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Project Number: ORL98-09
Wireless Luminescence Integrated Sensors (WLIS)
Project Final Report
November 7, 2003

Abstract

The goal of this project was the development of a family of wireless, single-chip, luminescence-sensing devices to solve a number of difficult distributed measurement problems in areas ranging from environmental monitoring and assessment to high-throughput screening of combinatorial chemistry libraries. These wireless luminescence integrated sensors (WLIS) consist of a microluminometer, wireless data transmitter, and RF power input circuit all realized in a standard integrated circuit (IC) process with genetically engineered, whole-cell, bioluminescent bioreporters encapsulated and deposited on the IC. The end product is a family of compact, low-power, rugged, low-cost sensors. As part of this program we developed an integrated photodiode/signal-processing scheme with an rms noise level of 175 electrons/second for a 13-minute integration time, and a quantum efficiency of 66% at the 490-nm bioluminescent wavelength. This performance provided a detection limit of <1000 photons/second. Although sol-gel has previously been used to encapsulate yeast cells, the reaction conditions necessary for polymerization (primarily low pH) have beforehand proven too harsh for bacterial cell immobilizations. Utilizing sonication methods, we have were able to initiate polymerization under pH conditions conducive to cell survival. Both a toluene bioreporter (*Pseudomonas putida* TVA8) and a naphthalene bioreporter (*Pseudomonas fluorescens* HK44) were successfully encapsulated in sol-gel and shown to produce a fairly significant bioluminescent response. In addition to the previously developed naphthalene- and toluene-sensitive bioreporters, we developed a yeast-based xenoestrogen reporter. This technology has been licensed by Micro Systems Technologies, a startup company in Dayton, Ohio for applications in environmental containments monitoring, and for detecting weapons of mass destruction (i.e. homeland security).

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	$125k	$250k	$250k	$125k

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Project Overview

Specific Project Objectives

The objective of this research was to develop a family of wireless, single-chip, luminescence-sensing devices to solve a number of difficult distributed measurement problems in areas ranging from environmental monitoring and assessment to high-throughput screening of combinatorial chemistry libraries. Integrated circuit technology was used to make these wireless luminescent integrated sensors (WLIS) small, low-power, inexpensive and rugged. We made the WLIS devices able to interface with a large number of luminescent (or some fluorescent) assays. However, in this research we developed and interfaced with whole-cell bioluminescent bioreporters engineered to be sensitive to xenobiotics. As a model system we interfaced the WLIS device to *Pseudomonas Putida* TVA8, a novel bioreporter sensitive to the environmental pollutants benzene, toluene, ethylbenzene, and xylene (BTEX). Additionally, we developed bioluminescent bioreporters sensitive to environmental estrogens and interfaced these to WLIS devices. The end uses of this technology include environmental monitoring and assessment, gene expression studies, energy exploration, discharge monitoring, water and food monitoring and inspection, industrial process control and monitoring, hazardous material identification and monitoring, and military and homeland defense applications. This technology has been licensed by Micro Systems Technologies, a startup company in Dayton, Ohio for applications in environmental containments monitoring, and for detecting weapons of mass destruction (i.e. homeland security).

Relation to DOE Mission and On-going Programs

This work will have a positive impact on several DOE programs. For example, The mission of the Natural and Accelerated Bioremediation Research (NABIR) program is to provide the scientific understanding needed to harness natural processes and to develop methods to accelerate these processes for the bioremediation of contaminated soils, sediments, and groundwater at DOE facilities. The devices produced from our work will be available to provide essential instrumentation toward these goals. Also, our work will support the Measurement Science Program research directed at meeting the needs for new measurement technology for selected environmental and life sciences, which has a thrust area in sensors and imaging chemical composition of contaminated subsurface environments. Finally, the rugged thin-film development work performed as part of our LTR-supported research will support DOE programs in material science which focus on increasing the understanding of phenomena and properties important to materials behavior.

