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SGER: Detection of Bioterrorism-Linked Microbial Pathogens Using Surface Acoustic Wave Liquid Sensors

Maruicio Pereira da Cunha
Principal Investigator; University of Maine, Orono, mdacunha@eece.maine.edu

Paul J. Millard
Co-Principal Investigator; University of Maine, Orono, paul.millard@maine.edu

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Project Participants

Senior Personnel

Name: Pereira da Cunha, Mauricio
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Millard, Paul
Worked for more than 160 Hours: Yes
Contribution to Project:

Graduate Student

Name: Bitla, Shivashanker
Worked for more than 160 Hours: Yes
Contribution to Project:
Shivashanker Bitla has investigated techniques for improving and quantifying the coupling of biomolecular receptors to surface acoustic wave and bulk acoustic wave devices. In cooperation with other graduate students, Bitla has contributed to the development of the testing system for the SH-SAW sensors and has carried out experiments to compare SH-SAW device responses with those of standard QCM devices.
Support: Bitla has been supported by Maine Sea Grant, a fellowship from University of Maine Graduate Association, and by this project.

Name: Berkenpas, Eric
Worked for more than 160 Hours: Yes
Contribution to Project:
Eric Berkenpas is working in this project on the fabrication, characterization, and test of the LGS Shear Horizontal SAW devices and biosensor. Together with Shiva Bitla (PhD student) and Tim Beaucage (2003 NSF REU student), Berkenpas has put together the biosensor testing systems during the project and initiated the characterization of the LGS SH SAW biosensor.
Support: Berkenpas is also GK-12 fellow and was able to share research results and topics with local high school students and teachers.

Name: Pollard, Thomas
Worked for more than 160 Hours: Yes
Contribution to Project:
Thomas Pollard graduated in electrical engineering in May 2003 and joined this project as an MSc student. Pollard's task was to model the SH devices. Pollard was supported as a NSF REU student during the summer of 2003.
Support: Pollard was supported by an industrial project until July 03 and since August 2003 he has been supported by this project.

Undergraduate Student

Technician, Programmer

Name: Bickerstaff, Lee
Worked for more than 160 Hours: Yes
Contribution to Project:
Lee Bickerstaff has carried out many of the fundamental experiments directed toward the use of molecular padlocks and rolling circle amplification for detection viral and bacterial DNA and RNA.
Support: Bickerstaff has been supported by the University of Maine.

Other Participant
Name: Gignoux, Henri
Worked for more than 160 Hours: Yes

Contribution to Project:
The project and lab facilities generated under this project also allowed the direct interaction of the PIs with rural Maine high schools through the NSF Research Experience for Teachers (RET) program. Prof. Da Cunha supervised Henri Gignoux, a high school teacher from Sumner Memorial High School, East Sullivan, Maine in a related LGX material characterization project. Sumner Memorial High School is located in geographically isolated, economically challenged areas.

Name: Alley, Lynn
Worked for more than 160 Hours: Yes

Contribution to Project:
The project and lab facilities generated under this project also allowed the direct interaction of the PIs with rural Maine high schools through the NSF Research Experience for Teachers (RET) program. Prof. Millard supervised Lynn Alley, a high school teacher from Jonesport-Beals High School in a project involving a sensor for okadaic acid detection. Jonesport Beals High School is located in geographically isolated, economically challenged areas.

Research Experience for Undergraduates
Name: Beaucage, Tim
Worked for more than 160 Hours: Yes

Contribution to Project:
Tim Beaucage was an NSF REU participant during the summer of 2003. He has worked in the implementation of an improved biosensor measurement system together with the graduate students Eric Berkenpas and Shiva Bitla.
Support: Beaucage has been supported by the NSF REU program and by this project.

