Grant Agreement no. 317916

LiPhos

Living Photonics: Monitoring light propagation through cells

INSTRUMENT: Collaborative Project (Small or Medium Scale Focused Research Project)

OBJECTIVE: ICT-2011.3.5

Final Report

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1. Final Publishable Summary Report

Executive Summary

Cardiovascular diseases (CVDs) are the major cause of mortality in western countries and therefore one of the main strategic directions of diagnostic research is aimed towards these disorders. A major challenge in CVD research and care is to determine the mechanisms that provoke chronic inflammation and endothelial dysfunction that promotes atherosclerotic disease, which will allow early CVD diagnosis and treatment.

The LiPhos project aims to combine cell biology, photonics, microfluidics and knowledge provided by cardiovascular researchers to realize a breakthrough diagnostic technology. Fundamental to this new approach is the dual role that cell cultures and tissues can exhibit, namely the ability to confine light whilst at the same time being interrogated by that light to yield unique optical responses that distinguish “healthy” from “non-healthy” status.

From a photonic point of view, a cell culture or tissue can be considered as a biomaterial with optical properties (spectral response, refractive index, etc.) which arise from its constituent elements, i.e. the cells and the surrounding extracellular matrix. The idea behind LiPhos is to exploit these characteristics to develop a new generation of biophotonic diagnosis tools (BDTs). Concretely, the primary scientific objective in this project is the development of biophotonic systems in which cells are used for defining the core of the waveguide, giving rise to the “Living Photonics” concept. That is, light will be coupled and confined within a cell culture so that they can be continuously interrogated. Hence, the cells will form the photonic system and will play the dual role of transducer and reporter element. The BDT will consist of living photonics systems coupled to auxiliary polymer/semiconductor micro-optical/integrated optical elements.

With this goal in mind, measurement protocols have been established for the determination of the Photonic Fingerprint (PIN) of the biological system under study. We define the PIN concept as the spectral response of the living photonics, consisting of the different inherent or acquired bands and peaks (scattering, absorbance and/or fluorescence) present in the spectra. Each cell type, in a given state and under identical experimental conditions, should yield a unique and identifiable PIN (reproducible spectral response). Moreover, healthy and non-healthy living photonics of the same cell type should exhibit different PINs. Comparison of these PINs will provide accurate diagnosis of CVD. Thus, we have successfully built a library of healthy and diseased cell PINs with primary human umbilical vein endothelial cells (HUVECs), porcine vascular smooth muscle cells (VSMCs) and macrophages obtained from diabetic mice, as well as macrophages and arterial segments from an atherosclerosis mouse model (apoE-KO mice). These studies with mouse and primary human cells will provide the basis for investigation of the PIN of human cells and arterial tissue from healthy donors and CVD patients. To our knowledge, this is the first attempt to harness the optical properties of living cells for diagnostics in the way explored by LiPhos.

The final objective of the project is to obtain reliable and specific “living photonics” information that could be used as a tool in basic research and eventually also diagnosis of cardiovascular diseases. The project involves, among other objectives: the design of microfluidic chips made of biocompatible materials for seeding cells, which, upon confluence, will form the core of a photonic system; the development of auxiliary photonic elements to couple light into the living photonics; and the realisation of BDT measurements of 3D biological elements (such as arterial segments or endothelial tubes).

Several BDT configurations have been developed and the most promising have been validated and benchmarked with the various cell types. An integrated proof-of-concept system has also been produced which will form the basis of a future “research instrument” which can be commercialised.

Summary description of project context and objectives

The specific objectives of LiPhos are to:

- Implement a new concept for diagnosis based on confining light in a cell culture to obtain the photonic fingerprint (PIN), providing an unprecedented level of detailed information for the diagnosis of CVD.
- Establish *in vitro* arterial models based on primary cell and tissue culture.
- Integrate Biophotonic Diagnosis Tools (BDTs) and fluidic microsystems in a system-level integration (photonic lab-on-a-chip, PhLoC) including the living photonics, the auxiliary photonic elements, the microfluidic network, the chip-to-world interface and the associated software, thus covering the full value chain.
- Demonstrate BDTs using a living cell culture as the sole constituent of a photonic system.
- Implement the PIN library in which healthy and non-healthy cell cultures (or tissues) are screened, considering their main differences in terms of variations of the overall optical profile, rather than in specific peaks.
- Perform pre-clinical validation of the PhLoC for diagnosing endothelial dysfunction, smooth muscle cell activation, and alterations in macrophages under different pathological conditions.
- Implement innovation management to ensure exploitation of the developed technology
- Promote dissemination of the project results within the international scientific community and eventually, within a wider medical community.
- Integrate multidisciplinary education, training and skills development with research activities.

