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14. ABSTRACT The goal of this proposal is to purchase the GWC Technologies, Inc. Horizontal Surface Plasmon Resonance Imaging (SPRi) System, Keyence Corporation of America Digital Microscope Controller System, and two Fisher Scientific FH100 Multichannel peristaltic pumps. The cadre of instruments will be used in two research projects integral to the mission of the Department of Defense (DoD) that will significantly enhance the quality of the STEM program at CSULA. Built on the productive research of Gomez in the development of microfluidics, SPR, and capillary electrophoresis (CE) separations, this equipment/instrumentation acquisition proposal aims at further					
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Report Title

Final Report: Acquisition of a Surface Plasmon Resonance Imager, Digital Microscope, and Peristaltic Pumps for Defense-Based Research

ABSTRACT

The goal of this proposal is to purchase the GWC Technologies, Inc. Horizontal Surface Plasmon Resonance Imaging (SPRi) System, Keyence Corporation of America Digital Microscope Controller System, and two Fisher Scientific FH100 Multichannel peristaltic pumps. The cadre of instruments will be used in two research projects integral to the mission of the Department of Defense (DoD) that will significantly enhance the quality of the STEM program at CSULA. Built on the productive research of Gomez in the development of microfluidics, SPR, and capillary electrophoresis (CE) separations, this equipment/instrumentation acquisition proposal, aims at further developing transformative research showcasing the high sensitivity and high throughput of SPRi in two research projects: 1) The Detection of Organophosphorus Compounds via an Enzyme Inhibition Concept, and; 2) The Development of SPRi-Based Detection of Explosives on a Microfluidic Platform.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received

Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received

Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received

Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Dr. Frank A. Gomez	0.00	
FTE Equivalent:	0.00	
Total Number:	1	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Andre Leon	0.00	Biochemistry
Hector Valadez	0.00	Biology
FTE Equivalent:	0.00	
Total Number:	2	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME

PERCENT SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment.

Technology Transfer

Final Report
Frank A. Gomez, Ph.D.
Award Number: W911NF-14-1-0040

Two major and one minor instrument were acquired in support of the proposal entitled “Acquisition of a Surface Plasmon Resonance Imager, Digital Microscope, and Peristaltic Pumps for Defense-Based Research”. This final report provides an overview of the research conducted utilizing this instrumentation.

The *GWC Technologies, Inc. Horizontal Surface Plasmon Resonance Imaging (SPRi) System* has been utilized in two projects. The first involved our on-going efforts to develop an assay for acetylcholinesterase (AChE) combining microchip capillary electrophoresis (MCE). The second involves the development of an assay for the detection of copper (Cu) in drinking water supplies.

Studies on Developing a Acetylcholinesterase (AChE) Assay

In this project two MCE-SPR platforms with multiple analysis regions were designed and fabricated. A patterned photomask for use with negative photoresist was designed using AutoCAD. Several attempts were made to successfully pattern the negative photoresist. Ultimately, modifying the traditional photoresist procedure was necessary for success. The resulting mold was used to create a poly(dimethylsiloxane) (PDMS) microchip. Additionally, energy dispersive X-ray spectroscopy (EDS) was used to verify the functionalization of a gold surface with 3-aminophenylboronic acid.

Capillary electrophoresis (CE), and MCE by extension, is a method of electrochemical separation. Using electric fields, molecules are separated based on their different charges and molecular masses. Instrumental design for CE is relatively simply and must consist of at least a capillary, electrodes and power supplies, input and output vials, and a detector. The ultimate goal of this work is to optimize an MCE system that utilizes SPR as its detector. By combining these two analytical techniques, users in the field and in the lab would reap the benefits of both. Since MCE-SPR was first reported in the literature, besides our group, little additional work has been published.

Experimental

Materials

AutoCAD Software was used to design photomasks. These designs were then manufactured by Output City. SU-8 2025, SU-8 2075, and SU-8 negative photoresist developer were obtained from Microchem. An AB-M Inc. mask aligner was used for photoresist exposure. Silicon wafers of variable diameters were used as the base for photolithography. PDMS oligomer base and Sylgard 184 curing agent were used for all microchip manufacture. SPR sensor chips (10nm gold vapor deposited on a chromium and glass base) were obtained from Biosensing Instruments.