    Years of schooling completed: Junior
    Home Institution: Same as Research Site
    Home Institution if Other:
    Home Institution Highest Degree Granted(in fields supported by NSF): Doctoral Degree
    Fiscal year(s) REU Participant supported: 2003
    REU Funding: REU supplement

Name: Scheinfeldt, Amanda
Worked for more than 160 Hours: Yes

Contribution to Project:
Amanda Scheinfeldt participated in the project as an NSF REU student during the Summer of 2003. Her research project involved preliminary studies of techniques for fabricating chemically regenerated analyte-binding surfaces to permit repeated use of SH-SAW sensors.
Support: Scheinfeldt has been supported by the NSF REU program.

    Years of schooling completed: Junior
    Home Institution: Other than Research Site
    Home Institution if Other: Tufts University
    Home Institution Highest Degree Granted(in fields supported by NSF): Doctoral Degree
    Fiscal year(s) REU Participant supported: 2003
    REU Funding: REU supplement

Organizational Partners
Research and Education Activities:

Shear Horizontal Surface Acoustic Wave (SH-SAW) Devices

The langasite (LGS) crystal propagation direction Euler angle (0?, 22?, 90?) was identified to have high electromechanical coupling and reduced temperature coefficient of frequency (TCF) based on the LGS material constants determined previously. The propagation properties of this LGS orientation were close to our reported LGT orientation (0?, 13.5?, 90?). A new microelectronic mask was designed and purchased for the SH-SAW device fabrication at UMaine. Both resonators and delay lines were fabricated and on-wafer tests were performed for electromechanical coupling, transmission loss, and temperature behavior. The LGS SH-SAW device performed with comparable characteristics to our originally described LGT device. The SH-SAW properties on this new crystal and propagation direction are summarized: (1) The SH-SAW device demonstrated strong guiding to the surface with reduced signal attenuation in liquids. (2) Temperature-compensated orientations diminished the influence of temperature on the sensor operation. The measured temperature coefficient of frequency (TCF) is ±3 ppm/°C around room temperature, in accordance with numerical predictions, and confirming the existence of temperature-compensated orientations, which minimize temperature cross effects. (3) High dielectric permittivity of the material avoided electrical short circuits in the presence of conductive aqueous solutions. (4) Eight-fold higher coupling when compared to equivalent SH quartz orientations, yielding an increased signal-to-noise ratio.

Nucleic Acid Sensing Surface

Marked progress has been made toward the development of sensitive, specific and robust molecular recognition for bacterial RNA. Specific accomplishments include: (1) The sequence specificity of padlock probes for synthetic target DNA oligonucleotides was demonstrated. (2) Data support the claim that padlock probes can detect single-nucleotide substitutions. (3) Padlock probes were able to recognize synthetic DNA target sequences or viral RNA in a greater than ten-fold excess of non-specific RNA. (4) Recognition and amplification of synthetic DNA targets was simplified and improved. Detection of specific sequences of viral RNA extracted from host cells has been successful. 

Experimental conditions for applying padlock technology to RNA sample analysis were improved. (5) Bst polymerase was shown to be more effective than phi 29 polymerase in rolling circle amplification of ligated padlock probes. (6) The method has demonstrated the ability of 100nM probe to detect synthetic DNA oligonucleotide target molecules in the fM to nM range. (7) Surface immobilization of molecular padlock/target via biotin-terminated rolling circle primer oligonucleotides was demonstrated using avidin-coated microspheres. (8) In order to further facilitate the rapid development of recognition/amplification procedures, real-time analysis of RCA/Hbr reactions were analyzed in a fluorescence-detecting thermocycler.

Testing of the Model Biosensor

The SGER effort was focused on SH-SAW delay line devices mounted in temperature-controlled microchambers for liquid testing. A reliable experimental test setup, including a test chamber for the introduction of fluids, has been designed and constructed. Fluids are introduced with an electronically-controlled Hamilton microsyringe through microbore tubing into a silicone chamber affixed to the top of the SH-SAW device. The experimental test setup implemented in this work allowed control of sensing parameters including temperature (to within 0.2 °C), fluid volumetric flow rate, and volume (to within 0.06 nL) applied to the sensor surface. The fluidic system implemented reduces the chance of introducing air bubbles into the testing chamber and therefore onto the LGS SH SAW surface, which would otherwise seriously compromise any sensor measurement performed. Computer automation of the liquid test setup improved the reproducibility of measurements and eliminated the introduction of human error during the device testing. The 150 mm I.D. capillary tubing and tube connections permitted a low fluidic system volume of about 30 mL, with future miniaturization and array development in mind.