Scientific and Technical Results

CMOS Microluminometer

The CMOS microluminometer portion of the WLIS device[1] is reported in several publications [1-4]. The WLIS employs a two-step chemical sensing strategy where a targeted substance interacts with a genetic regulatory system in a genetically modified bacterial cell, thereby leading to the production of bioluminescence. The second step in the process is the detection of this bioluminescence signal using an integrated CMOS microluminometer. The magnitude of the bioluminescent signal is proportional to the concentration of the targeted substance and the number of cells deployed on the WLIS. Therefore, both for the detection of low concentrations or for the use of a relatively small number of cells on the WLIS, the detection of very small bioluminescent signals is required. We found that a very low photodetector reverse bias maximizes the signal-to-noise ratio when leakage current variations due to temperature changes are considered as components of low-frequency noise. Accordingly, we focused on the detection of signals near the minimum detectable signal limit of the CMOS microluminometer. The complete block diagram of the microluminometer optimized for very low-level dc luminescence is shown in Fig. 1. Fig. 2 is a microphotograph of this microluminometer chip realized in a 0.5-μm n-well bulk CMOS process. The chip measured 2 mm × 2 mm with the photodetector consuming approximately 37% (1.47 mm^2) of the total chip area. This chip was packaged in a 40-pin ceramic dual inline package for testing, and was used to obtain the results reported here. A minimum detectable signal of 0.15 fA (937 electrons/sec.) was measured for a 1,510 sec. integration time. The measured photodetector quantum efficiency was ~64%.

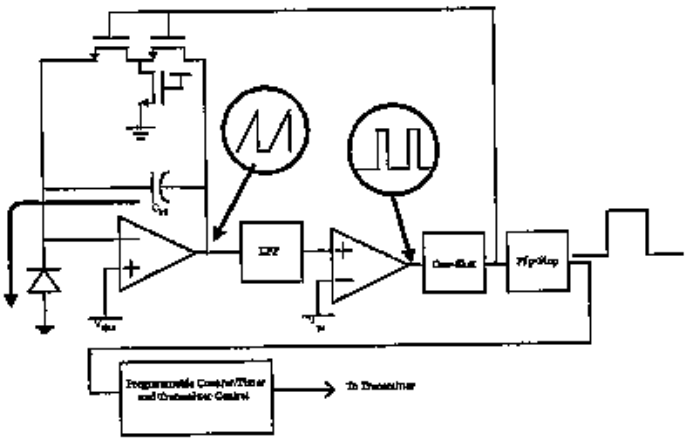

Fig. 1. Block diagram of CMOS microluminometer.

[1] In the literature, the WLIS device is most often referred to as a bioluminescent bioreporter integrated circuit (BBIC).

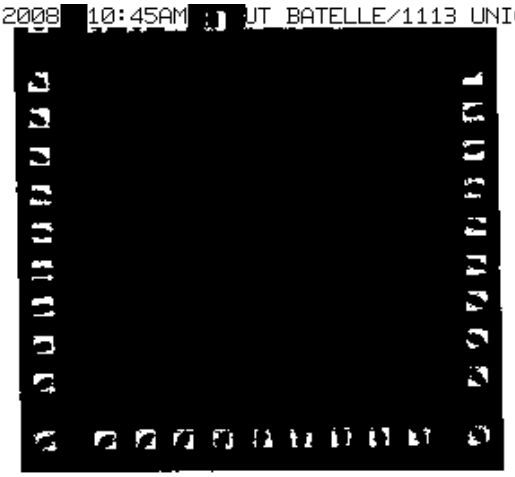

Fig. 2. Micrograph of CMOS microluminometer.