Methods

Mold creation began with proper cleaning of the silicon wafers: either 10 minutes of Piranha etching or 10 minutes of acetone suspension. Wafers were cleaned under an N₂ stream and dried in a 150°C oven for 25 minutes. SU-8 2075 was spin-coated onto the wafer surface at a height of approximately 150um following established protocol. In a deviation from protocol, the wafer was then heated on a hot plate for only 3 minutes at 65°C and 8 minutes at 95°C. These time frames were found heuristically after the protocol proved too extreme for the reagents. Following this round of heating, the wafer was exposed to UV light for 90 seconds under the photomask. This insured only the patterned channels were exposed and cross-linked. Following exposure, in another deviation from protocol, the wafer was heated for 2 minutes at 65°C and 5 minutes at 95°C. The wafer was then developed with SU-8 developer to remove unexposed photoresist. The mold was transferred to a glass petri dish and was then ready for use.

The PDMS for the chip was made by mixing PDMS oligomer with PDMS elastomer at 10:1. This was poured over the mold, placed in a vacuum to degas, and allowed to harden. After solidifying, the area around the desired channels was excised. The appropriate inlets and outlets were punched with either a 100um or a 300um hole-punch. The second PDMS layer of the MCE-SPR chip was made by following the appropriate PDMS thin-layer procedure for a 10uM thin-film. The two PDMS layers were plasma oxidized and joined at the appropriate regions for irreversible sealing. The PDMS layers were then overlaid onto the gold chip (the gold chip having been Piranha etched prior to sealing). The closed system was heated at 90°C for 15 for stronger sealing. To make the chip channels permanently hydrophilic, a modification solution was prepared as described in the literature. The only deviation was the use of 7.000kD SnakeSkin dialysis tubing instead of 3.500kD.

We have had great difficulty in derivatizing AChE onto the alkanethiol on the Au substrate. We do have preliminary data (not shown) that shows the formation of the thiol layer onto the Au. Standard derivatization procedures of enzyme onto the thiol were performed both on the SPRi instrument and off-instrument. Unfortunately, when binding experiments were conducted to confirm derivatization of enzyme onto the thiol, evidence of binding was inconclusive. Classic sensorgrams indicative of binding, were unrepeatable. Current work is on-going to improve the derivatization procedure and to develop the assay for AChE.

Detection of Copper

The GWC Technologies, Inc. Horizontal Surface Plasmon Resonance Imaging (SPRi) System has been used in developing an assay for the detection of copper (Cu) in drinking water supplies. Our work is based on creating a self-assembled monolayer (SAM) on a gold sensor surface where the analyte (Cu) can bind and the change in the refractive index can be measured. Figure 1 depicts the modification of the gold sensor and the process of the experiment.

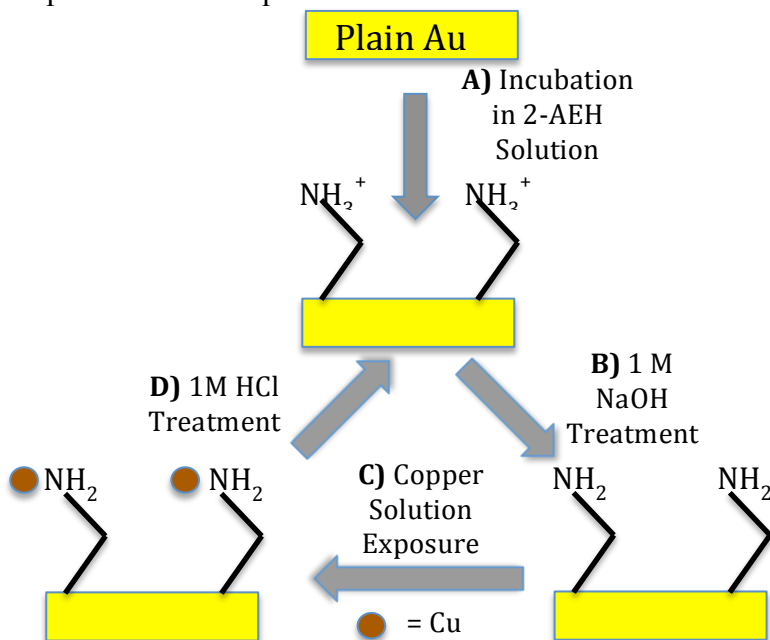


Figure 1. Copper detection experiment.