More detailed objectives for each Work Package are described below.

WP1: Targets and specifications

The objectives of WP1 are to:

- Determine the target analytes, the cell type and the biomarkers to be used
- Determine the requirements of the BDTs: microfluidic elements, materials, active and passive components, integration of transducers, storage
- Determine protocols for laboratory evaluation of the BDTs
- Build and maintain the PIN library

WP2: Artificial Arterial Models

The goal of this WP is to establish *in vitro* arterial models based on primary cell and tissue culture which are at once a relevant representation of the *in vivo* situation and compatible with the new living photonics paradigm. The objectives of this work package are thus:

- Controlled culture of arterial vessel segments
- Controlled 2-D and 3-D vascular endothelial cell cultures in microfluidic devices (monolayers on microchannel walls)
- Controlled self-assembled 3-D endothelial/VSMC culture approach under simulated microgravity
- Comparison of *in vitro* and *in vivo* endothelial, smooth muscle cell, and macrophage phenotypes
- Benchmark assays for cell dysfunction or drug response for comparison with living photonics diagnosis tools

WP3: Development of living integrated photonics

The goal of this WP is the development, validation and optimization of BDTs in which the waveguide core is defined uniquely by cells. The main objectives are as follows:

- Understanding light propagation and confinement in the living photonics concept, where the waveguide core is formed by cells
- Develop several BDTs according to the partner requirements and taking into consideration the available equipment and the cells to be used
- Select the more developed/promising BDT configurations, in the three different architectures (SLLP, MLLP and SCA) which could provide the PIN of the cell cultures under study
- Obtain a preliminary characterization of such BDTs, prior to being transferred to WP5 for benchmarking
- Implement a living database accessible to the consortium for easy reading of the BDT performance and partners involved
- Determination of the PIN as key parameter for CVD diagnosis
- Further develop the selected BDT configurations, enhancing their performance or user friendliness, so as to obtain robust and reliable platforms suitable to be integrated in a readout system

WP4: Microfluidics and System-Level Integration

The goal of WP4 is to investigate the required materials so as to obtain the cladding material with the required optical and biocompatibility properties. Additional goals are to develop and validate the microfluidic system and the system-level integration of the three different BDT configurations. The main objectives are:

- Selection and development of materials suitable to be used as cladding layer, including measurement of the optical properties, patterning and biocompatibility
- Geometries for the system-level integration of three different BDT configurations
- Development of the upstream elements
- Experimental validation of the various BDT configurations developed in WP3
- Transfer of these BDT configurations to WP5 for benchmarking
- Obtaining PINs from each of the BDT configurations and transferring the obtained PINs to the PIN library in WP1
- System level integration of the most suitable BDT(s) into the respective opto-fluidic network configurations

WP5: Benchmarking and pre-clinical validation

The goal of WP5 is the benchmarking and pre-clinical validation of the PINs and results obtained in living normal and diseased cells in the three BDT configurations. The main objectives are:

- Obtain the necessary ethical permissions for use of tissue samples from animals and patients
- Develop strategies towards benchmarking and pre-clinical validation of the different photonic BDTs
- Apply PINs to real-time measurement of endothelial, macrophage and smooth muscle cells
- Living photonics concept used for real-time measurement of endothelium-derived factors
- Measurement of light propagated through confluent HUVEC monolayers on TF-SLLP (“microcuvette”)

- Comparison of photonic measurements in ECs, VSMCs, and macrophages with well-known phenotypic parameters associated with CVD analysed by flow cytometry, histology, and immunofluorescence (expression of adhesion molecules, chemokine receptors, etc.)
- H-SLLP and TF-SLLP BDTs benchmarked and characterized using gene expression analysis of important biomarkers for inflammation
- Benchmarking of PINs obtained with FO-MLLP and arterial segments by haematology, histology, intra-vital microscopy, flow cytometry, etc.
- Pre-clinical validation of the FO-MLLP in arterial segments from WT and apoE-KO mice
- Comparison of endothelial progenitor cells from patients with CVD, control and breast cancer for photonics in monolayers (using H-SLLP) and single cell analysis
- Pre-clinical validation showing the use of the BDTs for diagnostics in animal and patient tissue

WP6: Exploitation and Dissemination

The objectives of this WP are to disseminate the results of LiPhos, establish a project website, identify and manage the intellectual property developed, and ensure that suitable exploitation plans are drawn up. Interest groups include: the scientific community, industry (medical, diagnostic and optical equipment suppliers), and regulatory authorities.

