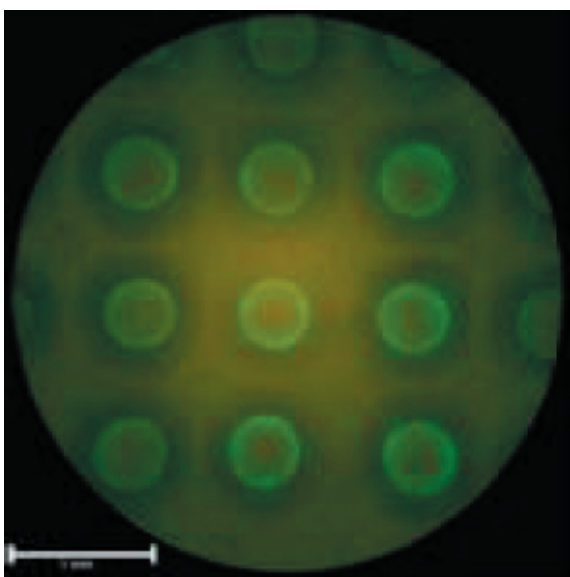


The Second year of the Toxichip project has been successful with all targets for the year being met. Prototypes of both the prokaryote and eukaryote biochips have been fabricated and analysis of these biochips has continued and will continue for the duration of the project. Bacterial reporter strains have been engineered to respond in a dose-dependant manner to several toxic chemicals. Hardware and software components have also been developed and work is continuing on improving these.

### Year Two in Summary:

**Work Package (WP) 1 (Project Management)** is progressing according to plan. Terri Wood has resumed her role as project co-ordinator. Project meetings and their respective minutes (which are available on the Toxichip website) have been well organized by partners and have as always been very constructive. All quarterly periodic activity reports have been submitted on time to the EU Project Officer. The deliverables for WP1 have all been achieved to date and no problems have been encountered.

**WP 2 (Surface Modification & Functionalisation for Cell Adhesion)** is progressing well. The work performed this year focused mainly on the optimization of the bacteria patterning method by using surface functionalisation and on the development of a piezoelectric microspotting technique to spot bacteria on the prokaryote sensor surfaces. Functionalized surfaces with APTS and poly-lysine micro-areas in polyethylene glycol matrix (anti fouling) have been successfully used producing well defined spots of bacteria with a good stability in time. The immobilization of bacteria in PDMS microwells has been successfully investigated. Experiment results with fluorescently labeled bacteria (SM110, green fluorescence) shows that the bacteria are trapped into the PDMS wells and that no bacteria are found in other regions.



A piezoelectric micro-spotting technique based on the sciFlexarrayer platform (Scenion GmbH) has been developed. New hard- and software components of the printer have been developed, the print nozzles redesigned and methods of surface coating developed to meet the specific requirements of cell spotting. Printing parameters such as the viscosity, the surface tension of the media to be printed, the wettability of the nozzle tip, and the nozzle parameters (the piezoelectric voltage) have

been optimized for better performance of the printer regarding the precision and reproducibility of cell micropatterning. Results show that this printing technology is suitable to allow an accurate sample loading in pre-structured supports as well as to generate micropatterns of spots of cells on smooth supports of different materials and surface functionalities.

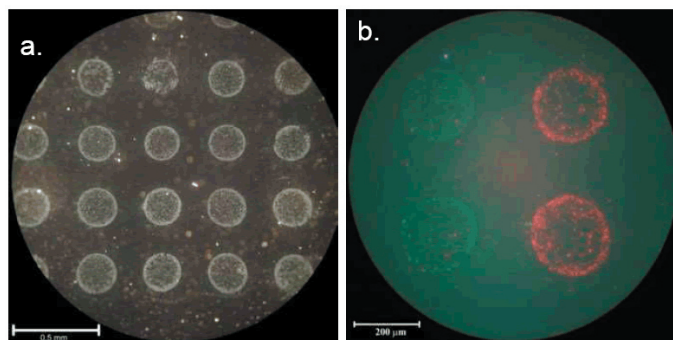
**WP 3 (Genetic Engineering of Prokaryote Cells)** is progressing well. A DNA chip array screening was performed and a list of candidate promoters has been chosen.

