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13. ABSTRACT (Maximum 200 words)

The original concept of the Phase II+ funding was to support integration of ICx Agentases's chemical weapons (CW) sensing materials into the Fido XT Explosives Detector system. It was determined however that despite marked advances in the enzymatic detection technology through the prototype, significant further work was needed before the Agentase materials would be sufficiently mature for integration. In place of CW detection, the radiation detection capabilities of ICx Radiation (another sister company of Nomadics under ICx Technologies) was selected instead as the intended integration of technologies.
The Interceptor is a mature, handheld radiation detector with gamma and neutron detection capabilities, and upgraded models also have the option of radionucleotide identification from an internal library. The size of the Interceptor was also considered more complimentary to the Fido XT. Given the maturity of the Interceptor, and the significantly lower expected cost of integration, funding was split between the Fido XT upgrade and further R&D efforts on the CW badge prototype, with a view to furthering the technology to a point where integration may be considered favorable.

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Final Project Report

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Date: July 31, 2008

The Fido® product is on the U.S. Munitions List (USML), as defined in the International Traffic in Arms Regulations (ITAR), 22 CFR 120-130, and this document may contain technical data subject to export control.

Introduction

The original concept of the Phase II+ funding was to support integration of the Agentase chemical weapons (CW) sensing materials into the Fido XT system. A prototype CW "badge" had been previously developed that utilized the enzymatic sensing in a liquid cell through which air samples could be drawn and interrogated. It was determined however that despite marked advances in the enzymatic detection technology through the badge prototype, significant further work was needed before the Agentase materials would be ready for integration with Fido.

The CW detector required reservoirs of 2 liquid reagents, in addition to pumps and the flow cell. As such, the size of the original prototype was prohibitive for integration to a handheld combination, and the flow cell of the CW system was susceptible to signal degradation from movement or interrupted liquid feeds with bubbles or disturbed reservoirs. It was deemed that there was too great a technological hurdle to overcome to produce an integrated Fido XT with CW detection under the scope of the initial funding.

Through discussion with the customer, it was proposed and agreed that in place of CW detection, the radiation detection capabilities of ICx Radiation (another sister company of Nomadics under ICx Technologies) would be selected instead as the intended integration of technologies. The Interceptor is a mature, handheld radiation detector with gamma and neutron detection capabilities, and upgraded models also have the option of radionucleotide identification from an internal library. The size and maturity of the Interceptor was also considered more complimentary to the Fido XT. Given the maturity of the Interceptor, and the significantly lower expected cost of integration, funding was split between the Fido XT upgrade and further R&D efforts on the CW badge prototype, with a view to furthering the technology to a point where integration may be considered possible.

Towards the end of the project cycle, some residual monies were directed, with the approval of the customer, towards additional efforts to further the development and advancement of the Fido XT.

Testing of the phenyl quinoline (PQ) based CW reporter, which was funded under the original parent Phase II SBIR, against additional explosive threat analytes including ammonium nitrate and other inorganics, was conducted with a view to expanding the suite of explosives detected by the Fido XT. Additionally, a platform for centralized storage and processing of Fido XT data files collected in house, targeted towards development of hit detection algorithms and system improvements was investigated.

All work on this funding award that deviated from the original Statement of Work was discussed and reviewed with the customer prior to proceeding. The CW work that was continued under this funding satisfied in large tasks 1 and 2 of the original Phase IIB+ SOW, namely identification of the CW detection technology that could be integrated for a CW/explosives detector system; and

miniaturization of the CW detection system for potential integration. The explosives/radiation integration was applied to task 3, the integration of the Fido XT with the orthogonal detection medium.

Summary of Work Undertaken

1. Explosives / Chemical Weapons Integration
2. Explosives / Radiation Integration
3. Expanded Suite of Explosives Analytes
4. Fido XT Data Bank and Algorithm Support

Explosives / Chemical Weapons Integration

Project Expenditure: $225k

Outline

The Agentase handheld CD prototype, provided highly sensitive detection of cholinesterase inhibitors, however, the dimensions of the device, and requirements of nearly 20 mL of liquid reagent to run for a day detracted from the viability of direct integration to the Fido XT. Further, the detection was not in real-time, as it required a 5 minute cycle to complete the analysis cycle of any detected CW agent. Accordingly it was accepted that focusing attention towards making the device both smaller and faster would result in a more useful product, potentially easier to integrate into a Fido or another detection platform.

The concepts that were considered to improve sensitivity, response time and miniaturize the device included;

- replacement of the pH indicator dye (bromocresol purple) with a pH-sensitive fluorophore.
    - fluorescence change should be easier to optically resolve than absorbance change, for a small amount of dye, enabling the use of a much smaller enzyme-laden polymer sponge, which in turn reduces reagent consumption and increased response time.
- replacement of the pH indicator dye with a fluorescent enzyme substrate.
    - fluorescent enzyme substrate is normally non-fluorescent, but becomes fluorescent when cleaved by an uninhibited enzyme, providing a more direct route for reading out an enzyme, using fluorescence.
- construction of smaller flow cells and miniaturized pumps to support the reduction of polymer sponge mass.
    - development of a micropump capable of very small flowrates was required to fully engage the reduced flow cell concept.

Preliminary Concept Work

Using a 490 nm LED, an appropriate filter set, and a large-core fiber coupled power meter, a buffered (pH 7) fluorescein solution was run through the original sponge flow cell, and a fluorescent emission power of roughly 40 nW was observed. Following the addition of substrate, the signal quickly dropped (in a few minutes) to approximately 4 nW. The active enzyme (AChE) in the sponge cleaved the substrate to form acetic acid which lowered the pH, and give that the fluorescent emission of fluorescein decreases with decreasing pH, the observed result confirmed that the fluorescein approach worked in principle.

To measure the fluorescent signal from a much smaller piece of polymer (1 mm diameter. by 3 mm long), it was sized such it would wedge in place in the tapered (~1 mm I.D.) section of a standard transfer pipette. This produced a flow cell sponge roughly ten times smaller (in volume) than the sponge used in the original prototype. Using a large core optical fiber (1 mm) for both excitation from the 490 nm LED (~5 mW of optical power) and for coupling to the power meter head, and an appropriate filter set, the measured fluorescent emission from the buffer solution was 5 nW of. Adding substrate, it was observed that the signal dropped to below 0.5 nW in a couple of minutes. This was the same magnitude of signal reduction from the original flow cell, but in one tenth the reaction volume.

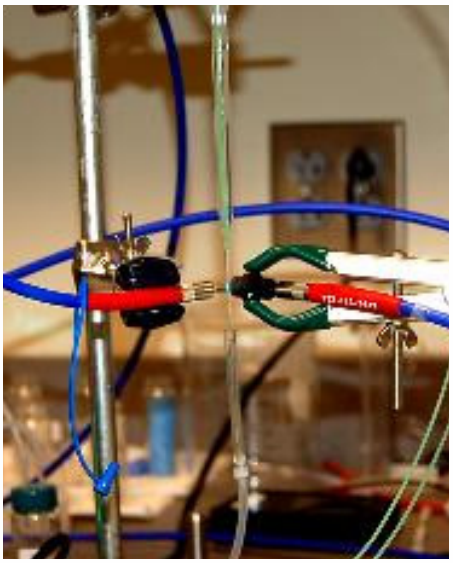

Figure 1 - The reduced volume (3mm^3) flow cell and optical couplings

It was decided to build a breadboard system that would maximize performance and flexibility to test a variety of fluorescent dyes and flow cell geometries The aim of this breadboard setup was to make it as simple as possible acquire quantitative data for a variety of test conditions, and to give the user flexibility to investigate the parameter space of an experiment.

Two Harvard syringe pumps were used to provide precise volumes of liquid reagents to the flow cell, and a small Sensidyne air pump was used to provide air flow. The optical system used an LED for excitation, and a Newport optical power meter to detect the emission. The LED power was computer controllable, and the readings from the power meter were logged via the software as well. The LED, excitation, and emission filters were easily changed to allow testing of different fluorophores. The software that controls the setup permitted both scripted and manual control of all components, allowing for complete user control.

Figure 2 - The breadboard system

The new flow cell was designed to minimize the volume of enzyme-laden polyurethane sponge required. It was theorized that fewer active enzyme sites should require less analyte to inhibit completely, and therefore, a smaller sponge should be more sensitive. Additionally, the smaller size of the sponge should take less time for substrate to diffuse through the sponge and find active enzyme sites, and should therefore be faster. Furthermore, the small volume should require significantly less liquid reagent per cycle, reducing the volume of total liquid consumable volume required.

The favored version of the flow cell featured a 1 mm ID capillary tube, and a ~1 mm^3 (1 µL) volume of polymer. The flow cell was optically accessible from several directions, allowing for flexibility in

probing fluorescence. With this configuration, the air pump (same as the air pump used in Fido) was able to provide several 100 mL/min of air flow, even when the sponge was wet.