WP7: Project Management & Coordination

The objective of this WP was the overall management of the project, including:

- Establishment of management committees and guidelines for their operation
- Establishment of technical and financial reporting guidelines
- Establishment of structures for execution of committee/co-ordination group tasks
- Provision of financial and technical monitoring and reporting
- Administration of Consortium Agreement
- Delivery of all necessary reports including periodic and final project reports

Description of the main S&T results/foregrounds

WP1: Targets and specifications

The starting point for the research project was a critical analysis of the user requirements and the technical and economic constraints (including pre-clinical validation) that the BDTs should satisfy when applied to the diagnosis of cardiovascular diseases. According to the DoW, there are three arrangements of living integrated photonics (referred to as “configurations”). These are: single layer of cells (Single Layer Living Photonics, SLLP), multiple layers of cells (Multiple Layer Living Photonics, MLLP) and single cells (Single Cell Analysis, SCA). However, within each “configuration”, different strategies may be applied. For example, within the SLLP configuration, biochips have been designed which will allow interaction of the cells with the evanescent field in one arrangement or with the full field in another. Similarly, in the MLLP configuration we have BDTs where multiple layers of cells are grown on a flat substrate, as well as systems for measuring either free-standing arterial segments or endothelial tubes.

In the first period, we summarised the specifications of various BDTs according to the three configurations for PIN detection. We have also evaluated how the three configurations can be applied, along with their advantages and disadvantages. Once identified, the key specifications and issues were transmitted to all the partners to establish the basic guidelines of the project.

There has been an intensive exchange of protocols and ideas between the biology and physics partners of LiPhos based on the experience of cell seeding on different chip prototypes. As a

result, some appropriate chip materials have been investigated regarding their optical and biocompatible properties while the chip designs have been refined to be suitable for the living photonics concept. In addition, steps toward an integrated microfluidic system have been taken and their validity has been experimentally demonstrated.

Cellular, chemical and physical parameters to be monitored for SCA have been defined. The photonic requirements for the SCA biochip have also been identified. System integration between the photonic and microfluidic systems for SCA has been designed and implemented.

In the second period, WP1 activities were primarily concentrated on completing the protocols for laboratory evaluation and the work on building the PIN Library. The optical setup to obtain PINs has been extended to include the UV and NIR ranges. This allows important additional information in the PINs and differences between them to be resolved.

Following discussion with all partners, we have defined key parameters to be included in each entry of the PIN library. The library has been implemented as a Microsoft Access database and populated with entries of experiments executed in WP2, WP3 and WP5. Various PINs have been measured for different types of cells/tissue and typical PIN profiles have been obtained for both healthy and diseased cells, including for differential levels of induced inflammation. These have all been added to the PIN library. Protocols have also been defined for the preparation of the various cells/tissue and their measurement within the different BDT configurations.

WP2: Artificial Arterial Models

In the first half of the project, different vascular biological models for biophotonic investigation were established. Protocols were defined for controlled endothelial cell, vascular smooth muscle cell, or macrophage culture in either microfluidic systems engineered to resemble vasculature or under conditions of microgravity. In the latter case, endothelial and vascular smooth muscle cells are co-cultured for a period of weeks to form vascular tubules. Arterial segments from mice have also been studied using one of the BDTs. Efforts to perform cell and arterial segment culture under different conditions to mimic both healthy and different disease states were then undertaken. These vascular cell cultures served as reference samples for recorded PIN spectra in the PIN library. Diagnosis of cardiovascular disease will involve comparing PIN spectra of vascular cell or tissue cultures of unknown disease state to these reference spectra. It was thus important that the protocols established in WP2 were robust and reproducible. A microscope-compatible incubation system was also finished in the first half of the project.

Work in the second half of the project was focused more on the comparison of *in vitro* endothelial cell phenotypes in microchannels versus well-plate cultures, with an eye to comparison with the *in vivo* phenotype as well. The RUG tested a magnetic bead-based protocol for isolation of endothelial cells from the kidneys and lungs of mice, followed by the seeding of these isolated cells into microchannels of 60 µm and 100 µm widths. Murine lung endothelial cells could be successfully cultivated in these microchannels. It was also possible to seed murine arterial endothelial cells (mAECs) into Vena 8 Endothelial+ chips from Cellix and image these cells after they had adhered to the surface using immunofluorescence (CNIC). Results were reported for the microgravity-assisted cultivation of endothelial tubes at AU, with researchers being able to show the formation of tubes from EA.hy926 cells after one week in microgravity.