The sensing region between the input and output interdigital transducers (IDTs), where the microchamber is placed, was gold metalized to avoid the cross measurement of variations in the liquid conductivity. The metalized sensing region also provided binding sites for recognition biomolecules. The microchamber for controlling fluid addition to the sensing surface consisted of a silicone gasket with an integral plastic window, which was drilled to create fluid entry and exit ports. Microchambers were placed on top of the metalized region between the IDTs. The sensitivity of the device to biomolecule binding in physiological solutions was tested by monitoring sequential binding of specific proteins to the modified sensing surface. A self-assembled monolayer of cysteamine was formed on the gold sensing region of the delay line and the resulting terminal amine moieties reacted with alkyl NHS-biotin to produce a biotin-rich sensor surface. The biotinylated sensor was then exposed sequentially to biotin-binding Neutravidin, biotin-modified rabbit IgG, and goat anti-rabbit IgG antibody. As each protein was bound
sequentially to the sensing surface, marked changes in the delay line phase were recorded. The data demonstrated the capability of these devices for biochemical recognition in aqueous saline solutions.

This project also included experiments with SiO2/LGS SH-SAW resonators. These devices showed that without effective electrical shielding the structure detect variations in the conductivity of buffered saline solutions several orders of magnitude more sensitively than any modification in the film structure or molecular adsorption at the surface. Development of shielded resonator structures, requiring research of other film structures to avoid reduction in the resonator quality factor, was considered to be beyond the scope of the proof-of-concept SGER project and will be addressed in the ongoing project.

Integration of Research and Education Component

This multidisciplinary SGER project resulted in the direct training of a Ph.D. student in Chemical and Biological Engineering, an M.Sc. student in Electrical Engineering (who is also a GK-12 fellow involved in local high schools), two NSF REU students, and two NSF RET participants.

Findings:

Shear Horizontal Surface Acoustic Wave (SH-SAW) Devices

In the context of the activities and results described in the previous sub item, the SH-SAW properties on this new crystal and propagation direction are summarized: (1) The SH-SAW device demonstrated strong guiding to the surface with reduced signal attenuation in liquids. (2) Temperature-compensated orientations diminished the influence of temperature on the sensor operation. The measured temperature coefficient of frequency (TCF) is $\pm 3$ ppm/$^\circ$C around room temperature, in accordance with numerical predictions, and confirming the existence of temperature-compensated orientations, which minimize temperature cross effects. (3) High dielectric permittivity of the material avoided electrical short circuits in the presence of conductive aqueous solutions. (4) Eight-fold higher coupling when compared to equivalent SH quartz orientations, yielding an increased signal-to-noise ratio.

Nucleic Acid Sensing Surface

Specific accomplishments towards the preparation of the nucleic acid sensing surface include: (1) The sequence specificity of padlock probes for synthetic target DNA oligonucleotides was demonstrated. (2) Data support the claim that padlock probes can detect single-nucleotide substitutions. (3) Padlock probes were able to recognize synthetic DNA target sequences or viral RNA in a greater than ten-fold excess of non-specific RNA. (4) Recognition and amplification of synthetic DNA targets was simplified and improved. Detection of specific sequences of viral RNA extracted from host cells has been successful. Experimental conditions for applying padlock technology to RNA sample analysis were improved. (5) Bst polymerase was shown to be more effective than phi 29 polymerase in rolling circle amplification of ligated padlock probes. (6) The method has demonstrated the ability of 100nM probe to detect synthetic DNA oligonucleotide target molecules in the fM to nM range. (7) Surface immobilization of molecular padlock/target via biotin-terminated rolling circle primer oligonucleotides was demonstrated using avidin-coated microspheres. (8) In order to further facilitate the rapid development of recognition/amplification procedures, real-time analysis of RCA/Hbr reactions were analyzed in a fluorescence-detecting thermocycler.

