# digitalMALDI: A single particle-based mass spectrometric detection system for biomolecules

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## Abstract

The development of a real-time system to analyze individual biomolecule-containing aerosol particles could provide a critical capability to diagnose lung disease rapidly. However, currently available technologies are mainly based on molecular assays, require costly reagents, and are relatively slow and challenging to multiplex. To address these limitations, we developed digitalMALDI™, a laser-based mass spectrometry-based system. In particular, digitalMALDI™ is a near real-time system that directly samples aerosols, eliminating the need for complex and time-consuming sample preparation and producing MALDI-MS spectra for individual aerosol particles. In this study, an intact insulin protein was used to demonstrate the feasibility of this approach. Results showed that digitalMALDI™ is sensitive to detecting ~1 picogram of insulin protein in single particles in real-time, suggesting that the system has a broad application for disease diagnosis, environmental monitoring, and biosecurity management.

KEYWORDS: Biomolecules, Aerosol particles, Digital, Mass spectrometry

## 1 INTRODUCTION

Biomolecule-containing aerosol particles, originating from diverse sources such as human activities, environmental pathogens, or processes, can directly impact respiratory health.[1] Therefore, developing a sophisticated aerosol particle analysis system could provide an essential tool for understanding aerosols and detecting diseases.[2] Beyond disease diagnosis, this system would fill a critical need for biodefense, environmental regulation, and public health management.[2] Its ability to identify and monitor various biomolecules, including human response factors and pathogens, is crucial in reducing their potential adverse effects.[2]

Current methodologies for biomolecule detection mainly combine aerosol sampling with detection systems, such as immunoassay or PCR-based techniques.[3-5] These methods, however, present significant limitations, notably their inability to monitor individual aerosol particles containing biomolecules autonomously and instantaneously. This shortcoming arises from the time-intensive nature of aerosol collection and the duration required for assay performance, whether immunoassay or PCR-based techniques. Furthermore, the costs associated with reagents or antibodies for these assays are an additional obstacle to their extensive application. Therefore,

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there is an urgent need for detection technologies that can characterize aerosol particles both in real time and with high sensitivity, thereby facilitating rapid and precise analytical results. Additionally, single-particle analysis is a powerful tool that can deconvolve complex mixtures.

Laser-based mass spectrometry is a highly sophisticated analytical tool to detect and characterize molecules accurately.[6] Its advantages, such as simple instrument configuration, reduced sample preparation, and high-throughput and automation compatibility, demonstrate its potential to fulfill these critical requirements, making it increasingly relevant in developing detection technologies for complex scenarios.[6] In this study, we presented a real-time aerosol particle detection technology, digitalMALDI™, that involves digitalizing aerosol particles into singular particles using a particle beam generator, followed by characterization through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This study details the design aspects and experiments, demonstrating the technology's heightened sensitivity in biomolecule detection in aerosol particles.

## 2 MATERIALS AND METHODS

### 2.1 Materials and chemicals

Rhodamine B powder was purchased from Sigma-Aldrich (Product number: R6626). MALDI matrix α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from Sigma-Aldrich (Product number: 70990). Full insulin protein was purchased from Sigma-Aldrich (Product number: I6634). HPLC-grade acetonitrile (ACN) and water were purchased from Fisher Chemical.

### 2.2 Aerosol particle generation

An Aeroneb® lab nebulizer unit controlled by an Aeroneb® module (NEB-7000, Kent Scientific Corporation) generated 1-2 μm aerosol particles. For aerosolization, 10 μL of rhodamine B (1 mg/mL in water) was mixed with 10 μL of CHCA matrix (9 mg/mL in 70% ACN), and the mixture was added in the nebulizer for aerosolization into a 500 mL laboratory wide-open bottle. The same procedure was followed to aerosolize intact insulin protein, except insulin was prepared at 1 mg/mL in water. After aerosolization, the bottle was connected to the aerosol orifice inlet on the digitalMALDI™ system.