These selected promoters were cloned upstream to the luxCDABE operon in a new and improved plasmid designed specifically for this project. After extensive genetic and physiologic optimization, a preliminary panel of bacteria strains that exhibit a dose-dependant luminescence response to chemicals in the Toxichip model list has been prepared (Table). The panel includes 16 bacterial reporters which consist of 14 different promoters expressed in 6 E. coli strains. Improvements in the performance of these reporter strains continue.

Strain name	Host strain	Plasmid	Toxicant
TC1	MG1655	pBR2TTS: <i>flu::luxCDABE</i>	Cadmium
TC2	RFM433	pBR2TTS: <i>znu::luxCDABE</i>	Chloride
TC3	RFM433	pBR2TTS: <i>zntA::luxCDABE</i>	
TC4	MG1655	pBR2TTS: <i>arsR::luxCDABE</i>	Sodium
TC5	RFM433	pBR2TTS: <i>marA::luxCDABE</i>	Arsenite
TC6	RFM433	pBR2TTS: <i>yjtS::luxCDABE</i>	Parathion
TC7	DH5 $\alpha$	pBR2TTS: <i>micF::luxCDABE</i>	Paraquat
TC8	RFM433	pBR2TTS: <i>gltT::luxCDABE</i>	Botulinum
TC9	RFM433	pBR2TTS: <i>ykgG::luxCDABE</i>	toxin A
TC10	DE112	pBR2TTS: <i>fnbA::luxCDABE</i>	
TC11	DE112	pBR2TTS: <i>grpE::luxCDABE</i>	
TC12	RFM433	pBR2TTS: <i>katG::luxCDABE</i>	
TC13	RFM433	pBR2TTS: <i>sulA::luxCDABE</i>	
TC14	AG1688	pBR2TTS: <i>sulA::luxCDABE</i>	
TC15	UTL2	pBR2TTS: <i>recA::luxCDABE</i>	
TC16	RFM433	pBR2TTS: <i>recA::luxCDABE</i>	

Above: ToxiChip panel of bacterial reporter strains

Immobilization through adhesion to different surfaces and viability experiences of the bacteria in small volumes (0.5-2.5 nl) has been performed using Scienion's sciFLEXARRAYER piezodispensing system. In addition, a representative panel of reporter strains have been immobilized in a polymeric matrix in small volumes (agar, 1  $\mu$ l) and a response to a set of toxicants was observed. This panel was exposed to several ToxiChip's model toxicants in order to characterize the unique response patterns ("biological signatures") of each chemical which will serve as a basis for the development of the recognition algorithm.

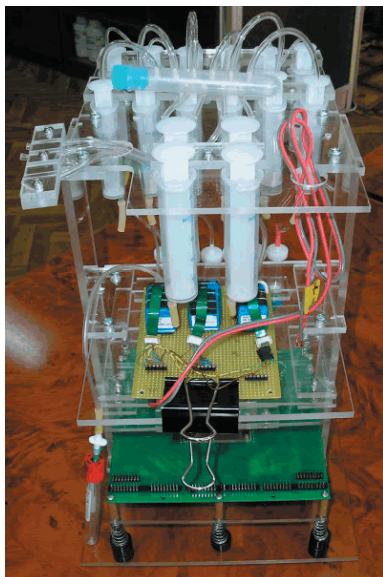


Above: (a) Attachment of 0.5 nl (top) to 2.5 nl (bottom) spotted PHL628 strain to APTS coated glass. (b) Magnification of four spots

**WP 4 (Development of Sensors Platforms)** in general has progressed well over the past year. Both Eukaryote and Prokaryote platforms are ready for testing and validation studies and it is envisaged that in the coming year plenty of data will be

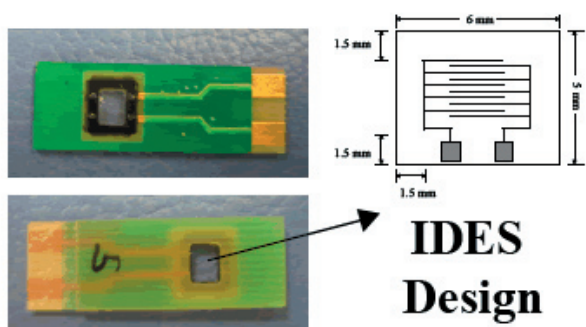
generated on the overall performance of both systems.

