REPORT DOCUMENTATION PAGE

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5c. PROGRAM ELEMENT NUMBER
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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14. ABSTRACT
During the period of 12/19/12 – 02/02/13 of Contract # W911NF-13-C-0017 we performed the following.

We developed a ROC (Receiver Operator Characteristic) curve for benzenethiol (BT) on a polyvinylidene difluoride (PVDF) substrate coated with gold nanoparticles (iFyber substrate). The ROC curve indicates detection levels around 2 x 10-8 ppm of the CWA agent simulant. This is ~ 200x above the target detection level in air. The

15. SUBJECT TERMS
ROC Curve, SERS, Raman, Surface Enhanced Raman, Aerosol Chemical Warfare Agents, Flexible Sensors, Portable Sensor, Portable Raman

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Keith Carron
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Report Title

Phase I Option Plasmonic Nanosensors for Chemical Warfare Agents Final Report

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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 Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

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

Technology Transfer

Phase I Option Plasmonic Nanosensors for Chemical Warfare Agents Final Report

Contract No. W911NF-13-C-0017

Contract Representative:
MKS Technology
Dr. Keith Carron
628 Plaza Lane
Laramie, WY 82070
(307) 460-2089

Title
Phase I Option Plasmonic Nanosensors for Chemical Warfare Agents Technical Report 1

Technical Report for the Period of Performance
12/19/2012 – 02/01/2013

Contract Amount
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Amount Paid to Date:
$16665.60

Total Amount Invoiced:
$49996.82

Number of Employees Working on the Project:
3 employees and 2 Subcontract this month

February 2, 2013

Technical Contact:

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Research Triangle Park, NC 27709-2211
Voice: (919) 549-4293
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CIN: 00102198280003

REQUISITION/PURCHASE REQUEST/PROJECT NO.
0010219828-0001

Issued by: US ARMY RDECOM ACQ CTR - W911NF
4300 S. MIAMI BLVD
DURHAM NC 27703

R&D Status Report
Effective Date of Contract: October 19, 2012
Plasmonic Nanosensors for Chemical Warfare Agents

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We developed a ROC (Receiver Operator Characteristic) curve for benzenethiol (BT) on a polyvinylidene difluoride (PVDF) substrate coated with gold nanoparticles (iFyber substrate). The ROC curve indicates detection levels around 2 x 10^{-8} ppm of the CWA agent simulant. This is ~ 200x above the target detection level in air. The difficulty and pathway to improved detection is discussed.

Figure 1 Experimental setup for data collection of ROC curve.

1. Data collection for the ROC curve for the SERS-DM substrates was initiated

In this section we discuss how we collected data and how a ROC curve for BT was obtained. The data were collected using the Raman reader proposed in Phase I. This device, a CBEx Chemical Biological and Explosive, is battery powered and designed for > 10 hour missions. The CBEx was mounted above a surface and collected SERS data from iFyber membranes. The experimental setup is shown in **Figure 1**.

Spectra were collected for 0.1 second for the new PVDF substrates. 10 averages were made for each measurement. The spectra were collected and stored with Snowy Range Instrument's PEAK software. The data were not smoothed.