### 2.3 Aerosol detection and characterization by digitalMALDI™

In the digitalMALDI™ system, aerosol particles are introduced at a 0.5 liters/minute flow rate. Upon reaching the detection chamber, particles collide with a 532 nm green laser, triggering ionization by a 349 nm blue laser. Ionized particles are then accelerated into a time-of-flight tube with a 19 kV potential.[7] An electron multiplier detects ions, and the resultant signals are recorded by a PicoScope operating at 1 GHz. Data for each excited particle is recorded as a time series in binary format. The conversion of time-of-flight to a mass-to-charge ratio (m/z) utilizes a calibration coefficient established through calibration peaks by the following formula: $m/z = (TimeTOF^2)*C$ where $C$ is the calibration coefficient established by calibrating the mass spectrometer using two CHCA matrix peaks, 190.1 m/z ($C_{10}H_7NO_3$, MH+) and 172.0 m/z ($C_{10}H_5NO_2$, MH+), rhodamine B (479.2 m/z, $C_{28}H_{31}ClN_2O_3$, MH+), and intact insulin chain (5732.6 m/z, $C_{254}H_{377}N_{65}O_{75}S_6$, MH+). The formula and corresponding calibration coefficients translate the time series data for each single particle within the digitalMALDI system into mass spectra.

## 3 RESULTS AND DISCUSSION

### 3.1 digitalMALDI™ design

Figure 1 illustrates the digitalMALDI™ single-particle mass spectrometer, an analysis system comprising an aerosol generator, a vacuum-sealed detection chamber, and a MALDI-TOF mass spectrometer.[7] The process begins with introducing aerosol particles containing biological molecules into the system (Figure 1A). These particles undergo focusing, producing a columnated

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stream when exiting the aerosol beam generator, enabling single-particle characterization. As particles proceed to the detection chamber, a dual laser system (a green laser for detection and a blue laser for ionization) operates under vacuum conditions, which allows for simultaneous detection and ionization, with the post-ionization particles analyzed by the MALDI-TOF mass spectrometer. The system was prototyped in the Zeteo Tech, Inc. laboratory and incorporates signal processing hardware and specialized software for single particle analysis (Figure 1B). Key to this system is the particle beam generator, featuring an orifice inlet, expansion chamber, and aerodynamic lens, which are crucial for the formation of individual particles (Figure 1C).

Critical advantages for aerosol particle analysis come from digitalMALDI's™ ability to individualize particles, allowing for digital characterization, and the dual laser system enabling simultaneous detection and ionization, enhancing real-time detection (Figure 1D). Additionally, its design, including an orifice inlet, expansion chamber, and aerodynamic lens, contributes to forming and accurately analyzing individual particles. This system represents an advancement in single-particle analysis, especially in fields requiring high sensitivity and specificity.