A decision on the assays for cell responsiveness to be pursued using the living photonics systems was made at Month 24. Measurements were made using several BDTs of *in vitro* cellular responsiveness and (dys)-function, using cell or arterial cultures / preparations under normal or imposed dysfunctional conditions. State-of-the-art analytical methodologies were used as reference. The information fed into further tests of living photonics platforms.

Cellix had previously developed the Vena8 chip and a series of different photonic platforms have been developed during the project. In addition, a series of different cell types including mainly endothelial cells, but also smooth muscle cells and macrophages as well as cell lines have been seeded and tested in these platforms mainly in the absence, but also in the presence of flow. The state-of-the-art analytical methodologies which were developed as reference methods for testing the cell cultures include immunohistochemistry, quantitative PCR, and intracellular calcium measurements.

WP3: Development of living integrated photonics

LiPhos has developed a large family of Biophotonic Diagnostic Tools (BDTs) which are suitable for interrogating cell cultures growing in the three most common situations, namely single layer (for which was developed the single-layer living photonics, SLLP), multiple-layer (Multiple-layer living photonics, MLLP) and single cell (single cell analysis, SCA).

Initially, the main objective of this WP was to understand and determine the optical conditions which resulted in light confinement in a confluent cell culture. This study not only involved the refractive indices of the materials involved, but also the surface roughness and the voids in the confluent layer.

Once it had been confirmed that light could be coupled in a confluent cell layer, the three previously mentioned BDT architectures were developed in parallel. This was done in order to address the different growing conditions of the various cells being examined (e.g. endothelial cells grow in confluent monolayers whereas smooth muscle cells can define a multilayer). Single cell analysis was also developed so as to provide extremely valuable and complementary information about the changes of the cells after drug inoculation, or the study of highly specific type of cells, such as the endothelial progenitor cells.

Prior to being transferred to WP5 for benchmarking, the different BDTs underwent preliminary characterization to confirm the working performance.

From the twelve BDTs developed in the project, six (two per BDT) were selected for further development and transfer to WP5 for benchmarking. This rigorous but necessary action was already planned in the DoW to focus all the efforts in those BDTs which showed the greatest potential. In this context, the H-SLLP based on glass technology and TF-SLLP, using microcuvettes and LioniX TriPlex waveguide were selected for single layer. The M-SLLP based on glass technology and FO-MLLP using 3D positioners and bare fibres were selected for the multilayer. Finally, the V-SCA-EC, and V-SCA-BC were selected for analysing, at the single cell level, endothelial and blood cells. These BDTs where developed until the end of the project, optimizing the geometry, fabrication and handling procedures so as to enhance their performance.

As a major outcome of this WP, it can be stated that for the first time it has been demonstrated that light can be confined in a cell culture. Similarly, the strategy used in the LiPhos project has been successful, allowing a reasonable number of Photonic Fingerprints (PINs) of different cell cultures (or cell profiles, in the case of SCA) to be obtained. The technology developed in LiPhos in no way can be considered to be at the final point, but rather the beginning of a new and exciting research line, further exploiting the diagnosis capability of the living photonics concept.

WP4: Microfluidics and System-Level Integration

All materials selected for the final BDT configurations (H-SLLP, TF-SLLP, TE-SLLP, FO-MLLP and V-SCA-EC) have been experimentally validated and meet the pre-defined requirements of biocompatibility, absence of leaks, long-term stability and optical transparency. Microfluidic networks for each BDT configuration have been experimentally validated and the results have been transferred for system-level integration and benchmarking.

After discussions within the consortium, system-level integration efforts focussed on the TE-SLLP and TF-SLLP configurations. The incubation system developed in WP2 by Cellix has been validated by assessing the reproducibility of endothelial cell culture, compatibility of silicon nitride and silicon oxide materials for protein coating and subsequent cell growth. The auto-alignment setup for TE-SLLP and TF-SLLP has been developed to the point of a proof of concept with a manual setup. The Kima pump cell culture system developed in WP2 by Cellix has been designed to work inside a CO_2 incubator and the same system has also been validated working outside the incubator with external temperature and CO_2 control. Integration of a stand-alone phase contrast microscope bench with optical BDT configuration and pumping system has also been undertaken. New software which controls all system modules has been designed, along with a prototype of the instrument casing.

A “chip-to-world” interface has been designed, fabricated and tested to be used in combination with the H-SLLP BDT configuration. Since it matches with the footprint specified in WP1, it is fully compatible with the hardware developed by Cellix, and closes the gap between the BDTs and the system-level integration as far as these two BDTs are concerned.