Testing of the Model Biosensor

In accordance to the research activities and results described above for the Model Biosensor, we have specifically found that: (i) The experimental test setup designed and constructed, which includes a test chamber for the introduction of fluids, is reliable, furnishing repeatable and consistent results. (ii) The experimental test setup allowed control of sensing parameters including temperature (to within 0.2 °C), fluid volumetric flow rate, and volume (to within 0.06 nL) applied to the sensor surface. (iii) The fluidic system implemented reduces the chance of introducing air bubbles into the testing chamber and therefore onto the LGS SH SAW surface. (iv) Computer automation of the liquid test setup improved the reproducibility of measurements and eliminated the introduction of human error during the device testing. The 150 mm I.D. capillary tubing and tube connections permitted a low fluidic system volume of about 30 mL. (v) The gold metalized sensing region between the input and output interdigital transducers (IDTs) which provided binding sites for recognition biomolecules, was also essential to avoid cross measurements due to variations in the liquid conductivity. (vi) A self-assembled monolayer of cysteamine was formed on the gold sensing region of the delay line and the resulting terminal amine moieties reacted with alkyl NHS-biotin to produce a biotin-rich sensor surface. The biotinylated sensor was then exposed successfully to biotin-binding Neutravidin, biotin-modified rabbit IgG, and goat anti-rabbit IgG antibody. As each protein was bound sequentially to the sensing surface, marked changes in the delay line phase were recorded. The data demonstrated the capability of these devices for biochemical recognition in aqueous saline solutions.

Integration of Research and Education Component

This multidisciplinary SGER project was instrumental in allowing the integration of research and educational activities on such diverse
levels of knowledge which included: the project's PIs, other faculty and technical staff involved in the discussions, undergraduate (including NSF REU) and graduate students (Ph.D. and M.Sc.), and high school teachers and students (in electrical, bioengineering, and integrative parts of the project).

**Training and Development:**
The project has served as a valuable source of cross-training for both Drs. Pereira Da Cunha and Millard, faculty members of the Department of Electrical and Computer Engineering and Department of Chemical and Biological Engineering at the University of Maine, respectively. Dr. Pereira da Cunha, whose area of expertise is microwave, telecommunications, and microwave acoustics, has been cross-trained in bioengineering, in particular regarding the techniques of biofilm preparation, growth, and usage towards a biosensor. Dr. Millard, whose area of expertise lies primarily in the areas of microbiology and cell biology, with an emphasis on quantitation of biological processes through the use of optical methods, has gained considerable experience in electrical engineering, materials science, and a variety of fabrication techniques. The joint work and weekly meetings between electrical, chemical, and bioengineers has provided the project participants (including faculty members, technicians, graduate and undergraduate students, and high school teachers) with a solid interdisciplinary base in a range of science and engineering subjects.

On the teaching side, Prof. Pereira da Cunha has included biosensor topics in the undergraduate course ECE 466 Sensor and Instrumentation Laboratory, and incorporated special shear horizontal acoustic wave topics in the graduate course ECE 663 Design and Fabrication of Acoustic Wave Devices, both courses taught in Fall 2003. The PI also lectured during one week of the interdisciplinary course CHE 598 - Microscale Bioengineering, under the responsibility of Prof. Millard. Specifically, Prof. Pereira da Cunha developed the topic Acoustic Wave-Based Detection in Biosensors. For the past two years Prof. Millard has incorporated sensor-related topics into his undergraduate course, CHB 460 â€” Biochemical Engineering. Microfabrication and bioengineering, and their use in sensor development, comprises a major fraction of Prof. Millard's graduate level CHE 598 â€” Microscale Bioengineering course, offered during the Spring semester of 2003 and 2004.