The microluminometer used to detect bioluminescence from whole-cell bioreporters. *Pseudomonas fluorescens* strain 5RL, containing the plasmid pUTK21, was designed to increase *lux* gene expression in the presence of naphthalene or the metabolite salicylate, and salicylate was chosen as the inducer (targeted substance) for those experiments. A flow system was used to bring the bioreporters in proximity with the microluminometer prototype. A light-tight enclosure with an inlet and outlet on either side was placed over the chip. A glass tube extended through the enclosure and was connected to a pump on either side by black rubber tubing. The growing culture was pumped through the flow system.

These tests were performed to determine how the WLIS prototype responded to an increasing population of induced bioreporter cells. *P. fluorescens* 5RL was exposed to 1 ppm salicylate solution and the bioluminescence of the growing cultures was determined as a function of time as shown in Fig. 3. The cells were induced (i.e. salicylate was added) at time = 0 hours. While it took about 3 hours for the cells to become maximally induced, the inset to Fig. 3 shows that a significant response was recorded within a few minutes of induction. The slight increase in detected light prior to time = 0 (visible in the inset of Fig. 3) was most likely due to basal (i.e. non-induced) expression of the *lux* genes. This basal signal increased at a low rate due to the increasing cell population.

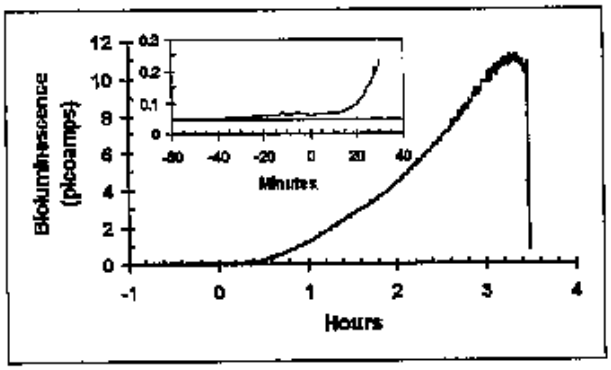

Fig. 3. Bioluminescence from a growing culture of *P. fluorescens* 5RL as measured by the microluminometer. At time = 0, salicylate (1 ppm) was used to fully induce the *lux* genes. The bioluminescence level increased for ~ 3.5 hours until the bioluminescent reaction became oxygen limited at a cell population of 1.53×10^8.

While it varies somewhat with the growth phase of the cells, we have measured that on average, a fully induced *P. fluorescens* 5RL cell produces a WLIS signal of approximately 3×10^{-20} A. Therefore, the MDS of 0.15 fA measured for this microluminometer (1,510 sec. integration time) can be expressed as approximately 5,000 fully induced *P. fluorescens* 5RL cells, a noise metric uniquely suited for this device.

Yeast Bioreporter

The *luxA, B, C, D,* and *E* genes from *Photorhabdus luminescens* were cloned and functionally expressed in *Saccharomyces cerevisiae* to construct a bacterial *lux*-based yeast bioreporter capable of autonomous bioluminescence emission. The bioreporter was engineered using a series of pBEVY yeast expression vectors that allowed for bi-directional expression of the individual *luxA, B, C,* and *E* genes. Requisite expression of *luxD*, as well as a flavin oxidoreductase gene (*frp*) for $FMNH_2$ synthesis, was achieved via novel fusions to yeast internal ribosomal entry site (IRES) sequences. The final construct, designated *S. cerevisiae* BLYEV, was capable of self-generating bioluminescence at levels exceeding 1×10^6 photons/sec/OD_{600} (approximately 0.1 photons/sec/cell). To establish its utility as a functional bioreporter, the genetic architecture of strain BLYEV was modified via chromosomal incorporation of a human estrogen receptor (hER-α) and plasmid based estrogen response elements (EREs) to generate a bioreporter (strain BLYES) for environmental estrogens. High throughput testing using 17β-estradiol, 17α-ethynyl estradiol, estrone, and 17α-estradiol demonstrated detection limits that diminished 5 to 10-fold in comparison to the *lacZ* based yeast estrogen assay (YES), but with corresponding detection times that decreased from 2 – 4 days to 2 – 4 h. Testing was performed with WLIS devices using this bioreporter interfaced to a flow-cell infused with 17β-estradiol contaminated wastewater effluent, with on-line response times occurring in less than 5 h.