WP5: Benchmarking and pre-clinical validation

Ethical permissions were obtained for the work on cells and arteries from animals and patients. Strategies were developed for validation and benchmarking of biophotonic platforms with other approaches. It was recognized that different approaches are required depending on BDT platform used. A robust amount of data was obtained with HUVECs cultured in the TF-SLLP BDT. The most significant findings were that the spectroscopic signal observed was due to forward light scattering, and that fluctuations in this signal were a direct consequence of cellular micromotion. Moreover, drugs known to interact with the cytoskeleton and thereby inhibit micromotion caused changes in recorded signal fluctuation and intensity. These changes were corroborated by actin staining experiments of HUVECs, which showed shifts in the ratios of different actin forms and associated cellular changes.

Porcine coronary vascular smooth muscle cells were seeded in the HF-SLLP BDT and consistent absorbance changes were observed in the absence and presence of tumour necrosis factor alpha (TNF-α). Also a calcium ionophore, known to induce pronounced changes in VSMC intracellular calcium induced characteristic changes in absorbance. Macrophages were cultured on the HF-SLLP BDT and revealed also specific absorbance changes associated with changes in cellular phenotype when cultured in the presence of IL-4, a known anti-inflammatory stimulus.

Segments of large arteries from control and an atherosclerotic mouse model (apoE-KO) were mounted in the FO-MLLP BDT and exhibited characteristic changes in the absorbance which was validated by staining and quantification of lipid-rich lesions, the presence of inflammation markers, and changes in blood circulating leukocytes.

A large series of experiments was performed in the V-SCA-EC BDT revealing characteristic changes in an endothelial cell line, EA.hy926 and in HUVECs exposed to TNF-α, and these changes were benchmarked with the changes detected by use of flow cytometry.

After testing a series of plasma values and endothelium-dependent vasodilatation in patients with CVD, control and breast cancer, endothelial progenitor cells were isolated for later seeding in the H-SLLP and initial measurements have been obtained in the V-SCA-EC. In a similar manner, macrophages have been examined in the V-SCA-EC. Thus, the single cell analysis in the V-SCA-EC BDT by itself provided valuable information regarding morphology and changes in auto-fluorescence bands that have a large potential in the diagnosis of CVD. Furthermore, the platform can also be used for benchmarking with, for instance, the H-SLLP platform.

In summary there are two major lines which have been developed to a stage allowing benchmarking: (1) Several BDT platforms can, in future, be used as an approach to evaluate pathophysiological changes in single cells, cell cultures, and isolated vascular preparations; (2) Several BDTs have achieved a level of performance which allows differentiation between healthy samples and samples characterized by changes exhibited in the development of CVD as well as to evaluate drug-induced changes. The use of the described systems for CVD diagnostics will require further development and studies involving larger numbers of patient samples.

Potential impact and the main dissemination activities and exploitation of results

Potential Impact

The development of photonic technologies with a proven industrial potential and high functionality, performance, component size and cost reduction may be considered one of the main challenges to be solved in the future. However, most of the efforts are currently focused on the improvement of already existing technologies: development of new materials, structures or components to improve technology performance. LiPhos provides a breakthrough photonic technology based on the use of high density cell cultures to confine and guide the light, thus acting as an active, living waveguide. Hence, LiPhos is NOT a merger of existing technologies for a high impact application. Rather, it presents an innovative approach in photonics for life sciences, in which the different roles of the biosensor (transducer, reporter elements) are combined in a photonic system. The BDT consists of a living photonic system together with auxiliary integrated microfluidics and micro-optical structures.

The main impacts of the project are grouped in four main areas:

- **High performance and functionality:** the cell-based photonic systems developed provide a revolutionary and game-changing tool for acquiring real time key information, which could be applied for investigating biochemical and metabolic processes related to physiological and pathological conditions or the effect of drugs. Although, this approach has been specifically targeted within the project towards cardiovascular diseases (CVD), this technology is expected to find application across a broad spectrum of areas within both the life science and photonics areas.
- **Novelty and potentiality:** even on the global scale, no other research groups or companies have already started developing this new generation of photonic systems and thus, the development of this approach is currently world-leading. The huge impact of this project has led to the European Photonic Industry Consortium (EPIC) showing great interest in LiPhos and supporting the work. EPIC has already contacted the LiPhos Coordinator with the aim of building a workshop around this concept as a potential dissemination/exploitation action. Hence, the cell-based photonic lab-on-a-chip systems developed in LiPhos will give Europe a pre-eminent position in the global market.
- **Multidisciplinarity, component size and cost reduction:** the synergistic combination of key research fields (photonics, microfluidics and cell biology) in a highly multidisciplinary approach has provided for the very first time an extremely compact, photonic lab-on-a-chip system suitable to perform real time multi-parametric screening of cell cultures and hence obtaining their photonic fingerprint (PIN).
- **Socio-economic impact:** CVDs are the main cause of death within the EU, being responsible for around 48% of the deaths every year and with overall estimated costs to the economy of €192 billion a year. [European Cardiovascular Disease Statistics, 2008 edition] This represents a total annual cost per capita of €391. Hence LiPhos can make a major economic and societal impact, putting Europe at the forefront of this new