This project has directly involved one PhD student, two MSc students, two NSF REU students, one lab technician, and two NSF RET teachers. As described, on a one to one basis in the Project Participant section, graduate and undergraduate students of diverse backgrounds such as electrical, chemical and biochemical engineering came together to design, fabricate, and test a preliminary LGS SH SAW biosensor and the respective testing system that led to the required proof-of-concept for the full scale ongoing project.

**Outreach Activities:**

**GK-12**
This NSF SGER program allowed the introduction of research topics, such as biosensors, electrical and bioengineering at high school level. Eric Berkenpas, a Master's student in Dr. Da Cunha's laboratory, with his research topic taken from this project on LGS SH SAW platform for biosensors, is also an NSF GK-12 fellow, and has integrated teaching at the high school level (Bangor High School and Sumner High School) into his research program through the NSF GK&#64979;12 program. As one of his high school activities, Eric has extracted DNA from vegetables, and related DNA extraction and his biosensing research work to the high school students.

Dr. Millard has hosted visits by numerous high school student groups to his research laboratory. During these visits, students are introduced to the fundamental concepts involved in biosensor function and in specific methods employed by his laboratory in this area.

**REU**
The SGER project also directly involved two NSF REU students during summer 2003. Tim Beaucage, an electrical engineering senior student from University of Maine, has worked with the MSc student Eric Berkenpas in the improvement in the biosensor testing system. This work has been reported in the 2003 IEEE International Ultrasonics Symposium and in the IEEE International Sensor Conference, and has been submitted as a paper to the IEEE Ultrasonics, Ferroelectrics, and Frequency Control Transactions. The research opportunity provided to Tim Beaucage, and the successful accomplishment of his tasks, sparked his interest in graduate studies, encouraging him to develop an MSc under the PI's supervision. He will continue this semester as an undergraduate student and be admitted as MSc student upon the completion of his EE degree in May, 2004.

The other NSF REU student is Amanda Scheinfeldt, a student majoring in Electrical Engineering at Tufts University, Boston, MA. Scheinfeldt developed a project directed toward improvement of surface attachment chemistry for ligand binding. Her work will serve as the basis for ongoing studies in this area.

**RET**
The project and lab facilities generated under this project also allowed the direct interaction of the PIs with rural Maine high schools through the NSF Research Experience for Teachers (RET) program. Prof. Da Cunha supervised Henri Gignoux, a high school teacher from Sumner Memorial High School, East Sullivan, Maine in a related LGX material characterization project. Prof. Millard supervised Lynn Alley, a high school teacher from Jonesport-Beals High School in a project involving a sensor for okadaic acid detection. Both Sumner Memorial High School and Jonesport Beals High School are located in geographically isolated, economically challenged areas.

**GRADUATE PRESENTATIONS**
In an effort to publicize the findings and research effort, the graduate students Shivashanker Bitla and Eric Berkenpas presented their work for
the University of Maine and the State of Maine research community in the Graduate Research Expo 2003 event that took place at Buchanan Alumni House, University of Maine, April 21st and 22nd, 2003.

HOMELAND SECURITY CONFERENCE
The findings of the project were presented in the 2003 International Conference on Advanced Technologies for Homeland Security, which took place at the University of Connecticut, on September 25 and 26, 2003.