Figure 3 - The reduced 1ul flow cell

Fluorescein Results

Using the new flow cell design with the breadboard setup, it was determined that complete acid production—fluorescence decrease—(with uninhibited enzyme) occurred in as little as 30-40 seconds, indicating that cycle times (time to detect analyte introduction) approaching 1 minute could be achievable. Liquid reagent volumes used in this test were 2 µL of substrate and ~10 µL of buffer (rinsing solution), and utilizing 5 minute cycles, the volume consumed in a 16 hour day would be about 2.3 mL. A 1 minute cycle time would require nearly 12 mL of liquid for a 16-hour day.

Fluorogenic Results

Several fluorogenic enzyme substrates suitable for testing were identified as potential reporters: resorufin butyrate, indoxyl acetate, N-methylindoxyl acetate, and 7-acetoxy-1methylquinolinium iodide. Due to availability, the first fluorogenic substrate tested was resorufin butyrate. Testing indicates that resorufin butyrate is not stable in aqueous solutions, and has a tendency to autohydrolyze and become fluorescent without any enzyme being present. It was therefore deemed unsuitable for the sensor as currently envisioned.

Figure 4 - Fluorescence from a vial of indoxyl acetate after with AChE sponge (left), BChE sponge (middle), and no sponge (right),

Indoxyl acetate was fairly stable in aqueous solution, however when tested in the flow cell, the generated fluorophore concentration could not be rinsed away with any reasonable amount of buffer solution. N-methyl indoxyl acetate, is more stable, but equally difficult to rinse out of the sponge.

Several different rinse solutions have been tried: organic solvents, high pH, low pH, etc, but the dye seems to have a very strong affinity for the polyurethane sponge. More aggressive treatments (such as bleach) have been ruled out, as they are likely to damage either the polyurethane sponge or denature the enzyme.

The fluorogenic approach was highly promising yet was incompatible with the polyurethane sponge, however, it was hypothesized that it may be appropriate to use a different matrix for holding the enzyme. Cleaved (fluorescent) substrate was run through the flow cell packed with fused silica glass wool (obtained from Restek). The fluorescent signal was easily washed out of the flow cell by a nominal amount of buffer solution, indicating that the fluorophore has no particular affinity for fused silica.

Ratiometric Fluorescent Dye Measurement

Given the difficulty with the affinity of the fluorogenic substrates to the polymer sponge, it was proposed to return to investigation of the fluorescent, pH-sensitive dyes. It was considered that a ratiometric measurement would provide the benefit of an internal control that would eliminate many concerns regarding intensity shift due to factors other than pH change, thus giving a higher confidence

measurement, These fluorescent dyes would be used in conjunction with the non-fluorescent AChE substrate, acetylcholine chloride, which is broken down to give acetate and choline, resulting in a change in pH.

In addition to repeating the fluorescein work, the use of pyranine, and the concept of using a combination of fluorescein/tetramethylrhodamine as the indicator dye were considered. However both fluorescein and pyranine have emission spectra with one peak, thus excluding them from consideration as it was desired to review the change in the ratio of two peaks / wavelengths with change in pH. The combination of fluorescein and tetramethylrhodamine however met the criteria; fluorescein shows a pH dependent fluorescence emission and tetramethylrhodamine has pH independent emission. Additionally, the combination works in the pH range of interest. The use of two separate dyes with two separate excitation maxima would require the use of two separate excitation sources however, and additional complications in the hardware and software were also likely, due to the requirement for switching between the excitation sources and the detection mode.

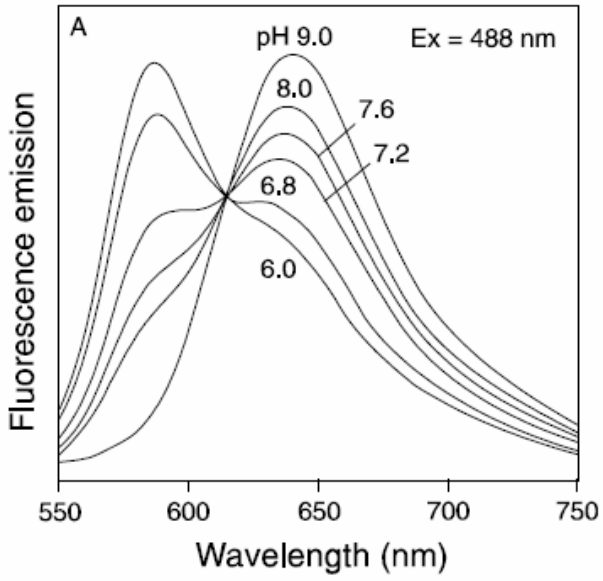

Figure 5 - Emission spectra of carboxy SNARF-1 in 50 mM potassium phosphate buffers at various pH values. Samples were excited at 488 nm (taken from Invitrogen literature on SNARF probes)

The need for alternative dyes led to consideration of SNARF-1 dyes from experience in unrelated research efforts on a separate ICx Nomadics project. It was proposed to proceed with the SNARF-1 dyes, as they were shown to be sensitive to pH change in the pH range of interest for this research, and because the emission spectrum has two separate wavelength maxima, both of which are sensitive to changes in pH (Figure 5). At pH≤6, the 580 nm peak has greater intensity than the 640 nm peak; when pH=6.8, the intensity of 580 nm peak decreases but is still stronger than then 640 nm peak; at pH>7.2, the intensity of the 640 nm peak is greater than the 580 nm peak.

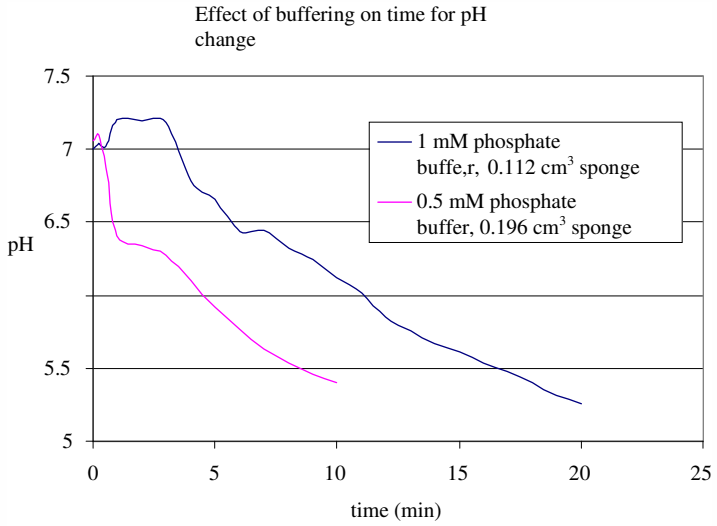

Figure 6 - Response of AChE sponge/SNARF1 at different buffer concentrations in bulk solution

The response of the AChE-containing sponge/SNARF1 dye indicator system on addition of substrate was observed in bulk solution with minimal mixing. A section of AChE-containing sponge was placed in ~2ml of buffer containing acetylcholine chlorine. Aliquots were removed at given time intervals and the pH determined. It took at least 11 min for the pH to change from 7.04 to 6.02 when the phosphate buffer concentration was 1mM, and over 5 min for the pH to change from 7.06 to 5.92 when the phosphate buffer concentration was 0.5mM. With the phosphate buffer concentration at 0.1mM, the pH of the mixture did not change from 6.52.

The observed change in pH was slow, however, the bulk solution environment likely resulted in diffusion limitation into the sponge—i.e. reduced mass transport rate—and therefore masked the anticipated fast reaction. It was anticipated that the response will become apparent more quickly when the interaction is set-up in a flow cell of small dimension.

Reduced Volume Flow-Cell

A new smaller-dimension flow cell was designed to further reduce the quantity of AChE-laden polyurethane sponge required, the quantity of consumable reagents required, and the time per detection cycle. The smaller the quantity of sponge, the fewer active enzyme sites inhibited by less analyte, and the more inherently sensitive the system could be. This also held true for the consumable reagents. The new flow cell dimensions were 0.75 mm ID X 8 mm length, giving a volume of ~3 mm^3. The smaller dimension small flow cell was implemented in the broad-application breadboard fluorescent interrogation setup and was adapted for use in this NCE (Figure 2). The Newport optical power meter used for emission detection in the previous "breadboard" fluorescent interrogation setup was replaced by an Ocean Optics spectrometer (model QE65000). The LED excitation source, and band pass filters were changed to support the SNARF1 dyes.