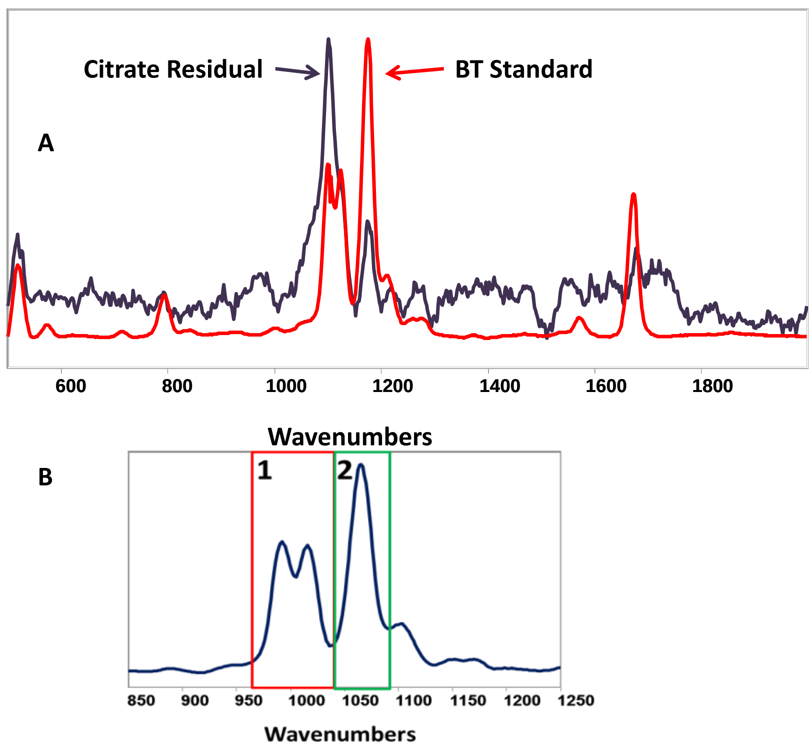

Figure 2 BT spectra on an iFyber gold DM substrate with the blank. A) Note the overlap between residual amounts of citrate from the AuNP synthesis and the BT spectrum, spectral subtraction was used to mitigate the overlap. B) Regions 1 and 2 of interest used for calculations.

2. Post-acquisition data management

A spectrum of 100% ethanol soaked substrates has peaks that overlap with the BT peaks. This is illustrated in **Figure 2**. The spurious peaks have been identified as citrate. Citrate is used in the process to reduce Au^{+3} to Au^{0}.

We used a spectral subtraction routine to remove the blank from every measurement. A baseline correction was also used to better illustrate the spectra.

Figure 3 shows the correlation as a function the concentration. An outlier was observed at concentration 3 (1 x 10^{-5} ppm). This point was repeated and the point below (2 x 10^{-8} ppm) was repeated. New solutions were also made and this "outlier" was still observed. We believe this could be due to a change in the structure of the monolayer. BT is known to tilt at a full monolayer and may be lying flat at the concentration. The preferential enhancement of the normal modes perpendicular to the surface could be the reason for this change.

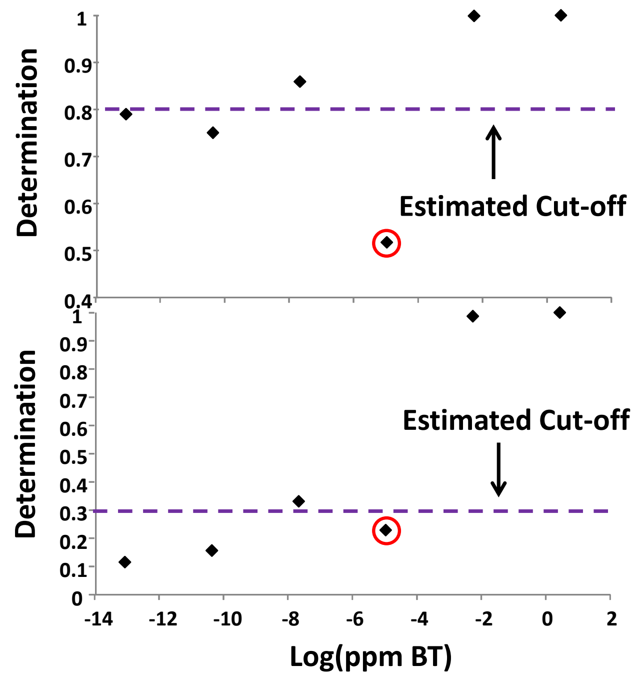

Figure 3 r^2 plot for BT vs concentration. The anomalous outlier is shown circled in red. An estimated (visual) cut-off is shown.

The top plot represents **Region 1**, the 1101-1124 wavenumber doublet, and the lower plot represents **Region 2**, the 1067 wavenumber singlet. Depending on the cut-off used the detection limit will vary. However it appears that 0.8 and 0.3 respectively are reasonable cut-off values, this assumption will be explained later.