Progress on the development of the sensor platform for the eukaryotic system, including the chemical sensors development and fabrication and fluidic system development, fabrication, heating and testing has been achieved by IMT. The development of the fluidic system by integrating the temperature control sensor and pH sensor into the channel and the development of the data acquisition system for the testing phase have been performed. Integration of sensors with the channels and the electronic system allow the cells temperature, pH and respiratory process to be monitored. It also enables the signals to be properly recorded, thus minimizing the biological noise.



Above: Fluidic platform with Eukaryote biochips inserted in plug & play mode.

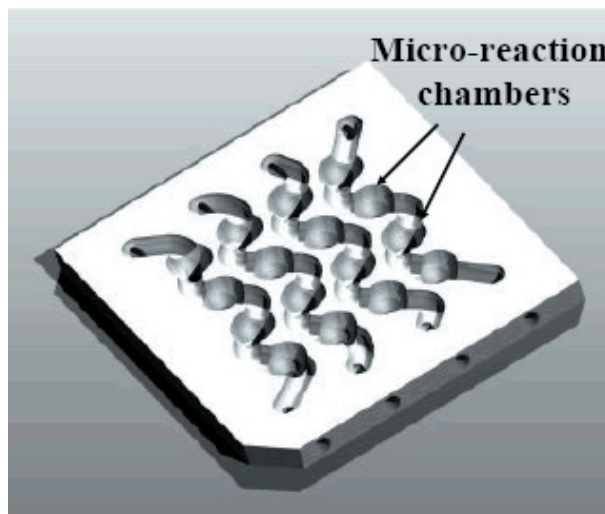
The performance report for the Eukaryote and Prokaryote biochip prototypes have also been realised during the past year by Tyndall. Biocompatibility of the packaged Eukaryote biochips and the electrical connect, showing impedance measurements have been studied. The materials used in the fabrication of these biochips are biocompatible with the cells under study, as investigations verified that growth of cells on the indium tin oxide (ITO) sensor surface was comparable with growth of cells under normal tissue culture conditions i.e. in tissue culture flasks.



Above: Fully packaged Eukaryote biochip.

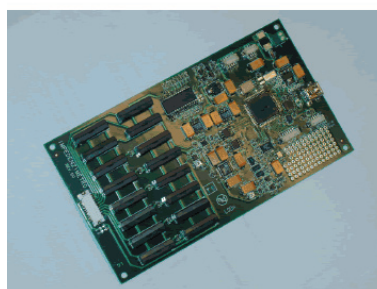
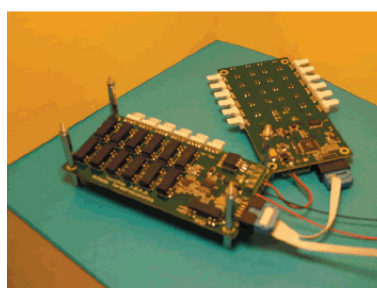
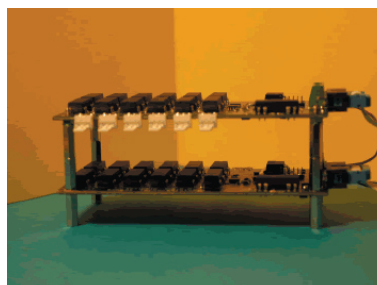
The performance of the Prokaryote biochips were also examined by TAU. Measurement of a bioluminescent signal by a) photomultiplier tube (PMT) under flow condition and b) photodiode (PD) under static condition has been studied. The performance of the Prokaryote biochips was better using the former as a better bioluminescent signal was obtained. An initial increase of bioluminescence can be detected after 25 minutes. For the PD setup using the reflective Aluminium chamber the achieved bioluminescent signal showed a good growing intensity after 2 hours. The optical setup is not fully

optimized and will be changed in future experiments in order to increase the PD signal.



Above: The layout of the Prokaryote biochip.