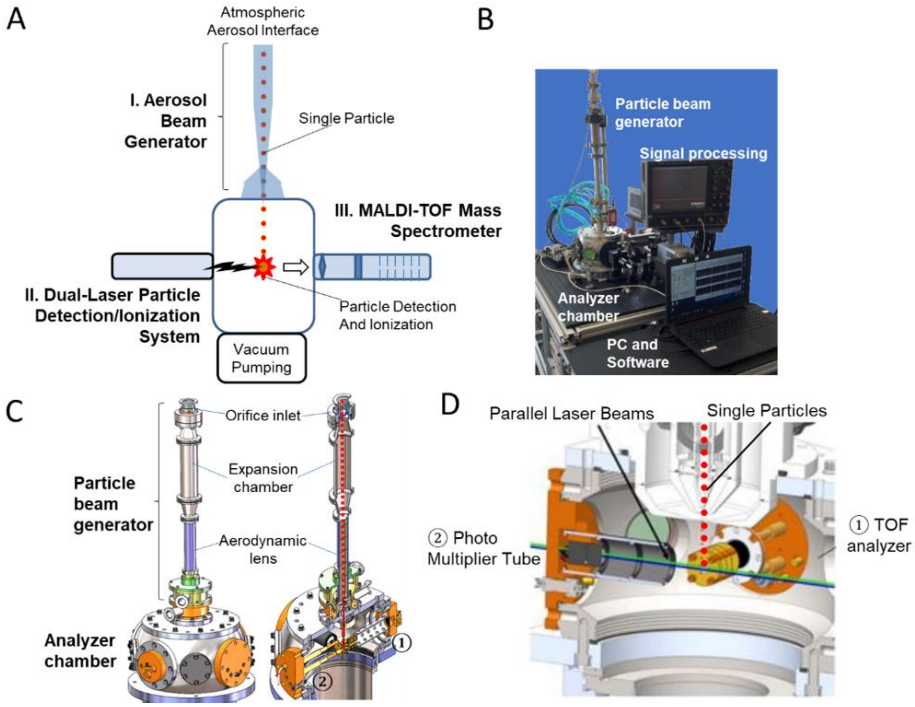


**Figure 1** Schematic representation of the digitalMALDI™ single-particle mass spectrometer. A. Composition and functionality. B. System configuration at Zeteo Tech laboratory. C. Particle beam generator design. D. Particle detection and ionization process in the analyzer chamber.

3.2 System feasibility testing using aerosol particles containing rhodamine B

This study tested the digitalMALDI™ system's feasibility using aerosolized rhodamine B to simulate biological molecules. Analysis of over 900 individual particle profiles revealed varying signal intensities of rhodamine B. 'Empty' particles, lacking biological molecules or MALDI matrices, exhibited flat line profiles with background signals but lacked rhodamine B peaks (Figure 2). Particles with rhodamine B showed significantly enhanced signal-to-noise ratios compared to averaged profiles where background noise, such as environmental factors (Figure 2B) suppressed rhodamine B's mass peak. Additionally, molecular profiles from background particles

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without rhodamine B were well characterized (Figure 2B). This figure demonstrates the system's ability to distinguish and quantify specific molecules within individual aerosol particles (Figure 2B).

Feasibility testing demonstrated several key advantages of the digitalMALDI™ system: its ability to analyze individual particle profiles with varying signal intensities, the distinct detection of rhodamine B characterized by enhanced signal-to-noise ratios, and the capability to differentiate particles containing specific molecules from 'empty' particles or background noise, which is essential for accurate molecular characterization in complex aerosol particle types.