technology. Considering that both the pharmaceutical and medical market is progressively tending towards biophotonics, the social impact of LiPhos is expected to be even higher in the medium term. Next to this, it will have its impact in the new paradigm of Personalized Medicine as a promising concept for Companion Diagnostics (CDx) that should complement the development of targeted drugs.

Dissemination

Within the first 6 months of the project, a project website (www.liphos.eu) was established and this has been maintained throughout the course of the project. The website contains the following public pages:

- A home page describing the aims of the project and hosting project vision, execution and technology development videos
- A partner page with specific links to their relevant websites
- A deliverables page containing any publicly available deliverables and reports
- A presentations page, where oral and poster contributions to conferences are detailed, together with copies of any presentations
- A publications page, where journal publications are listed
- An events page, detailing events where LiPhos has been disseminated

The dissemination activities in LiPhos achieved their goals in various ways; by collecting, collating and managing the information developed during the project, through the creation of a project flyer, and via a project Twitter account. More specifically, these goals have been achieved through the organisation of a LiPhos Workshop in conjunction with the Scandinavian Physiological Society annual meeting taking place from 18th – 20th September 2015. Furthermore, a dedicated booth was organised at the huge (30,000 visitors) Laser World of Photonics trade fair in Munich at the end of June 2015. In addition, various publications have been produced, as described in section 2, and several more are currently in preparation.

Exploitation

For the fruitful consideration of the exploitation aspects of LiPhos, it is important to understand the functionality and opportunities that are enabled by the project. Most importantly, the LiPhos project is aimed at the development of biophotonic systems in which cells are, for the very first time, used for defining the core of the waveguide, giving rise to the “Living Photonics” concept and the development of an innovative biophotonic diagnosis tool (BDT) suitable for cardiovascular disease (CVD) diagnosis.

The basic principle of LiPhos is to utilise a living cell culture as a waveguide (either as individual cells, monolayers of cells or multi-layered growth) and the recognition of their spectral response for diagnosing a CVD pathology. Here, LiPhos is an example of a significant development in biophotonics with broad potential for future applications.

As a consequence it was of particular importance to the project to undertake a coordinated effort to understand the marketplace, the barriers to entry and the competitive position, with the ultimate aim of producing a confidential LiPhos Final Exploitation Plan and Technology Roadmap. IP protection has also been performed for certain aspects of various BDTs.

The Final Exploitation Plan was prepared which contained an overview of the potential for LiPhos as a research instrument, along with details on the category drivers and competitors. Some of the main issues that need to be overcome were discussed and potential business models presented. Final commercialization recommendations were included and these, along with details of exploitation plans, were presented to the European Commission at the final Technical Review.

Address of the project public website

The address of the project website is www.liphos.eu. The website will remain active for at least 2 years after the end of the project.

2. Use and Dissemination of Foreground

Section A (public)

The table below lists all of the scientific (peer reviewed) publications relating to the foreground of the project, starting with the most important ones.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[1] (if available)	Is/Will open access[2] provided to this publication?
1	Optical detection strategies for centrifugal microfluidic platforms	King, D.	Journal of Modern Optics				2014		http://dx.doi.org/10.1080/09500340.2013.873496	No
2	Optical detection on centrifugal microfluidic lab-on-a-disc platforms	King, D.	Encyclopædia of Microfluidics & Nanofluidics				2014		ISBN: 978-3-642-27758-0, http://dx.doi.org/10.1007/978-3-642-27758-0_1785-1	No
3	Photonic profiling towards monitoring endothelial cell dysfunction at single cell level	King, D.	Proc. 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2014)				2014		http://www.rsc.org/Publishing/Journals/LC/news/uTAS_Abstracts_2003_to_Present.asp	Yes
4	Monitoring endothelial cell dysfunction at single cell level utilising "Living Photonics" (LiPhos) concepts	King, D.	Proc. 4th European Conference on Microfluidics				2014			No
5	Modular optofluidic systems	Ackermann, T	Proc. 18th International Conference on				2014		http://www.rsc.org/Publishing/Journals/LC/news/uTAS_Abstracts_2003_to_Present.asp	Yes

