Final Report
For
CRADA Program No. ORL-SC-95-09
With
PerSeptive Biosystems, Incorporated

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Laser Desorption Time-of-Flight Mass Spectrometer DNA Analyzer

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- I. **Principal Investigators:** C. H. Winston Chen (ORNL) and Steve A. Martin (PerSeptive Biosystem Inc.)
- II. **Budget:** \$50,000 at ORNL
- III. **Goal:**

The purpose of this Cooperative Research and Development Agreement (CRADA) between Martin Marietta Energy Research System Inc. and PerSeptive Biosystem, Inc. is to develop a laser desorption time-of-flight mass spectrometer DNA analyzer which can be broadly used for biomedical research.

IV. **Background:**

DNA analysis is critically important for biomedical research, disease diagnosis and forensic applications. At present time, gel electrophoresis is the only method for routine DNA analysis. However, gel electrophoresis is a very time-consuming and labor extensive process. Thus, it is very desirable to develop a faster and less expensive technology for DNA analysis.

Since DNA analysis by gel electrophoresis is based on the different drift velocities for different sizes of DNA, it is natural to consider the use of a time-of-flight mass spectrometer to do DNA analysis. However, "soft" desorption and ionization of DNA is required since a mass spectrometer can only detect ions in gas phase. In 1987, Hillenkamp and his co-workers discovered that large protein molecular ions can be produced by laser desorption without much fragmentation

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if these biomolecules are mixed with organic compounds that serves as matrices for strong absorption of a laser beam. This process is now called matrix-assisted laser desorption (MALD). The typical preparation technique of MALD is to dissolve biomolecular samples in solution, then prepare another solution that contains small organic compounds such as sinapinic acid. These two solution are subsequently mixed and a small amount of solution is placed on a metal plate to dry. After the sample crystalizes, the sample plate is placed in the mass spectrometer for analysis. With the MALD process, matrix materials strongly absorb the laser energy, thus "soft" desorption can be achieved. Furthermore, it has also been found that protein ions can be produced during the MALD process in addition to the expected neutral molecules. The production of ions is speculated to involve a proton transfer process. The process involving ionization and matrix-assisted laser desorption at the same time is called matrix-assisted laser desorption and ionization (MALDI).

Since the discover of MALDI, many research groups have succeeded in measuring various proteins by MALDI. Observation of protein ions with molecular weight greater than 100,000 Daltons without significant breakup has been reported. MALDI has also been applied to DNA detection, but until recently the success has been limited to very small segments. In 1994, we succeeded in using MALDI to detect single-stranded DNA the size of 500 mer and double-stranded DNA with 500 base pairs. However, the resolution is poor. In this program, we try to improve the resolution, sample preparation, and detection sensitivity for MALDI for DNA analysis.

PerSeptive Biosystem Inc. has been a major manufacturer of time-of-flight biomolecule mass spectrometer for years. Their mass spectrometers have been well known for high resolution, good sensitivity and high reliability. Their mass spectrometers has also been known suitable for protein analysis. In this program, we like to extend the capability to DNA analysis.

V. Approach:

The approach of this program is to develop and test several innovative ideas to improve the resolution, detection sensitivity and reproducibility for MALDI. Major tasks include (1) pulsed ion extraction (PerSeptive) to improve resolution (2) Two-component matrices to enhance ionization, and (3) solid phase DNA purification. We also applied the developed technology to do disease diagnosis and DNA sequencing.

VI Results:

The direct application is to develop a better Time-of-Flight mass spectrometry technology for fast DNA analysis. Further applications include DNA fingerprinting, disease diagnosis and DNA sequencing. The research effort of this CRADA was performed under the following tasks which are described in the scope of the work in the proposal.

Task 1 Standardization of Sample Preparation and Comparison of results from linear and reflectron time-of-flight mass spectrometer.

Detailed procedures for sample preparation have been established. We found the resolution for protein from reflectron time-of-flight mass spectrometer is usually better than results from a linear TOF device. However, the signal of large DNA ions from reflectron TOF compared to linear TOF can be significantly less due to the existence of metastable DNA ions. However, the quantity of metastable ions produced also strongly depends on the laser fluence, matrix and voltage setting in ion production zone.

Task 2 Improvement in Sample Quality.

