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FINAL TECHNICAL REPORT

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Streamlining Physical Evidence Collection and Forensic Analysis

Submitted by:

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I. Introduction

The focus of research under Contract 2004-LP-CX-K048 was directed at two major thrust areas: (1) Molecular Tools targeting improved methods and approaches to obtaining and characterizing trace levels and/or degraded DNA for forensic uses; and (2) Analytical Tools targeting the use and development of emerging or new tools in biotechnology that could be directed at improving, streamlining, and enhancing the processing of physical evidence of a biological or chemical origin. The key personnel involved and major accomplishments in both of these areas are summarized in this report (Section II) since experimental details were provided in the quarterly reports. In addition to the key personnel more than 10 undergraduate students were involved in different aspects of the research conducted. In Section III, we provide details on the Work Product that includes publications (published, in press and in review), oral presentations, poster presentations and patents that have resulted from the work effort to date. We also have made significant progress in establishing partnerships/collaborations with forensics groups both in the state of Florida and elsewhere (e.g., Forensic Laboratory, Fort Lauderdale, FL, Armed Forces DOD Armed Forces Institute of Pathology, DNA Identification Laboratory, Washington, DC, and Tennessee Bureau of Investigation, Knoxville, TN; See Section IV and attached letter).

II. Summary Accomplishments

A. Molecular Tools

Key Personnel: Dr. D. Brown, Dr. S. Coticone, Dr. A. Goebel, and Dr. M. LePuil

Major Accomplishments

- Developed new and rapid method for the isolation of trace levels of severely degraded DNA from bone utilizing modulated and focused acoustics (Covaris™ E200) that yields amplifiable DNA suitable for individual identification using standard methods
- Streamlined DNA extraction, processing, and minimized sample handling
- Developed new primers set or degraded for mtDNA that target hypervariable regions outside the D loop
- Developed a collaboration with the DOD Pathology Laboratory, Armed Forces DNA Identification Laboratory to identify soldier's DNA from burned bone fragments

The specific deliverables addressed in this focus area of the proposed studies are the development of use of novel, modulated and focused acoustics technologies to rapidly and efficiently isolated trace amounts of DNA from bones that have been burned or otherwise degraded. Our studies addressed the need to be able to isolate trace levels of both nuclear and mitochondrial (mtDNA) or trace levels of degraded DNA (in particular in burned bones) in a state that is suitable for PCR amplification and individual identification. We have develop a DNA extraction and processing methodology that minimizes sample handling using a very new technology that exploits modulated and focused acoustics. This technology is provided in an instrument made by Covaris™ Instruments (Waltham, MA) that allows for rapid DNA isolated from burned bones without degradation of the DNA do to heat and/or sheer common to all other DNA isolation technologies. This technologies yields high quality DNA from burned bones even when DNA is only present at trace levels and is badly degraded. The isolated DNA from the burned bones obtained in this manner was suitable for amplification. The DNA was readily amplified using commercial STR primer sets for diagnostic genes for DNA-based human identification, allowing for individual identifications. Several presentations have been made on the progress and accomplishments in this thrust area and manuscripts are in preparation. These accomplishments are listed in Section IV.

B. Analytical Tools

Key Personnel: Dr. R. Alberte, Dr. J. Barreto, Dr. T. Beatty, Dr. J Biggerstaff, Dr. C. Coates, Dr. S. Coticone, Dr. R Coty, Dr. T. Dubetz, Dr. M. LePuil, and Dr. H. Walsch-Heany

Major Accomplishments

- Developed the use of multi-spectral inferometry for rapid and sensitive body fluid analyses on fabrics and other materials
- Expanded the utility of the ASI multi-spectral optics system to achieve multi-color (>7 colors simultaneously) FISH detection and 3-D imaging for trace detection with collaboration from the optics group at DOE ORNL
- Utilized modulated focused acoustics with modern spectroscopic methods and 4th derivative analyses to provide a rapid and sensitive means for trace fiber analysis – dye composition fingerprinting – that minimizes sample handling that reduces time of processing analysis from days to minutes
- Utilized new high resolution Time-of-Flight mass spectrometry (TOPF-MS) technology combined with Direct Analysis in Real Time (DART) to rapid (minutes) identify, fingerprint and established longevity of arson accelerants
- Utilized DART TOF-MS technology to rapidly fingerprint and characterize body fluids (salvia and blood) in collaboration with Jeol USA marker of the DART TOF-MS system
- Utilized DART TOF-MS technology to rapidly fingerprint and characterize inks compared with standard forensic analytical techniques