[1] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[2] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

			Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2014)							
6	Effect of Channel Width on Human Umbilical Vein Endothelial Cell (HUVEC) Culture in Microfluidic Channels	Grajewski, M.	Proc. 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2014)				2014		http://www.rsc.org/Publishing/Journals/LC/news/uTAS_Abstracts_2003_to_Present.asp	Yes
7	Nanoscale Photonic/ Fluidic Device For Monitoring Cellular Micromotion Using Forward Light Scattering Through a Cell Monolayer	Grajewski, M.	Proc. 19th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2015)				2015		Proceedings will appear on RSC site (mentioned above) in the spring of 2016	Yes
8	Label-free, single-cell optical multi-parameter monitoring of apolipoprotein E-null differentiated macrophages on a centrifugal microfluidic platform	King, D.	Proc. 19th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2015)				2015		Proceedings will appear on RSC site (mentioned above) in the spring of 2016	Yes
9	Systems biology in single cells	King, D.	Essentials of single-cell analysis: Concepts, applications and future prospects, Series in BioEngineering.		Springer, Berlin, Heidelberg, New York, 2015		Accepted for publication 2015		ISBN: 978-3-662-49116-4	No

The table below lists all of the other dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

No.	Type of activities[3]	Main leader	Title	Date/Period	Place	Type of audience[4]	Size of audience	Countries addressed
1	Web	iXscient	Project website (www.liphos.eu)	23/2/13 to date	-	General public	-	Worldwide
2	Flyer	iXscient	LiPhos project flyer		-	General public	-	Worldwide
3	EC Concertation	CSIC	Biophotonics Concertation meeting	3/12/12	Brussels, Belgium	FP Project Coordinators	-	Europe
4	Poster presentation at µTAS 2014 conference	CSIC	Modular Optofluidic Systems	26-30/10/14	San Antonio, Texas, USA	Scientific community	1500	Worldwide
5	Poster presentation at µTAS 2014 conference	DCU	Photonic Profiling Towards Endothelial Cell Dysfunction at Single Cell Level	26-30/10/14	San Antonio, Texas, USA	Scientific community	1500	Worldwide
6	Poster presentation at µTAS 2014 conference	RUG	Effect of Channel Width on Human Umbilical Vein Endothelial Cell (HUVEC) Culture in Microfluidic Channels	26-30/10/14	San Antonio, Texas, USA	Scientific community	1500	Worldwide
7	Oral presentation at 4th European Conference on Microfluidics (µFlu '14)	DCU	Monitoring Endothelial Cell Dysfunction at Single Cell Level Utilising Living Photonics (LiPhos) Concepts	10-12/12/14	Limerick, Ireland	Scientific community	500	Worldwide
8	Oral presentation at SPIE Microtechnologies conference	CSIC	Living photonics: monitoring light propagation through cells (LiPhos)	4-6/5/15	Barcelona, Spain	Scientific community	50	Worldwide
9	Oral presentation at SPIE Microtechnologies conference	DCU	Single cell analysis biophotonic diagnosis tools	4-6/5/15	Barcelona, Spain	Scientific community	50	Worldwide

[3] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.
[4] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

10	Poster presentation at SPIE Microtechnologies conference	CNIC	Fiber optics, label-free biophotonic diagnostic tool for cardiovascular disease	4-6/5/15	Barcelona, Spain	Scientific community	50	Worldwide
11	Project booth at World of Photonics Trade Exhibition	LioniX	Project overview	22-25/6/15	Munich, Germany	Scientific community, industry	30,000	Worldwide
12	Workshop	All	LiPhos Workshop	18/9/15	Aarhus, Denmark	Scientific community	35	Worldwide
13	Oral presentation at LiPhos Workshop – The Year of Light, SPS 2015 conference	RUG	Microengineering Advanced *In Vitro* Technologies for Cell and Tissue Studies	18/9/15	Aarhus, Denmark	Scientific community	35	Worldwide
14	Oral Presentation at LiPhos Workshop – The Year of Light, SPS 2015 conference	DCU	Living Photonics (LiPhos) Single Cell Analysis – Label Free Detection Methods	18/9/15	Aarhus, Denmark	Scientific community	35	Worldwide
15	Poster Presentation at LiPhos Workshop – The Year of Light, SPS 2015 conference	DCU	Label-free, single-cell level optical monitoring of the apoE-KO mouse model of atherosclerosis towards an early-stage diagnostic for CVD	18/9/15	Aarhus, Denmark	Scientific community	400	Worldwide
16	Poster Presentation at LiPhos Workshop – The Year of Light, SPS 2015 conference	DCU	Label-Free Optical Multi-Parameter Monitoring of Endothelial Cell Dysfunction at Single Cell Level	18/9/15	Aarhus, Denmark	Scientific community	400	Worldwide
17	Poster presentation at 19th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2015)	DCU	Label Free, Single Cell Multi-Parameter Monitoring of Apolipoprotein E-Null Differentiated Macrophages on a Centrifugal Microfluidic Platform	25-29/10/15	Gyeongju, South Korea	Scientific community	1500	Worldwide
18	Poster presentation at 19th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS 2015)	RUG	Nanoscale Photonic/ Fluidic Device For Monitoring Cellular Micromotion Using Forward Light Scattering Through a Cell Monolayer	25-29/10/15	Gyeongju, South Korea	Scientific community	1500	Worldwide