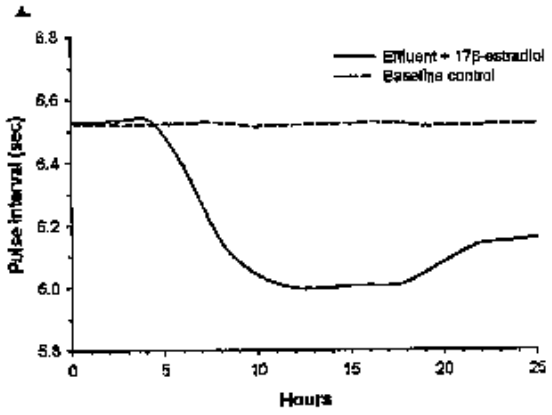

Fig. 4. Bioluminescence profile established with the WLIS detection system. Alginate encapsulated *S. cerevisiae* BLYES bioreporters exposed to wastewater devoid of 17β-estradiol produced pulse intervals averaging 6.5 sec (dashed line). Bioluminescence induction from BLYES encapsulated cells in response to flow through wastewater effluent artificially contaminated with 17β-estradiol at 8 ppb (solid line) produced a pulse interval response that significantly differed from the baseline within 4.8 h of addition and maximizing at approximately 6.0 sec (the pulse interval is inversely proportional to measured bioluminescence) (n = 2).

For the WLIS experiments, strain BLYES at an OD_{600} of 0.8 was encapsulated in 2 mm diameter alginate beads and loaded into a 7 cm^3 flow cell chamber embedded with the WLIS chip. Wastewater effluent artificially contaminated with 17β-estradiol at 8 ppb was infused through the chamber at a rate of 2 ml/min. A microcontroller with a 16-bit timer/counter input measured the interval of the WLIS digital pulse output and serially transmitted this data to a remote computer using a commercially available spread-spectrum radio telemetry system. Results are shown in Fig. 4.

Conclusions

All of the technical objectives of the WLIS program were met. A viable device technology was developed and demonstrated both for the detection of BTEX compounds and xenoestrogens. Although the CRADA partner, the Dynamac Corporation, declined to commercialize this technology, it has since been licensed by Micro Systems Technologies, a startup company in Dayton, Ohio for applications in environmental containments monitoring, and for detecting weapons of mass destruction (i.e. homeland security).

References

[1] E. K. Bolton, G. S. Sayler, D. E. Nivens, J. M. Rochelle, S. Ripp, and M. L. Simpson, "Integrated CMOS photodetectors and signal processing for very low-level chemical sensing with the bioluminescent bioreporter integrated circuit," *Sensors and Actuators B-Chemical*, vol. 85, pp. 179-185, 2002.

[2] M. L. Simpson, G. S. Sayler, B. M. Applegate, S. Ripp, D. E. Nivens, M. J. Paulus, and G. E. Jellison, "Bioluminescent-bioreporter integrated circuits form novel whole-cell biosensors," *Trends in Biotechnology*, vol. 16, pp. 332-338, 1998.

[3] M. L. Simpson, G. S. Sayler, J. T. Fleming, and B. Applegate, "Whole-cell biocomputing," *Trends in Biotechnology*, vol. 19, pp. 317-323, 2001.

[4] M. L. Simpson, G. S. Sayler, G. Patterson, D. E. Nivens, E. K. Bolton, J. M. Rochelle, J. C. Arnott, B. M. Applegate, S. Ripp, and M. A. Guillorn, "An integrated CMOS microluminometer for low-level luminescence sensing in the bioluminescent bioreporter integrated circuit," *Sensors and Actuators B-Chemical*, vol. 72, pp. 134-140, 2001.