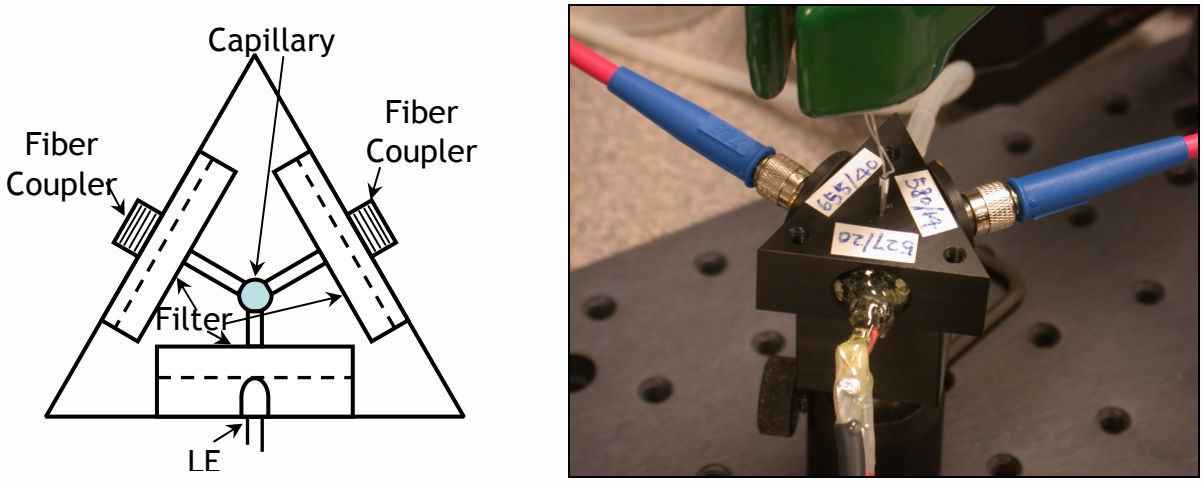

Figure 7 - Reduced volume flow-cell desgined for ratiometric measurement

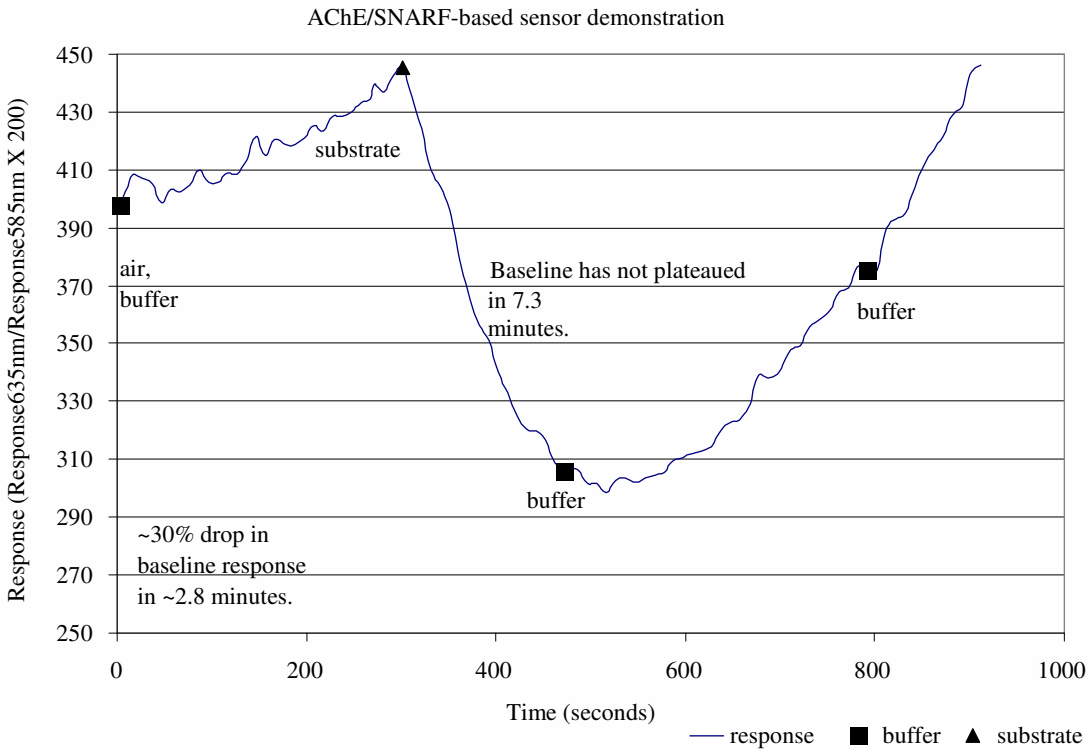

Figure 8 – SNARF-based flow cell demonstration

As the funding of the project drew to a close, during the initial test of the system air was passed through continuously, as movement of micro-volumes of fluids through the system was inadequate in the absence of this additional force. Buffer (0.5 µM phosphate buffer, pH 7.4, containing 1 µM SNARF-1) was then passed through the system at a rate of 6 µl/min. 30 µl of buffer was passed through the system before the "buffer" pump was switched off and the "substrate" pump was switched on. 10 µl of substrate (buffer containing 1.7 µM acetylcholine chloride) was passed through the system at 6 µl/min. The system was then returned back to buffer flow. A further 30 µl of buffer was passed through at 6 µl/min, before an additional 30 µl of buffer was pushed through at 15 µl/min.

Figure 8 details the ratiometric response to pH change during the system operation. While far from optimized, a 10% drop in baseline response could be observed within 45 seconds, although the recovery and return to "baseline" after switching from substrate to buffer was slower than desired. This system was successful at demonstrating that faster responses can be achieved than with the AChE-containing sponge in the bulk solution, when the fluidics are miniaturized.

Future Efforts Considered

Looking forward towards production of a miniaturized "handheld" instrument, a block diagram of the required system component structure (Figure 9), and the sensor flow cell designs (Figure 10) were generated The assembly depicted has a replaceable capillary tube attached to a removable cap (shown in red), to permit the ease of replacement of the sponge media as required.

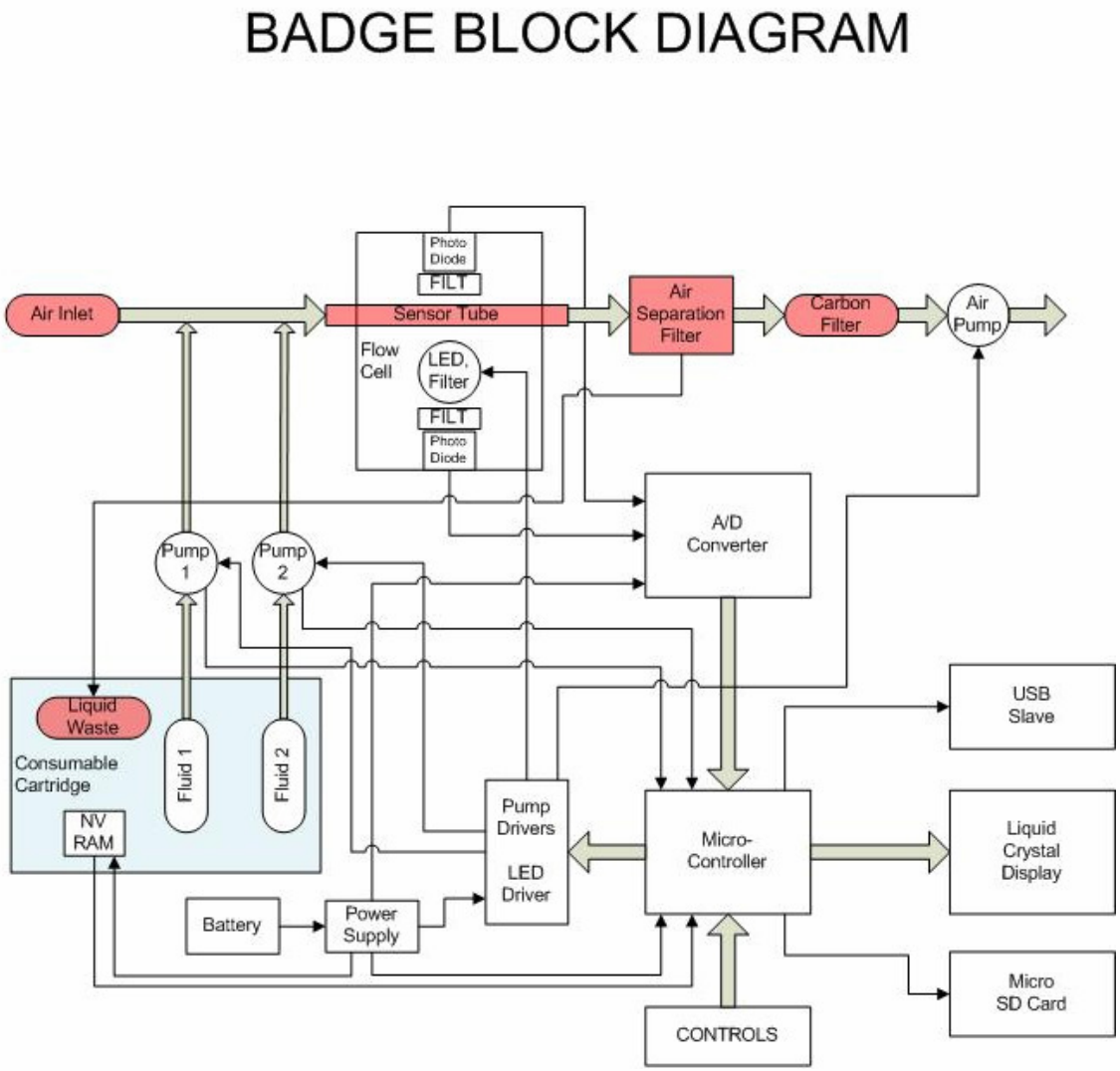

Figure 9 - Block diagram of planned miniaturized AChE sponge/SNARF-1—based sensor