After incubation of the gold sensor in 2-aminoethanethiol hydrochloride (2-AEH), the nitrogen on the deposited SAM is positively charged. Treatment of the sensor with NaOH strips a hydrogen off the amino group resulting in a pair of non-bonded electrons on the nitrogen. Cu in the solution of interest can then complex with the nitrogen. Treatment with HCl removes Cu and regenerates the NH_3^+ on the SAM.

Every step in the process is monitored via the SPR imager. After treatment of the SAM modified sensor with NaOH (pH 11) the SPR angle is expected to decrease from the baseline. Regeneration of the system with HCl (pH 3) results in an increase in the SPR angle and regeneration of the baseline. Figure 2a shows the SPR signals generated by treating the SAM functionalized sensor with both NaOH and HCl. Figure 2b shows the SPR signals of a plain gold sensor after exposure to NaOH and HCl. The signals of the plain gold sensor are opposite that of the SAM modified sensor. That is, treatment with NaOH results in an increase in the SPR angle while treatment with HCl causes a decrease in the angle. The differences in signal between both sensors indicates that the changes in angle observed are specifically due to structural changes in the amine group due to deprotonation and protonation brought on by changes in pH.

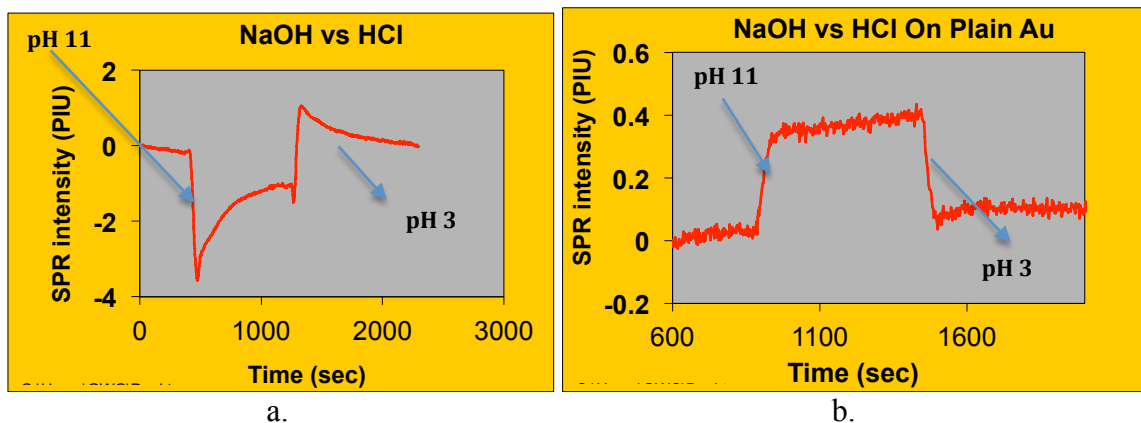


Figure 2. a) NaOH and HCl SPR response on SAM modified sensor. b) NaOH and HCl SPR Response on plain Au Sensor.

When a solution containing Cu is introduced into the system, an increase in SPR angle is expected due to the copper-amine complex on the SAM. An increase in the angle with increase in copper concentration was not obtained while performing the experiments (Figure 3). The reason for this is thought to be that the amount of SAM deposited on the sensor is insufficient resulting in a significant decrease of sites available for Cu to bind.

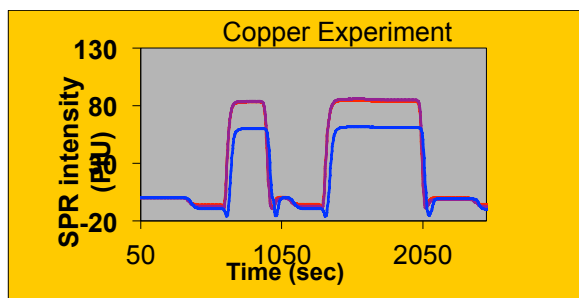


Figure 3. SPR signals for Cu Solutions.

Our current focus is on increasing the amount of SAM on the surface of the sensor by modifying multiple variables in our methodology. One technique we have been using to monitor surface modification is measuring the contact angle on the surface of the

gold (Table 1). Addition of the SAM on the surface of the gold increases the hydrophobicity of the surface. Therefore, the more SAM that is deposited on the surface will result in an increase in the contact angle.

Table 1. Water $\frac{1}{2}$ angle measurements taken at various positions on SAM functionalized Au sensors.