Mitigation of Difficulties: We will explore the anomaly at 1.1 x 10^{-5} ppm level in further experiments. A high sensitivity bench top system will be used to better understand this data point. iFyber will examine methods to remove residual citrate with Raman inactive materials. Residual citrate from silver-based SER-DM substrates can be removed using a saline treatment (this replaces citrate anions for chloride anions); however, in the gold-based SER-DM which have vastly improved performance with respect to detection sensitivity, citrate is bound much more strongly and saline treatment is ineffective. iFyber has successfully used Piranha solution (3:1 H_2SO_4:H_2O_2) to remove the citrate background from Au SER-DM, but they have determined that this strong chemical treatment reduces the detection limit achievable by using the resulting substrates. Nevertheless, in the current application of SER-DM for detection of nerve agent analytes, iFyber will develop analyte specific coatings that will remove the adventitiously bound citrate during manufacturing leaving a clean substrate prior to being exposed to nerve agent, and thus, this citrate background is not expected to be an issue.

We estimated where we believe the cut-off for detectability might be, 0.8 and 0.3. This conjecture was made from the current data by determining where the curve appeared to level off. This would be the point where signal to noise is 3 (for a yes/no) or signal to noise = 10 (for quantitation). Since we determined that the SERS materials had an interference from citrate we did not continue to make multiple measurements to determine the statistics necessary to make a statistically justified cut-off point.

4

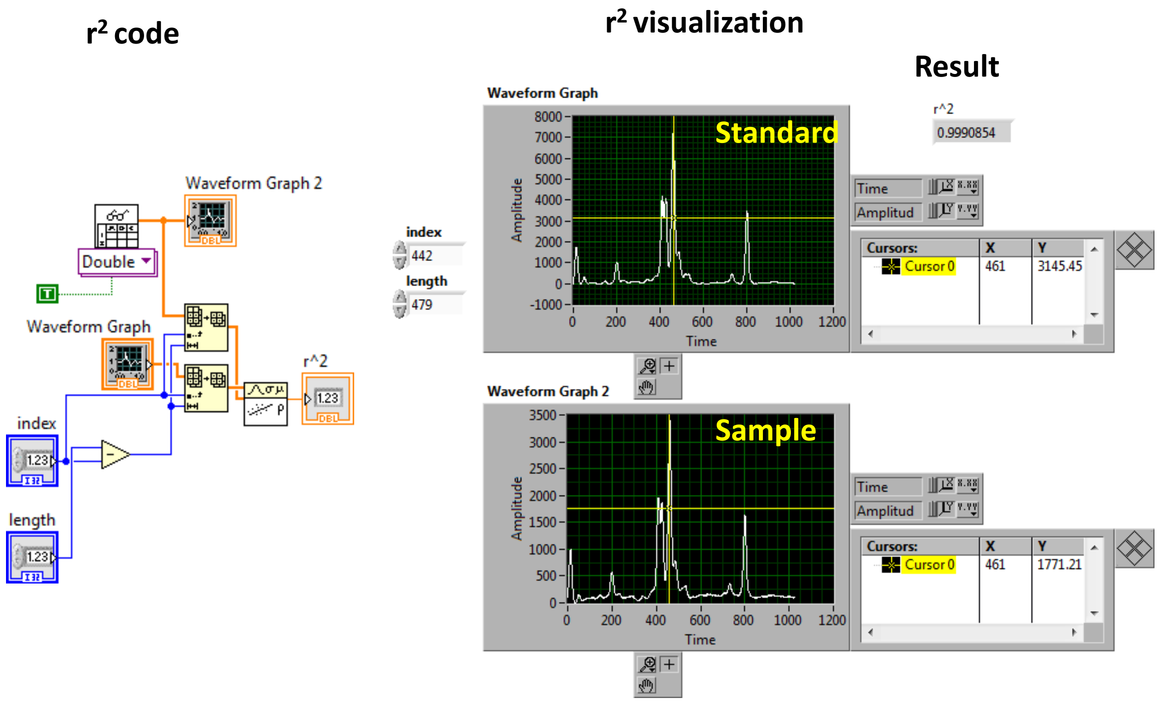

Figure 4 LabView code to calculate correlations against a standard (library) entry and sample spectra. The code allows the user to select a specific region for the correlation.