Focused on the flow cell capillary tube are a 525nm LED excitation source, and two photo-detectors band-pass filtered to detect 580 and 655nm respectively for the two emission wavelengths. The two liquid streams (buffer and substrate) shall be supplied to the capillary tube, and air will flow through the tube, and the presence of nerve agents in the air would be indicated by a change in the ratio of outputs from the two photo-detectors. The present configuration is significantly smaller compared to the current bench-top set-up, or original prototype model.

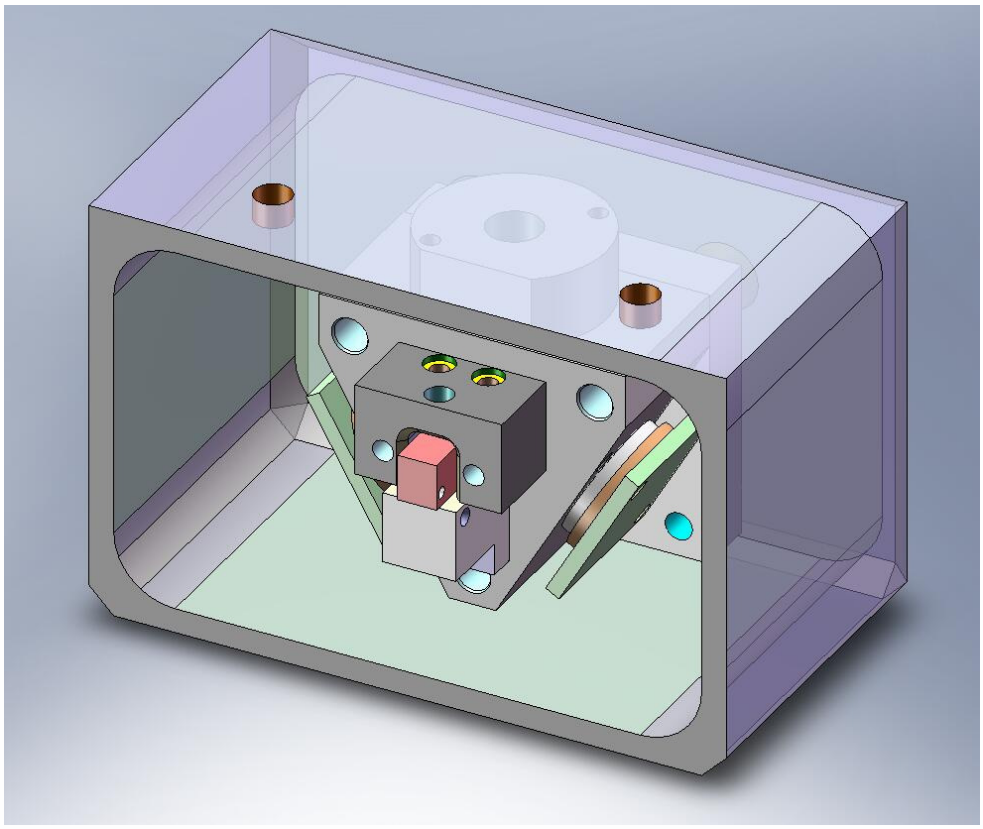

Figure 10 - Assembly drawing of planned, miniaturized AChE sponge/SNARF-1—based sensor

Explosives / Radiation Integration

Project Expenditure: $175k

Outline

The original concept of the Phase II+ funding was to support integration of the ICx Agentase chemical weapons (CW) sensing materials into the Fido XT system. It was observed however that despite marked advanced in the enzymatic detection technology through other funded work developing a handheld prototype, further work was needed before the ICx Agentase materials would be ready for integration with Fido XT. It was then put forward that in place of CW detection, the radiation detection capabilities of ICx Radiation would be employed instead as the intended integration of technologies.

Accomplishments

In discussion with ICx Radiation in Oak Ridge TN, regarding the various technologies available in the product line, it was concluded that the Interceptor, a CZT crystal based detector offered the best combination of size, power consumption and sensitivity. A subcontract with ICx Radiation for a modified gamma detector with supporting NRE was processed. The Interceptor components were configured to operate under a Windows CE processor environment, and to fully integrate to the PIC controller of the Fido XT, some supporting circuitry was required to accompany the CZT crystal assembly. In a parallel effort, the Fido XT head components were modified to accommodate the dimensions of the gamma radiation detector.

An interface module was developed to permit the connection of the CZT module. The interface module consisted of a custom circuit board assembly and supporting firmware, to collect data from the CZT board on a schedule, and present that data to the Fido XT's central processor upon request. The data was processed and simplified, such that the Fido was not dependent on radiation implementation details. The data stream delivered by the CZT system consists of both a gamma ray rate (counts per second) and a radiation dose rate (microsieverts per hour). The gamma rate responds quickly and in real time whereas dose rate responds to radiation changes more slowly, but with added sensitivity and a more accurate evaluation of the health risk of low-intensity radiation sources.

Figure 11 – The orginal concept design (left) ad the actual ombined explosives / gamma radiation detector product (right). Note the marginally deeper Fido XT body to accommodate the dimensions of the attached CZT assembly.

The Fido XT, interface module, and Interceptor were successfully integrated and demonstrated to work together. The assembled prototype is shown in Figure 11, both as the concept design and finished article. The firmware for both the integrated Fido and CZT interface module was completed to allow side by side operation, and it was demonstrated that the addition of the CZT circuitry did not create any electromagnetic interference issues with the original operations of the Fido XT. For safety reasons, the integrated system was only tested with low-intensity sources (around 1 microcurie), as any further testing required at higher intensities needs safety and licensing issues addressed out with the capabilities of ICx Nomadics' Stillwater facility. Figure 12 provides screen shots of the firmware of the modified Fido, which show not only the usual Fido XT explosives detector status, but the new, additional status screen for the CZT detector. The CZT detector was tested against low dose gamma sources such as ^{22}Na, ^{60}Co, ^{133}Ba and ^{137}Cs. The standoff versus dose curves obtained for each nucleotide demonstrated classic first order responses.

Figure 12 - Screen captures for the added CZT gamma detector (top) showing real-time counts and dose rate, and the existing Fido explosives detector (bottom)

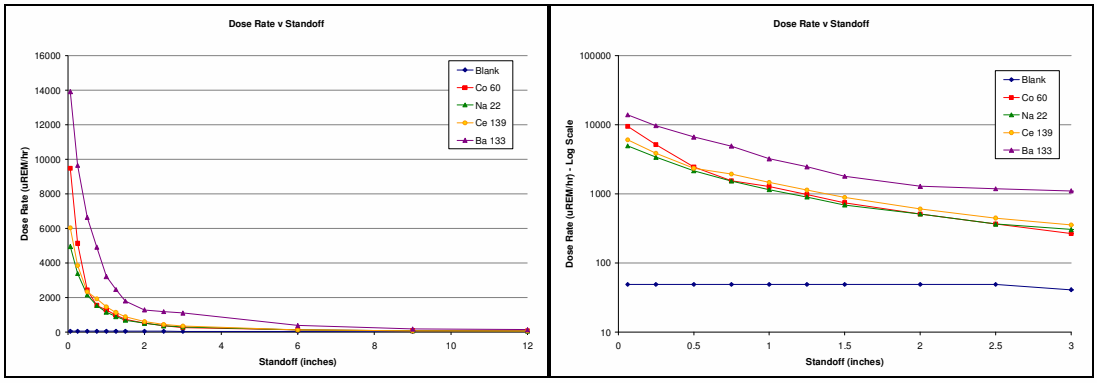

Figure 13 - Dose rate versus standoff curves for selected nucleotides

Discussion

The first planned integration involving the addition of the CZT to deliver real-time gamma count and dose rate readings to enhance the Fido was achieved, and as such satisfied Task 3 of the original SOW if taken into account the change in project direction. It was originally planned that ongoing efforts between ICx Radiation and ICx Nomadics could further enhance the Fido XT with the neutron detection and radionucleotide identification capabilities of the Interceptor product line. Unfortunately there was an engineering and parts bottleneck at ICx Radiation, such that they could not guarantee the timely

delivery of the modified neutron detector to support the CZT. Furthermore, there were significant concerns regarding the viability of including libraries to support the isotope identification, based heavily on the processor limitations of the Fido XT, which was already stretched to support the CZT dosimeter. Additionally the isotope library on the Interceptor was software based for a Windows CE environment, and modification to ensure compatibility with the PIC controller of the Fido, a chipset of the library would need to be built on a daughter board. This was out with the original budget and timeframe of the funding, and as such was discontinued in place of the CW reporter and additional sub-projects detailed below.

