detection Platform. As the industrial partner of the consortium, **COSINGO** plans to exploit the outcome of the project to offer a new generation of sensing devices for biology research laboratories.

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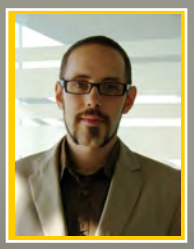

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