3. Method of ROC Curve Calculation

A software package was created to choose the region to be analyzed and to compute the r^2 value. The code shown in **Figure 4** loads the spectra as a single column of intensities and it creates a subset in both the standard and the sample. For example, as shown in the visualization we are using the region from pixel **442** to pixel **479**. The r^2, in this case, is between the standard and the 5.5×10^{-3} ppm sample.

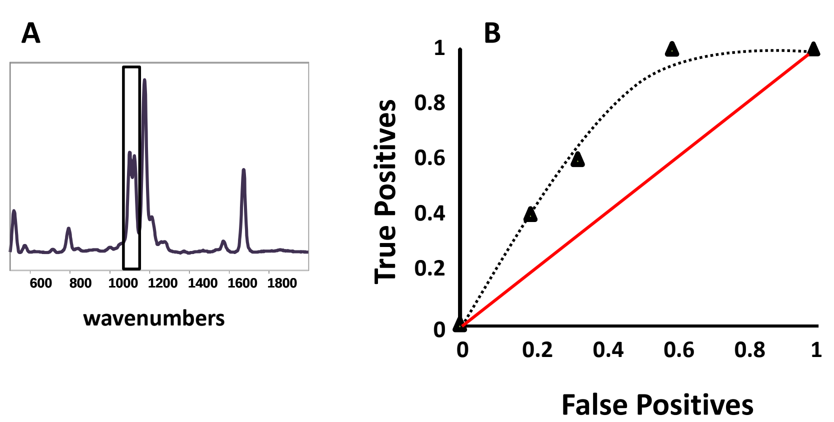

Figure 5 ROC curve produced by a specific spectral region. A) illustrates the region used to form the ROC curve. B) shows the ROC curve. This figure proves that this region and method produces a valid assay.

4. The ROC Curve

Figure 5 illustrates a ROC curve produced with the iFyber substrates and BT in ethanol. Figure 5A illustrates the region used for the analysis. Figure 5B illustrates the ROC curve. Note that the points are above the red line. This indicates that this method is a valid assay for BT. In other words, the number of false positives is always less than the number of true positives. As we improve our method and reduce interferences the curve should improve.

5. Progress in SER-DM substrate performance and production

iFyber SERS substrates have been further refined for improved detection limits and improved substrate-to-substrate reproducibility. Detection limits have been improved by selection of a PVDF base substrate, which resists burning to give interfering background signal and allows for use of greater laser power during sampling. Further, gold nanoparticle (AuNP) size and overall loading amount onto the porous membrane have been optimized (**Figure 6**).

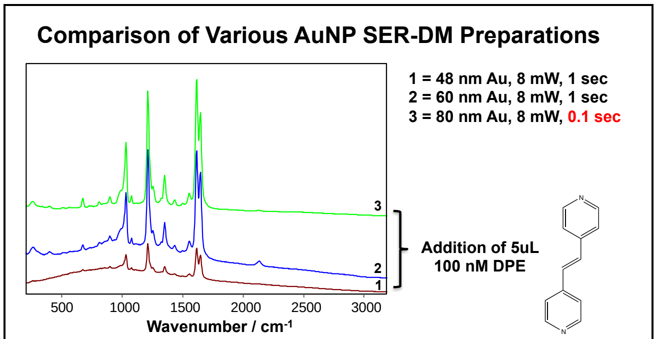

Figure 6 Significant improvements in enhancement factor made to AuNP SER-DM. Various AuNP sizes were assembled onto PVDF porous membranes and assayed a CBEx reader and trans-1,2-bis(4-pyridyl)ethylene (BPE) as a model analyte. AuNP with an 80 nm diameter clearly out perform 50-60 nm particles [note the much lower integration time used for the 80 nm substrates]. It is also important to point out that as particle size is increased, the #AuNPs/substrates decreases (a function of AuNP synthesis). So, enhancements with 80 nm AuNP occur with a much lower overall surface area, and thus, less BPE absorbed onto the AuNP relative to the smaller AuNP sizes.