Expanded Suite of Explosives Analytes

Sub-Project Expenditure: $35k

Outline

In Phase I of this program, ICx Nomadics demonstrated the concept of surface decontamination assurance via fluorescence-based reporter materials that are reactive with chemical weapons. During Phase II, development continued on the technology for detecting fluorescence changes on surfaces using these reactive fluorescent reporters (RFRs). ICx Nomadics RFRs were designed to provide very high sensitivity and a unique selectivity to the agents.

The RFRs provide dark field detection, meaning that the material is non-emissive in the absence of agent and fluorescent when agent is present. This onset of a fluorescent signal is easily detectable with a simple optical system. The original RFRs were designed to detect G-class nerve agents such as sarin, soman, and tabun, however a modified reporter, with a high sensitivity to acid, and that is easier to synthesize, was developed at ICx Nomadics.

This molecule, shown in Figure 14, has a phenyl quinoline (PQ) group and is protected by a siloxane (thus called PQS). The sensitivity of this PQS RFR to acid has been used for detection of acidic toxic industrial chemicals (TICs) such as HCl, HF, HBr, HNO_3, and H_2SO_4. This sensitivity is due to the protonation of the PQS, as shown in Figure 14, which also generates a fluorescent product due to a change in the structure of the molecule. Much effort, under other funding, has been devoted to characterizing the performance of PQS as a reporter for the detection of acids. Testing with acidic TICs shows strong responses at permissible exposure level (PEL) concentrations with a response time of less than ten seconds for the fluorescence intensity to reach a 100% signal increase. These rapid and large responses at PEL concentrations for five acidic TICs on the USACHPPM Toxic Industrial Chemicals Info Card are shown in Figure 16.

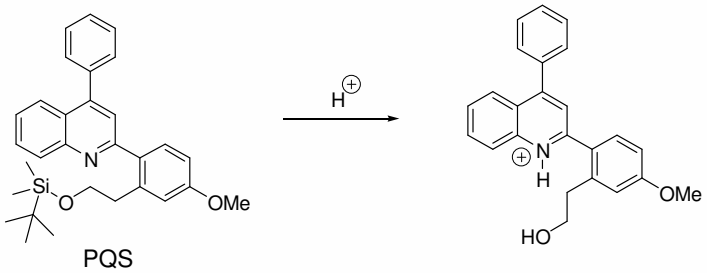

Figure 14 - PQS Acid Reporter undergoing protonation to fluorescent species

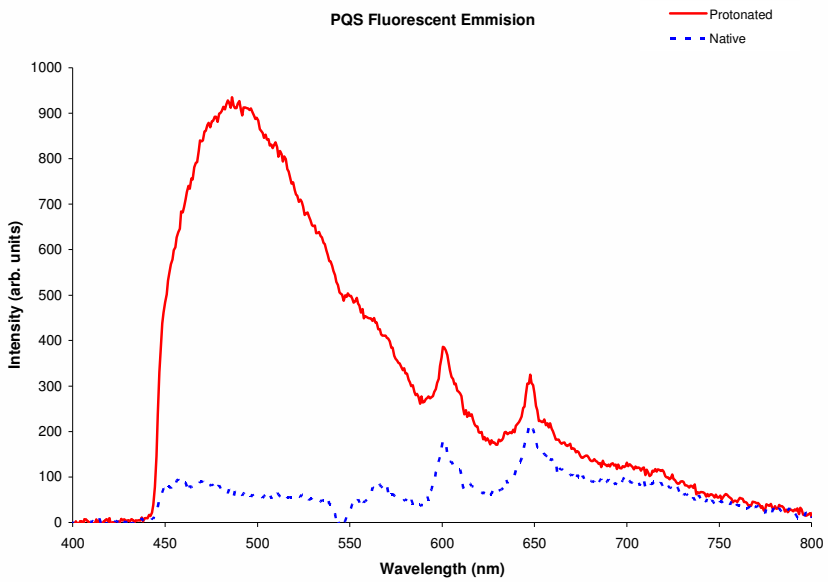

Figure 15 - The Fluorescent emission spectrum of PQS in protonated form

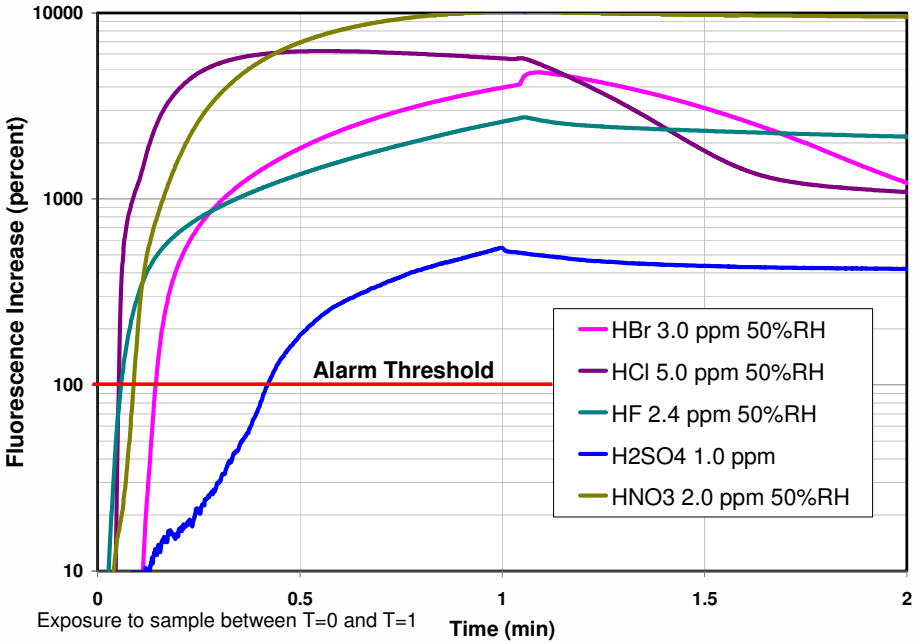

Figure 16 - PQS responses to 5 strong acids

During testing of alternative polymers and reporters for sensing of explosives undetected by the AFP structure currently utilized in the Fido XT, the PQS showed promising results for detection of the

Ammonium Nitrate based explosive ANFO (Ammonium Nitrate Fuel Oil). Thus, it was considered to evaluate one of the PQ-based reporters developed in Phases I and II (originally for direct CW detection) of this contract; for its capacity to detect Ammonium Nitrate, estimate its Limits of Detection (LODs), and to perform some preliminary screening for possible interferents to the reporter. When used in conjunction with Ammonium Nitrate Fuel Oil (ANFO), the suspected reaction is that PQ responds to Nitric Acid (HNO_3) that is produced by the vaporization of Ammonium Nitrate within the ANFO compound. ANFO is composed of ~94 wt% Ammonium Nitrate, 6 wt% Diesel Fuel.

Accomplishments

The PQS reporter was prepared in a chloroform solution at 25mg/ml. Phenylmethyldimethlsiloxane (PMDMS) was also included at 100mg/ml to increase analyte retention and improve coating characteristics on the silanized glass capillaries. Sensing elements were spun coat with the PQS/PMDMS solution. As a dark field reporter, PQS increases in brightness (flares) when activated. Sequential flares dropped in intensity, and with each repeated exposure, the baseline decreased more rapidly. After exposure, baselines did not return to their initial brightness (~150K-350K counts). Ongoing work has focused upon increasing the capacity of PQS to recover back to its non-emissive form after exposure

Initial PQS Concentration Series for HNO_3 and AN:

To demonstrate the reporter's sensitivity to analyte levels of known mass, known volumes of HNO_3 and Ammonium Nitrate mixed at varied concentrations in methanol were dosed ontoswipes and presented to the Fido. By spiking a know volume of a fixed concentration onto a swipe surface, and allowing the methanol solvent to dry, a residue of known mass could be generated. Each capillary had a series of 5 to 10 exposures of a given mass. The exposures were ~3 minutes apart except for the first and second 100ng HNO_3 doses and the 8^{th} and 9^{th} 10ng HNO_3 doses. The Fido was operated at elevated temperatures (Tip=145ºC, Polymer Zone=60ºC), and the swipe presentation duration for each exposure was approximately 10 seconds. Ch2 is not graphed because it is typically less sensitive; however, Ch1 versus Ch2 sensitivity depends on the thickness of the PQ coating throughout the capillary.