We pursued the developed of several new analytical tools for trace detection and as a means to streamline physical evidence processing. These included the development of rapid and sensitive means for fiber analyses, ink analyses, accelerant detection and characterization and body fluid detection and analyses. More recently we expanded our investigations of fiber and ink analyses and these data are provided below in Section III.

The Applied Spectral Imaging Microscopy system provides the technology for addressing the issues discussed above. By acquiring the full spectral signature of common surfaces (i.e. ***background***) and the full spectral signature of several substances sought after in crime scene investigation (i.e. *specimen*), it is possible to build a library that can be used as a reference when analyzing an unknown sample. The ability of spectral analysis with the support of this library allows accurate detection of important trace substances in-situ, with significantly reduced sample preparation.

We showed direct detection of body fluids on fabrics and identified such fluids as blood and saliva using multi-spectral inferometry imaging techniques couple with FISH technologies. In order to develop and refine the multi-spectral systems we exploited well characterized bacterial systems and FISH technology to expand the number of markers than can be determined in forensic samples. We demonstrate multi-color imaging with up to sever color markers at the same time without extraction or significant sample processing. We anticipate broader applications of this technology for forensics. Body fluids like blood, semen, saliva and vaginal fluids are naturally fluorescent. Pattern wounds or lividity marks present different colors that penetrate at different depths within the skin of a victim. Bones and enamel of teeth will strongly fluoresce under blue light in range of 425-500 nm. Certain toxins and antibiotics such as tetracyclines that accumulate in bone osteons are also fluorescent or can fluoresce when properly excited. These examples demonstrate the potential expanded of multi-spectral inferometry in forensic science and this technique will find applications that would allow detection of trace substances such as those mentioned in-situ on suspected surfaces such as fabrics, flooring materials (e.g. concrete, linoleum).

We also explored the use of DART TOF-MS technology for analyses of body fluids to address the need to rapidly identify body fluids, often on fabric or other materials, without extraction or processing. We were able to show that salvia and blood could be rapidly (<2 minutes) identified based on marker chemistries resolved by the DART mass spectrometry, on solid substrates or as liquids. The fingerprints of the chemical composition of the fluids were reliable for identification of sources – this, it was readily detectable whether the salvia was from two different individuals. These efforts will continue to establish their broader utility in forensics (manuscripts in preparation).

The key findings and accomplishments on fiber analysis aimed at streamlining sample processing has revealed that the Corvaris acoustic technologies described for trace DNA isolation is also very effective for the rapid extraction of dyes (<1 min) from small fibers, and that thee dye compositions can be profiled and compared by absorption and 4th devastative spectrophometry in less than 1 hr. Various traditional methods (sample extraction, HPLC, etc.) and the acoustic and optical methods were compared. Rigorous statistical analyses were conduced on 4th dx data to ascertain whether, with certainty this analysis could be used to provide 99.9% confidence in establishing similarity or difference in two samples that were comprised of dye mixtures that varied in composition by 1-2% (wt/wt). The analyses revealed that this was not possible, however the statistical test have not been conducted on actual fibers to determine whether fibers which appear identical in color and structure represent different or the same dye lots. These efforts are still underway and require additional mathematical analyses and variations in the use of the 4th dx algorithms.

The DART TOF-MS technology was also exploited to assess the ability to rapidly identify and characterize various accelerants that might be used in arson cases. The DART technologies allows for rapid chemical fingerprinting of chemicals typically used as accelerants and can readily distinguish different gasoline sources. The studies reveal that different accelerants could be rapidly – minutes –identified and characterized, and their stability or persistent over time assessed. This technology has great promise for arson investigations (see Manuscripts in review).