Ultimately, iFyber aims to produce large volumes of SER-DM to improve on sample-to-sample variability. Currently, using a small-scale laboratory production method that mimics the large scale coating methods planned for Phase 2 (using Kodak facilities), SER-DM can be produced having relative standard deviations of <15% (RSD) in measured signal of adsorbed analytes.

Roll-to-roll processing can potentially offer the best solution to SER-DM substrate-to-substrate variability; however, this type of processing is only practical if hundreds of square feet of material are made in a single run due to the costs associated with operating the equipment and the volumes of base substrate (i.e., based porous membrane) needed to begin processing. For example, 10-15 ft^2 of material is often wasted in roll-to-roll coating just to begin the process. Thus, iFyber is also working on several form factors that allow for the production of highly reproducible substrate arrays (e.g., 96-well arrays for high throughput screening of analytes (**Figure 7**). Depending on the required volumes of SER-DM for a given application, production of substrate arrays may offer a convenient method

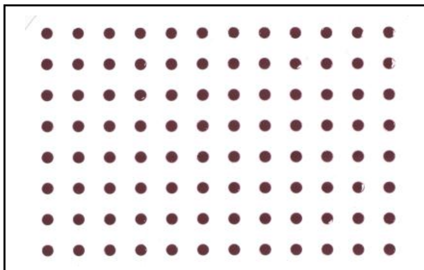

Figure 7 SER-DM substrate array. Current achievable RSD values obtained using this 96 well array is ~30%; however, we anticipate significant improvements in RSD after fixing problems associated with how analytes are delivered to the substrate and how the substrate assayed (both of which are highly variable at this time).

for producing reproducible substrates in a batch type process – a potential alternative to roll-to-roll processing for large volumes of SER-DM substrate. These arrays can be used for screening large amounts of samples in an array reader format, or the substrates can be singulated through a cutting die and used individually.

December 19 – February 2 Budget

			Project Total		Month 1	Month 2	Month 3
Category and/or Individual:	Rate/Hour	Est.Hours	Cost				
PI Keith Carron	45	15	675		225	225	225
EE Mark Watson	60	15	900		300	300	300
ME Shane Buller	52	15	780		260	260	260
Subtotal Direct Labor (DL):			2,355.00		785	785	785
Fringe Benefits, if not included in Overhead, (rate 30.0000 %) x DL =			706.5		235.5	235.5	235.5
Labor Overhead (rate 0.0000 %) x (DL + Fringe) =			0		0	0	0
Total Direct Labor (TDL):			3,061.50		1020.5	1020.5	1020.5
DIRECT MATERIAL COSTS:					0	0	0
iFyber Materials			20,000.00		6666.667	6666.667	6666.667
MKS parts for reader testing			1,190.00		396.6667	396.6667	396.6667
Subtotal Direct Materials Costs (DM):			21,190.00		7063.333	7063.333	7063.333
Material Overhead (rate 0.0000 %) x DM:			0		0	0	0
Total Direct Materials Costs (TDM):			21,190.00		7063.333	7063.333	7063.333
OTHER DIRECT COSTS:					0	0	0
Northwestern University subcontract			17,000.00		5666.667	5666.667	5666.667
Subtotal Other Direct Costs (ODC):			17,000.00		5666.667	5666.667	5666.667
Direct Cost Overhead (rate 0.0000 %) x ODC			0		0	0	0
Total Other Direct Costs (TODC):			17,000.00		5666.667	5666.667	5666.667
G&A (rate 20.0000 %) x (base: TDL+TDM+TODC)			8,250.30		2750.1	2750.1	2750.1
Total Cost:			49,501.80		16500.6	16500.6	16500.6
Fee or Profit (rate 1.0000 %)			495.02		165.0067	165.0067	165.0067
TOTAL ESTIMATED COST:			49,996.82		16665.61	16665.61	16665.61