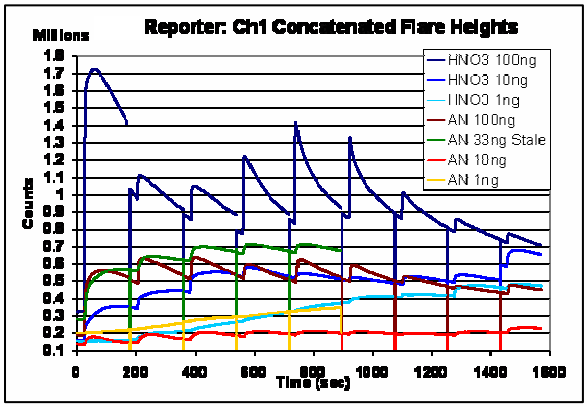

Figure 17 - PQS responses to HNO3 and AN samples

Analyte, Date, #Flares / #Samples	Ch1 Avg Start Height	Ch1 Start Height Stdev	Ch1 Avg Flare Height	Ch1 Flare Height Stdev	Ch1 Avg NDA (%F)	Ch1 NDA (%F) Stdev	Ch1 Maximum % Flare
HNO3 1ng 20070823 7/10	345862	132062	19405	12935	5.23	3.78	**10**
HNO3 10ng 20070823 10/10	442788	112703	54842	50042	19.55	31.00	**105**
HNO3 100ng 20070910 10/10	769469	178321	359200	409933	70.04	127.56	**428**
AN 1ng 20070821 1/5	272501	0	882	0	0.24	0.00	**0**
AN 10ng 20070821 9/10	180700	28099	23499	12923	15.00	10.07	**30**
AN 100ng 20070910 10/10	451801	97064	107295	102717	34.61	57.06	**195**

Table 1 - Summary of LOD data for HNO3 and AN

Generally, the series responses seen from 100ng of Ammonium Nitrate (AN) were closest to the responses of the 10ng of Nitric Acid (HNO_3) series, indicating the conversion from AN to HNO_3 is ~10%. However, if based on first hit magnitude, the conversion appears closer to 30% since the 33ng AN and 10ng HNO3 first dose responses are closest in magnitude. For these PQ tests, at concentrations greater than 1ng for HNO3 or AN, the first exposure to PQ gave the largest flare.

PQS Sensitivity to ANFO and UN

To verify that the reporter was sensitive to ammonium nitrate mixed with diesel, Ammonium Nitrate Fuel Oil (ANFO) was mixed in typical ratio (94% w/w AN: 6% w/w diesel fuel). Figure 10 (below) displays responses from PQS to varied masses of ANFO dissolved in methanol, then dosed on swipes, allowed to dry, and presented to a Fido XT running the PQS-coated, modified sensing elements. For each mass, a separate capillary was used. PQS was also tested for sensitivity to Urea Nitrate (UN), another nitrate based binary explosive. The following table and figures demonstrate the response capacity seen. Since the reporter was not freshly prepared, information from the Maximum response from each mass tested is most applicable; fresh reporter solutions may even have increased response.

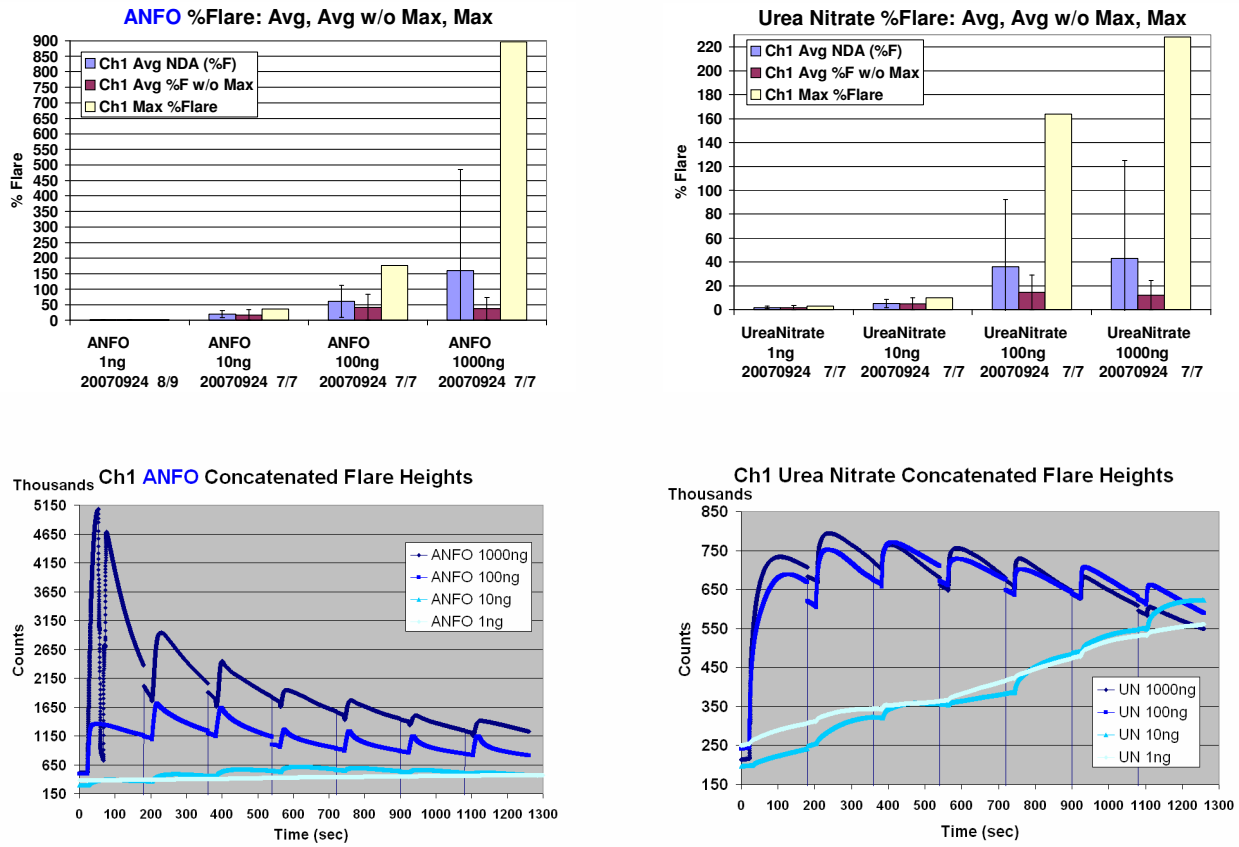

Figure 18 - PQS responses to ANFO (left) and UN (right)

If the results from all series of Ammonium Nitrate, Nitric Acid, ANFO and Urea Nitrate tests are compared on the basis of the Max % Flare; Nitric Acid causes the highest response, with responses of ANFO, AN, and Urea Nitrate being ~ 30% as responsive at the 100ng level.

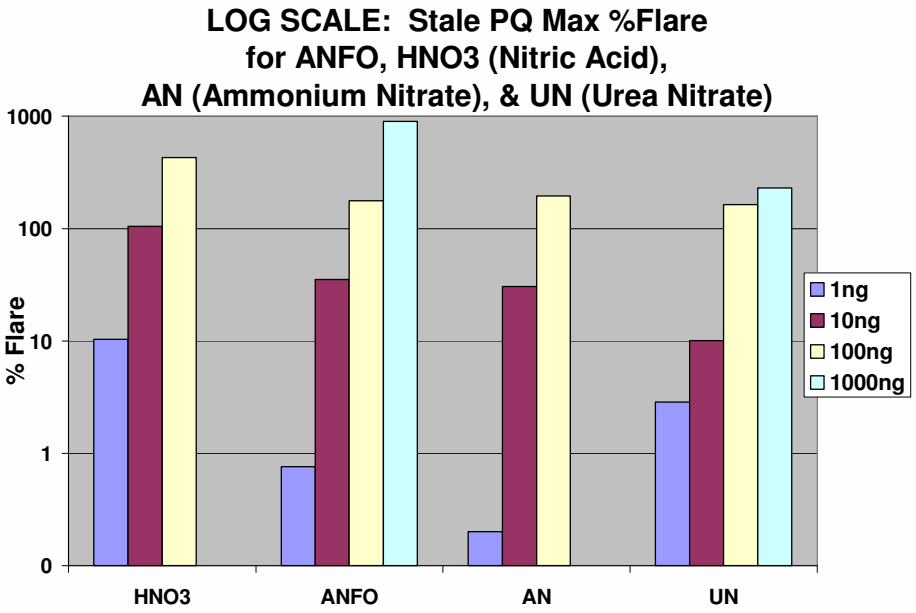

Figure 19 - Comparion of PQS maximum response

Analyte Mass	**HNO3** Nitric Acid	**ANFO** Ammonium Nitrate Fuel Oil	**AN** Ammonium Nitrate	**UN** Urea Nitrate
1ng	10	**0.8**	0.2	2.9
10ng	105	**35**	30	10
100ng	428	**176**	195	164
1000ng		**897**		229

Table 2 - Comparion of PQS maximum response

Data Consistency – Aging of Reporter:

Much of the preliminary data collected for PQS thus far, was taken with solution that was later determined to be stale. The stale solution was mixed in May 2007 by combing two stock solutions (Phenyl Quinoline in $CHCl_3$ and PMDMS in $CHCl_3$) that had been sitting for an unknown period of time. It was later discovered that the May 2007 mixture had gone bad, in that the starting counts for the solution were much higher than freshly mixed solutions and that the "stale" solution's sensitivity and capability to recover were severely decreased from those of freshly mixed solutions. The following figures depict the response from the stale PQS solution, with which most of the data presented in this report, was taken, along with a freshly mixed solution from freshly stocks. While the recovery is clearly different, the absolute sensitivity observed is with a factor of 2.