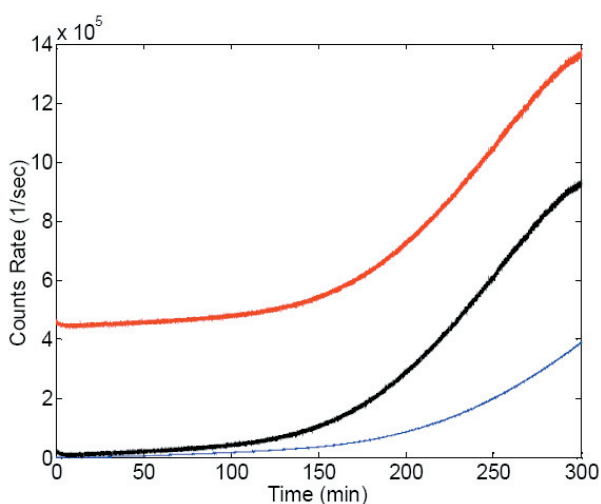
WP 5 (System Integration for Continuous On-line) is on schedule with the first prototypes of hardware and software interfaces being developed. The structure of the system was designed with the cooperation of several partners (ISMB, IMT, JRC and Tyndall) and ISMB developed the electronic circuitries with the management software. The Eukaryote cell biochip system is analysed by using different modular boards, so it is possible to integrate or improve the different system parts. The system allows several parameters to be measured in real time. These are impedance of the cell culture, temperature, oxygen percentage and pH. The software developed is LabView based and this will be available for use on various platforms (Windows, Linux, Macintosh).



Above: A comparison of the SPAPD performance relative to a Photomultiplier Tube (PMT)

**WP6 (Sensor Platform Analysis)** is on schedule. New prokaryote biochips have been designed and these consist of four main channels which will be used for four experiments/tests: sample test, positive test, negative test and constitutive test. The first three tests consists of four strains with a repetition of three bacteria wells for each strain. The constitutive test consists of one strain which works in "Normally On" mode. The biochip will be made of PDMS.

Several solid state photodetectors have been tested. It was found that the best performance was achieved by a Single Photon Avalanche PhotoDiode (SPAPD). A comparison of the SPAPD performance relative to a Photomultiplier Tube (PMT) is shown at end pf last page. Here the sample consists of bacteria responding to 16ppm of NA. The blue curve is the PMT signal, the red curve is the SPAPD signal and the black curve is the SPAPD signal shifted down by its dark current. It can be seen that although the SPAPD has a much larger dark current, its quantum efficiency and its signal are larger.



Above: Comparison of SPAPD to PMT

In relation to the Eukaryote biochips, it was decided that the use of NHK cells for experimentation/analysis be abandoned due to the slow growth of these cells. A549 cells will be used for analysis purposes instead. Work is underway at the moment examining the biocompatibility of the ITO IDEs. Hoechst is being used to quantify and determine the distribution of the cells on the biochip surface using epifluorescence microscopy. As Hoechst is a nuclei stain it allows cell numbers to be counted on a surface. To assess biocompatibility, cells will be grown on control substrates and biochips for a few days and cell numbers analysed to see if there are differences in cell numbers on the two surfaces.

**WP 7 (Performance Evaluation & Biochip Validation)** is progressing well. To enhance productivity, the compliance with regulatory guidelines and comparison with the final Toxichip module, it was decided to switch evaluation experiments from the usual 24-well culture plates to 96-well plates. Cellular plating densities ranging from 2.10<sup>5</sup> to 6.10<sup>5</sup> and from 1.10<sup>6</sup> to 4.10<sup>6</sup> have been tested on various types of 96-well plates, followed by morphological observations, doubling-time evaluation and verifications of IC50s using compounds such as CdCl<sub>2</sub>, NaAs<sub>2</sub>O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, paraquat, camptothecin and erythromycin and at least two different viability tests (XTT formation and LDH release). The protocol for Balbc/3T3 cells was changed in order to have a protocol which follows the standard one recommended by ICCVAM.

**WP 8 (Dissemination and Exploitation)** is also on track. Tyndall's promoting the project through the dedicated website and the elaboration of a newsletter, while VigiCell is the designated commercial partner of the project and product and hence responsible for exploitation plans and marketing studies/action. VigiCell have drawn up a market analysis as well as drafted a preliminary version of the Exploitation plan for Toxichip.

For further information please contact:  
terri.wood@tyndall.ie