Section B (Confidential)

This section is confidential.

3. Report on Social Implications

A	**General Information** *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	317916
Title of Project:	Living Photonics: Monitoring light propagation through cells
Name and Title of Coordinator:	Dr Andreu Llobera, Consejo Superior de Investigaciones Científicas

B	**Ethics**

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***YES*** ***YES***
2. Please indicate whether your project involved any of the following issues (tick box) :	
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	✓
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	✓
• Did the project involve Human data collection?	
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	✓
• Were those animals transgenic small laboratory animals?	
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education tc..)?	
DUAL USE	
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	2
Work package leaders	1	6
Experienced researchers (i.e. PhD holders)	5	21
PhD Students	2	3
Other	8	15

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	5
Of which, indicate the number of men:	4

D Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project**?	○ ●	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective → Very effective
❑	Design and implement an equal opportunity policy	○ ○ ○ ○ ●
❑	Set targets to achieve a gender balance in the workforce	○ ○ ○ ○ ○
❑	Organise conferences and workshops on gender	○ ○ ○ ○ ○
❑	Actions to improve work-life balance	○ ○ ○ ○ ○
○	Other:	

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

● No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

● No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

● No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

Main discipline[5]: 1.2

Associated discipline[5]: 1.5 | Associated discipline[5]: 2.3

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	○ ●	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[5] Insert number from list below (Frascati Manual).

11c	**In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?**	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture Audiovisual and Media Budget Competition Consumers Culture Customs Development Economic and Monetary Affairs Education, Training, Youth Employment and Social Affairs		Energy Enlargement Enterprise Environment External Relations External Trade Fisheries and Maritime Affairs Food Safety Foreign and Security Policy Fraud Humanitarian aid		Human rights Information Society Institutional affairs Internal Market Justice, freedom and security Public Health Regional Policy Research and Innovation Space Taxation Transport	

13c If Yes, at which level? ○ Local / regional levels ○ National level ○ European level ○ International level			
H Use and dissemination			
14. How many Articles were published/accepted for publication in peer-reviewed journals?			**9**
To how many of these is open access[6] provided?			
How many of these are published in open access journals?			**5**
How many of these are published in open repositories?			**0**
To how many of these is open access not provided?			**4**
Please check all applicable reasons for not providing open access:			
✓ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ✓ no suitable open access journal available ✓ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[7]: ……………			
15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*			**2 (plus 1 in progress)**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark		**0**
	Registered design		**0**
	Other		**0**
17. How many spin-off companies were created / are planned as a direct result of the project?			**Too early to say**
Indicate the approximate number of additional jobs in these companies:			
18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project: ✓ Increase in employment, or ✓ Safeguard employment, or ❑ Decrease in employment, ❑ Difficult to estimate / not possible to quantify	✓ In small & medium-sized enterprises ❑ In large companies ❑ None of the above / not relevant to the project		
19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs: Difficult to estimate / not possible to quantify			*Indicate figure:* **31**

[6] Open Access is defined as free of charge access for anyone via Internet.

I	Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ● No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes ● No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

✓	Press Release	✓	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	✓	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
✓	Brochures /posters / flyers	✓	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

✓	Language of the coordinator		English, Spanish
❑	Other language(s)		

Question F-10: Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

[7] For instance: classification for security project.

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)
3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)
3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES
4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)
4.2 Veterinary medicine

5. SOCIAL SCIENCES
5.1 Psychology
5.2 Economics
5.3 Educational sciences (education and training and other allied subjects)
5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES
6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)
6.2 Languages and literature (ancient and modern)
6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]