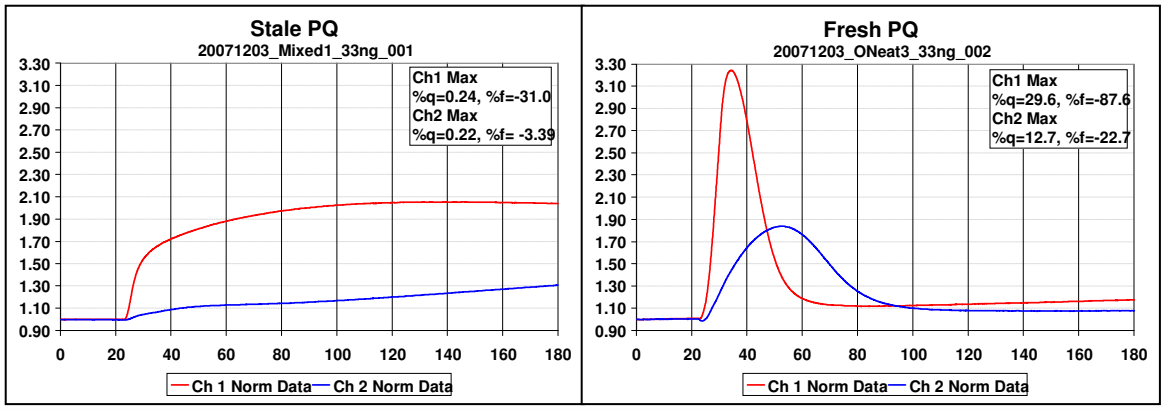

Figure 20 - Effects of aging upon response characteristics of PQS solution

To date, it has not determined whether the increase in starting baseline counts, decrease in sensitivity, and longer rise/fall times of the reporter is due to cyclization of the PQ in solution, reaction of the PQ with $CHCl_3$, reaction of the PMDMS with $CHCl_3$, or some other cause. However, recent work continued on alternative funding suggests the reaction causing decreased sensitivity and longer response and recovery occurs even in the stock solutions of the PQ in CHCl3 or PMDMS in CHCl3 or both, not simply in the combined mixture of the PQ and PMDMS.

Another indication of aging in the reporter is that since the baseline does not recover after the first exposure to target materials; the first dose to a stale reporter almost always causes the largest increase in brightness, with a large drop off in sensitivity for following responses. For freshly made, freshly mixed solutions, the flare magnitudes are much more repeatable, and the first flare is not typically significantly larger.

Fido XT Data Bank and Algorithm Support

Sub-Project Expenditure: $50k

Outline

The purpose of this effort was to make available an organized data repository of unique explosive trace files and blank trace files from Fido XT and Fido S systems currently being used in the Nomadics laboratories. The database was intended to house extensive information about each trace file collected including such information as analyte, experiment goal, sampling method, sampling surfaces, and over twenty other parameters. The final end goal of the repository was to provide a gold data set for the evaluation of automatic detection algorithms.

The following steps were planned to achieve the purpose above:

1. Create an organized data storage structure,
2. Create the necessary database for the structure.
3. Derive a method for uniquely identifying trace files.
4. Create an application for entering data into the database while simultaneous moving the files into the unique storage area.
5. Add modify/update functionality into the tool created in Step 4.
6. Create an application to allow to query against the database and allow a potential algorithm developer to select a subset of samples
7. Deploy sample algorithms that have been deployed in house
8. Test algorithms against the database categories.

Accomplishments

Given the short duration of effort and the limited funds, significant work was accomplished on the project, with the results detailed below. The basic organization of the database dictates the top level object of the database as the experiment. From there, each experiment has multiple runs, each of which has documented instrument settings, an associated trace file, and links to the main analytes table. Figure 21 below shows the database structure in its entirety.

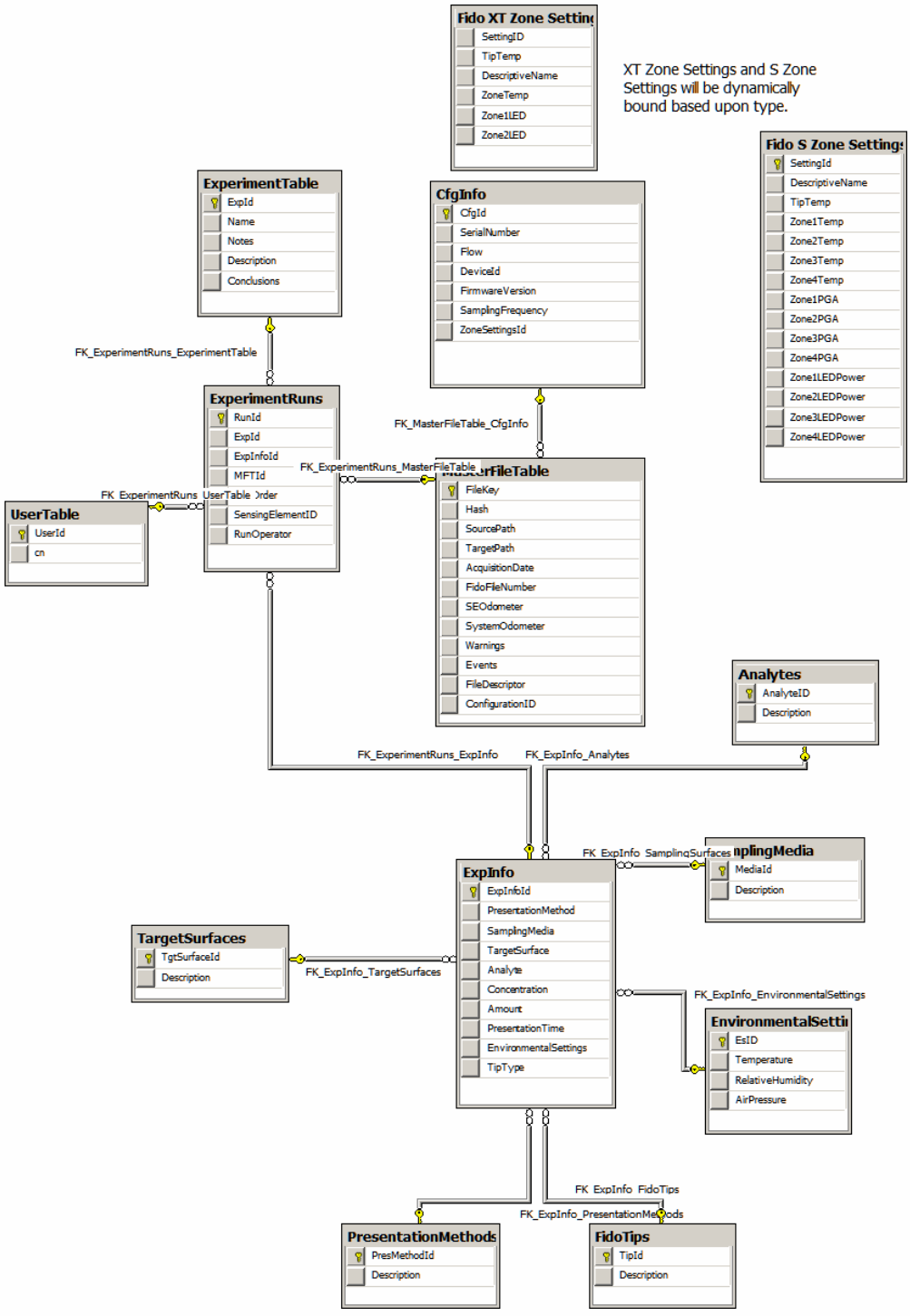

Figure 21 - Fido Data File Storage Structure

To uniquely identify and store the individual files, the entire contents of the data file was hashed, and then moved them into a secure location on the central file server. The hash was stored in the Master File Table (MFT) in the database, and all queries to get a copy of the file should come from that location. Figure 22 details the file database structure, noting the CSV file called Master.CSV which stores a backup copy of all the information about each file that was entered into the database. This approach to data redundancy ensures that any loss to the database should be insured, with the majority of the data will still available for recovery. Currently, there are over 3800 files in the database, with more being added every day.