Water $\frac{1}{2}$ Angle $^{\circ}$ of Au-SAM Sensors	
Chip 1	Chip2
48 $^{\circ}$	72 $^{\circ}$
68 $^{\circ}$	72 $^{\circ}$
45 $^{\circ}$	72 $^{\circ}$
70 $^{\circ}$	64 $^{\circ}$
55 $^{\circ}$	83 $^{\circ}$
72 $^{\circ}$	67 $^{\circ}$
62 $^{\circ}$	65 $^{\circ}$
60 $^{\circ}$	71 $^{\circ}$

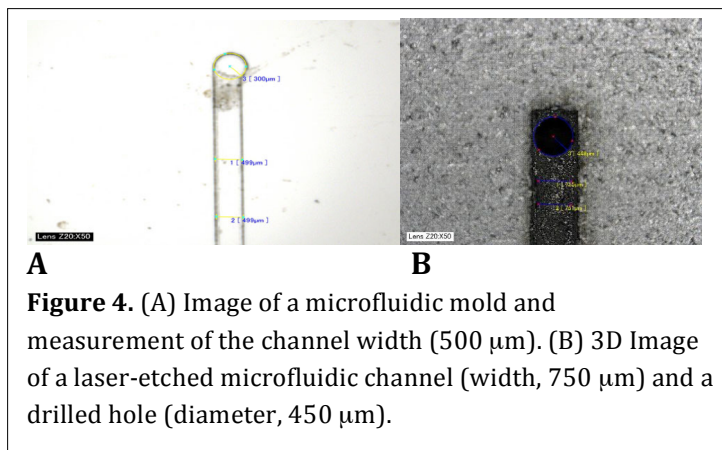
The *Keyence Microscope* has found great usage in our research program due to its capabilities and especially its 3D options. Detailed below are examples of the quality of the pictures that can be taken using the Keyence instrument.

Energy underpins all aspects of modern life. Energy use is directly correlated with broad measures of people's well being, status, and health. The scale and importance of global energy use are matched only by environmental factors.

The future of portable electronics requires efficient and small power sources to operate them. We have been developing microfluidic formate, methanol, and hydrogen fuel cells (FCs). The use of these fuels entails one of the most promising mobile technologies by which such power can be provided. FCs can be considered chemical reactors designed to convert

chemical reactant streams into electrical energy and chemical products. Students involved in this project have included Lenny Sanchez [MS], Chris Darakjian [MS], Mary Arrastia [BS], Mark Aguilar [BS], Samantha Sotez [BS], Kryls Domolaon [BS], Vicente Galvan [BS], Ani Avoundjian [BS], Catherine Tang [BS], Alex Mendez [BS], Santino Valiulis [BS], Franky Bernal [BS], Ricardo Ortiz [BS], Usama Tohid [MS], and Hector Gomez [MS]. Several novel prototypes built on both a paper and poly(dimethylsiloxane) (PDMS) platform and have been developed. Focus has been on optimizing the design of the FCs that has involved a myriad of parameters that will be elaborated below as well as computational and numerical simulations.

In the design and development of FCs, the ability to fabricate them is highly desirable or there is little opportunity for commercialization.



A

B

Figure 4. (A) Image of a microfluidic mold and measurement of the channel width (500 μm). (B) 3D Image of a laser-etched microfluidic channel (width, 750 μm) and a drilled hole (diameter, 450 μm).

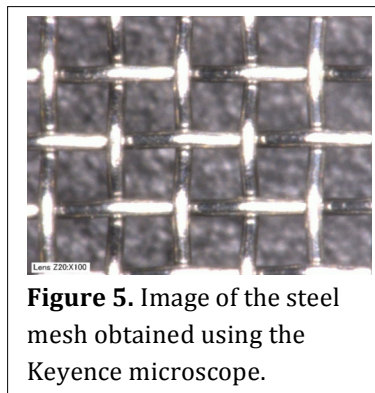


Figure 5. Image of the steel mesh obtained using the Keyence microscope.

Figure 4A shows the image obtained using the Keyence microscope verifying the channel width of a single microfluidic channel. Figure 4B is a 3D image of a laser-etched microfluidic channel containing a hole that has been drilled at one end. Many of the FCs require the use of a mesh to serve as a current collector. In these cases the steel was directly covering part of the surface of the catalyst. In order to calculate the total area (and, thereby, calculate the accurately the power density), it was required to determine the approximate proportion of the total steel mesh in the “open area” compared to the total area. Figure 5 is a 3D image of the steel mesh in a direct methanol FC.