We found more homogeneous samples can be produced with alcohol as part of the solvent. When ammonium citrate is added, ionization efficiency can be enhanced significantly. A paper regarding the effect of ammonium salt on MALDI was submitted and published.

Task 3 Purification Methods for DNA Analysis by Mass Spectrometer

PerSeptive Biosystem Inc. has put major effort in achieving fast purification of DNA by using solid phase approach. Magnetic beads coated with streptavidin for strong interaction with biotinylated DNA has been used for DNA purification. It increases the speed of purification. The purity of DNA is also better. When DNA sequencing is pursued, this process can also eliminate the interference of primers and templates.

Task 4 Pulsed Ion Extraction

An innovative approach to use delayed pulsed ion extraction was developed at PerSeptive Biosystem Inc. for compensation of the velocity spread of desorbed ions in MALDI process. This device is now incorporated into the new models of

time-of-flight mass spectrometers produced by PerSeptive Biosystem Inc.

Task 5 Various Matrices for Mass Spectrometer Analysis

Several two-component matrices were tried for MALDI. We found 3-aminopicolinic acid, sinapinic acid-ammonium citrate, and HCCA-ammonium citrate are good matrices for MALDI of DNA.

Task 6 Resolution Improvement

We found the velocity spread, inhomogeneity of sample distribution, inhomogeneous laser beam distribution, too high laser fluence and production of secondary ions are the major factors to cause poor resolution. Delayed pulsed ion extraction was developed to significantly improve the resolution.

Task 7 Evaluation of the Improved Instrument for DNA sequencing

DNA sequencing with standard Sanger's enzymatic method for DNA ladders preparation and cycle sequencing approach has been pursued. Successful sequencing of DNA has been demonstrated.

In addition to the above achievements, we also worked on detection of point mutation for cystic fibrosis disease. The successful results have been accepted for publication.

VII. Conclusion:

We consider this program is very successful. We achieve all major tasks listed in the scope in the proposal. Six papers are either published or accepted for publication. Four presentations were given in international conferences. This program is certainly beneficial to Human Genome Project in DOE and NIH. Since DNA analysis can become a multi-billion dollar business in the near future, improvement in the speed and reliability of DNA detection is very important to economy. However, it is worth to put additional time and effort to resolve the key reasons for relatively poor resolution of large DNAs even when delayed pulsed ion extraction approach is used.

VIII. Publication and Patent:

No patent application are resulted from this program. Six papers are published

with complete or partial support from this program. The list is in the follows.

- (1) Chung-Hsuan Chen, Nelli I. Taranenko, Yi Fei Zhu and Steve L. Allman, " MALDI for Fast DNA Analysis and Sequencing" Laboratory Robotics and Automation, 8, 87-99 (1996)
- (2) Y. F. Zhu, C. N. Chung, N. I. Taranenko, S. L. Allman, S. A. Martin, L. Haff and C. H. Chen, "The study of 2,3,4-Trihydroxyacetophenone and 2,4,6-Trihydroxyacetophenone as Matrices for DNA Detection in Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry" Rapid Comm. Mass. Spectrom. 10, 383-388 (1996)
- (3) C. H. Chen, N. I. Taranenko, K. Tang and S. L. Allman, " Laser Desorption Mass Spectrometry for DNA Analysis and Sequencing", SPIE 2386, 13-23 (1995)
- (4) C. H. Chen, " Mass Spectrometry High Speed DNA Fragment Sizing " Encyclopedia of Molecular Biology and Molecular Medicine Vol. 4, 1996 edition pp 1-11
- (5) N. I. Taranenko, K. J. Matteson, C. N. Chung, Y. F. Zhu, L. Y. Chang, S. L. Allman, L. Haff, S. A. Martin and C. H. Chen " Laser Desorption Mass Spectrometry for Point Mutation Detection " Genetic Analysis: Biomolecular Engineering (in press)
- (6) Y. F. Zhu, N. I. Taranenko, S. L. Allman, S. A. Martin, L. Haff and C. H. Chen " The Effect of Ammonium Salt and Matrix in the Detection of DNA by MALDI-TOF Mass Spectroscopy" Rapid Comm. Mass Spectrom. (in Press)

IX. Appendix: Reprints and preprints are attached.

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