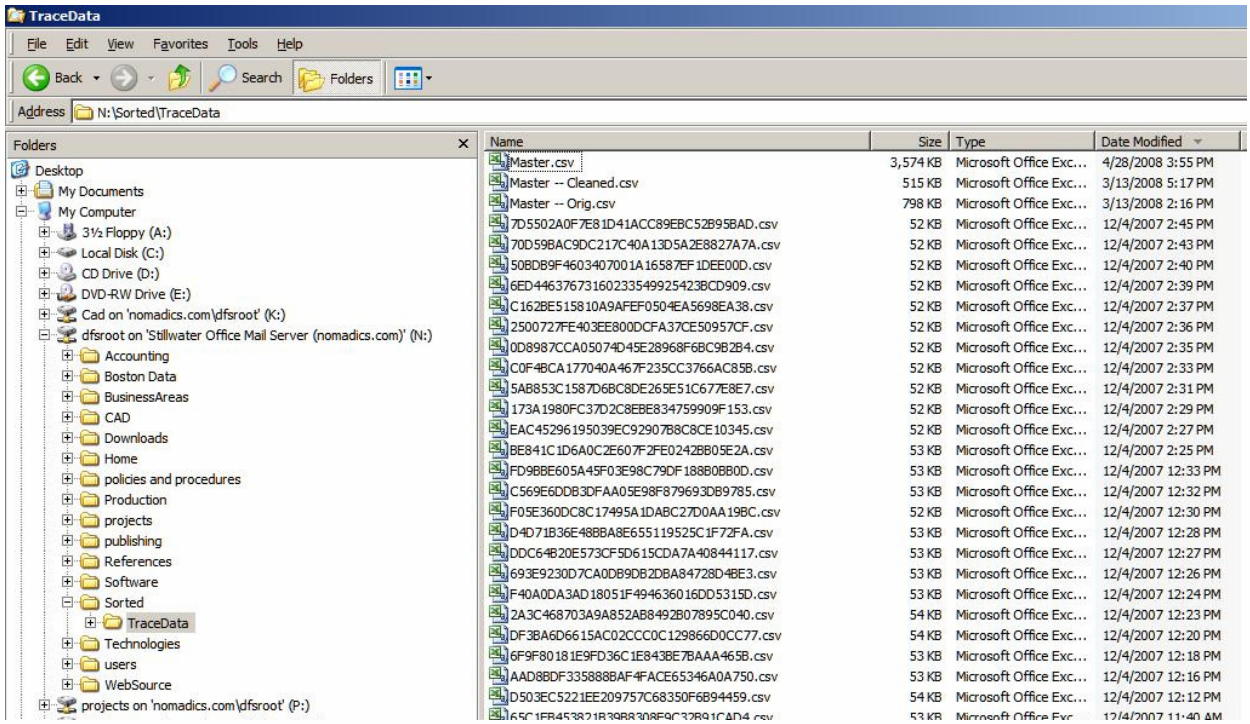

Figure 22 - Hashed File Database

The application created for entering the data was named *Fido File Sorter*, and provides multiple tools to facilitate simple entering and gathering of data about the file. The software provides a regular expression engine for extracting information directly from the file name, and the tool itself opens the files and harvests as much of the information as possible; to directly fill in the metadata fields for each entry.

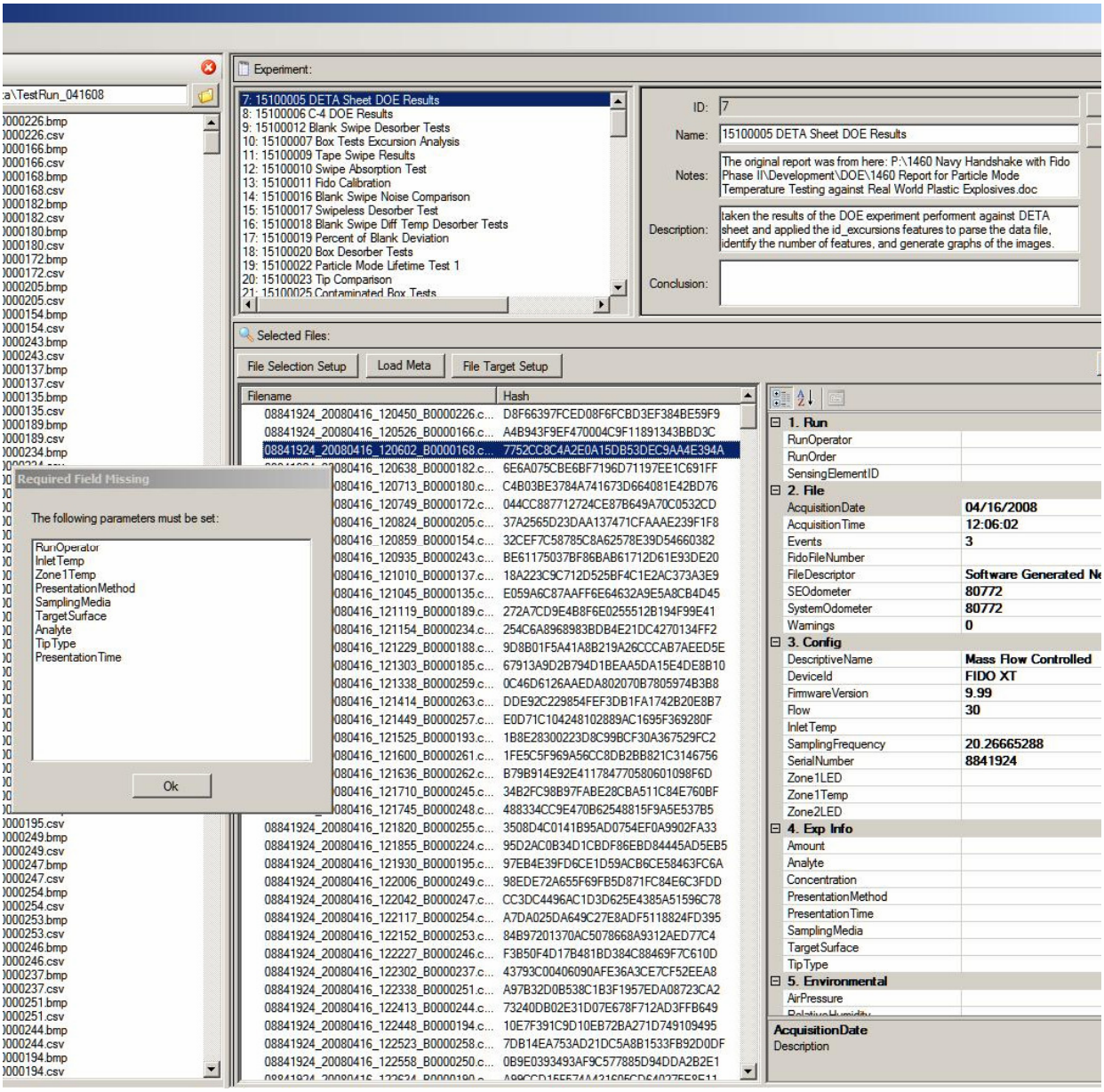

Figure 23 - Fido File Sorter

Discussion

Given the late nature of the application's creation with the remaining funds, the work on the database was stopped after the creation of the database and the import of the data files to hand. There was insufficient funding to support development and testing of algorithms, however it is hoped to continue

the efforts and maintain the database with the information such that it will remain a resource for further and future tests.

Discussion and Further Work

The CW prototype has undergone significant improvements as a result of this funding. The new system is smaller, faster to respond, and has lower reagent requirements because of the reduced flow-cell. The core technology is less complex and show require lower power draw as a results of these achievements. The real-time, sensitive, and portable detection of CW is possible with the technology that has been developed with the assistance of this funding.

The combined radiation/explosives Fido XT is functional both for explosives capabilities on par with a regular Fido XT, and gamma detection from the Interceptor. There is still the opportunity to upgrade the radiation specifications to that of a full Interceptor should the interest remain.

The additional work on expanded analyte suite and data processing have both furthered the sustaining engineering of the Fido XT product line, and are both key interests in the new developments of future products. Work on the PQS reporter for ANFO and UN has continued on alternative funding, given the interest it has generated. Interferent testing and additional inorganic species are currently undergoing evaluation.