Journal Publications


Books or Other One-time Publications

Collection: IEEE 2003 International Ultrasonics Symposium Proceedings
Bibliography: October 5-8, Honolulu, Hawaii, 2003, pp. 1404-1407

Editor(s): IEEE Sensors Society
Bibliography: in press

Editor(s): Meeting of AIChE
Bibliography: Proceedings

Editor(s): IEEE International Ultrasonics Symposium Proceedings
Bibliography: Proceedings

Editor(s): University of Connecticut
Bibliography: 2003 International Conference on Advanced Technologies for Homeland Security

S. Bitla, "Development of Biosensor for pathogen detection in liquids", (2003). Graduate Research Expo 2003, Buchanan Alumni House, University of Maine, April, Published
Editor(s): University of Maine
Bibliography: Graduate Research Expo 2003
Contributions within Discipline:
The findings and techniques from this project contributed to both principal disciplinary fields: electrical engineering and biological engineering. Primary accomplishments of the project were:
1. Proved the validity of using an LGS SH SAW delay line device as a liquid and biochemical sensing platform using immunochemicals for differentiation of analytes.
2. Established the use of molecular padlock nucleic acid recognition in conjunction with rolling circle amplification as diagnostic method for the detection of virus-derived RNA.
3. Developed an automated test system to fluidics, including sample introduction, and to acquire data from the SH SAW device.

This work is important to the advancement of research in acoustic wave-based biosensors and is directly relevant to the fields of electrical engineering and bioengineering. These accomplishments represent important advancements in this specific combined research area and are likely to provide tools for other researchers in the field.

Contributions to Other Disciplines:
This 12-month SGER project has integrated research and education from several disciplines from its inception, including electrical, chemical, mechanical, and bioengineering. The PIs have collaborated with both faculty and students from these diverse disciplines through their affiliation with the University of Maine's Laboratory for Surface Science and Technology (LASST). From the beginning, this effort has provided multidisciplinary training of undergraduate and graduate students through laboratory research and course units. For example, graduate students admitted through the NSF IGERT (Integrative Graduate Education and Research Traineeship) program, which offers a Ph.D. program in Functional Genomics, have been exposed to our methods and findings through a new course entitled CHE 598 - Microscale Bioengineering. As we continue to develop new approaches to sensor design, we have also introduced our ideas to other UMaine faculty, e.g. to colleagues in marine science, for detection of problem algal species such as Red Tide, and to researchers in aquaculture, for identification of pathogenic agents in cultured organisms.

Contributions to Human Resource Development:
This multidisciplinary SGER project resulted in the direct training of one Ph.D. student in Chemical and Biological Engineering, two M.Sc. students in Electrical Engineering, two NSF REU students, and two NSF RET participants (high school teachers), a lab technician, in addition to the PIs, the technical support team, and other faculty at the Laboratory for Surface Science and Technology at the University of Maine. The project, therefore, contributed significantly to human resource development in science, engineering, and technology.

Contributions to Resources for Research and Education:
This project has augmented the individual research programs and labs of both PIs. Dr. Pereira da Cunha's laboratory has expanded its range of activities to include fluidics, molecular biology, and digital imaging microscopy. Dr. Millard's laboratory has expanded its range of activities to include acoustic wave devices, analytical instrumentation, and microfabrication. As a result, this project has given rise to a common research laboratory with combined resources and capabilities. This facility is essential for multidisciplinary training of graduate and undergraduate
students as it permits interaction between students and faculty with different areas of expertise and provides integration of facilities not available in any of the previously existing research laboratories.

**Contributions Beyond Science and Engineering:**
This project determined the validity of integrating biomolecular technology with a novel SH-SAW sensor platform. This NSF SGER initiative permitted proof-of-concept verification of the LGS SH-SAW biosensor device and the results gave rise to a full proposal. It is expected that research and development of this particular sensor will permit the further creation of sensors for use in a wide range of environmental, medical, industrial, homeland security, and military applications. By providing a rapid, reliable and ultimately, inexpensive sensor, the development of this technology will contribute to general well-being of the population at large, and serve as a starting point for the development of an important new class of biosensors.

The Bangor Daily News featured an (online and print) editorial (Sept. 11, 2002) on this research, indicating that University faculty and students can play an important role in protecting the nation against bioterrorism events. While it is difficult to evaluate at this point in time whether such an editorial informed regulatory policy of any kind, the newspaper is widely read by business and public policy leaders across Maine.

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**Categories for which nothing is reported:**

Organizational Partners
Any Product