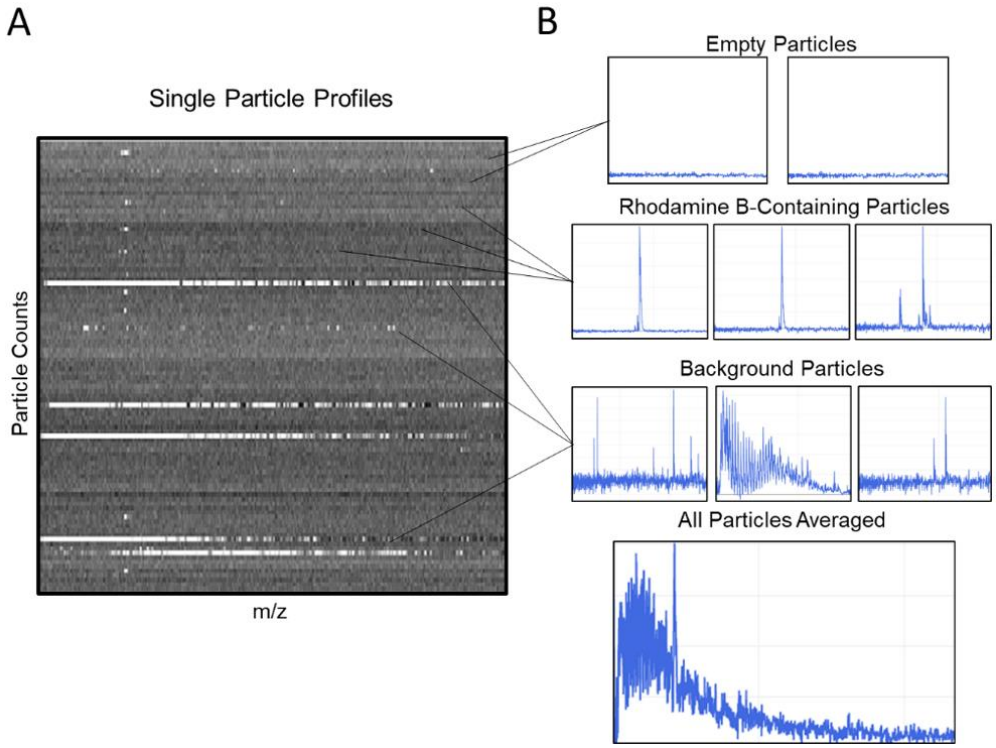


**Figure 2** Single Particle Profiles from the digitalMALDI System. A. Single particle profiles using containing rhodamine B. B. Mass spectra of single particles containing different molecular profiles.

3.3 Detection of insulin protein-containing aerosol particles using digitalMALDI™

The digitalMALDI™ system's ability to detect biomolecules was next evaluated using intact insulin protein. The system demonstrated sensitivity in identifying proteins within single aerosolized particles, about 1 µm in size, each estimated to contain approximately one picogram of insulin. This highlights the system's capability to detect extremely low quantities of intact proteins, showing its potential in advanced biochemical and clinical diagnostics. This performance is particularly significant in disease diagnosis and monitoring, where such sensitivity is crucial.

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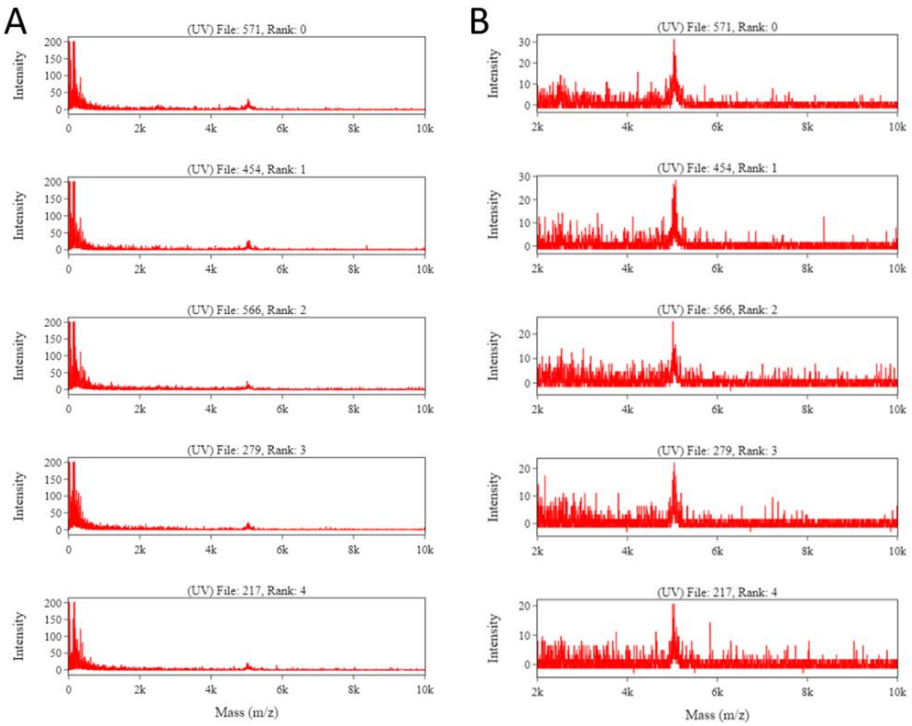


**Figure 3** Analysis of Single Particle Profiles of Intact Insulin Protein via digitalMALDI. A. Mass spectra of intact insulin in single particles. B) Selected mass ranges from the profiles of five single particles containing intact insulin protein.

## CONCLUSION

This study demonstrated a novel detection system for real-time characterization of biomolecule-containing aerosol particles. With sample digitalization to generate single particles and MALDI-TOF mass spectrometry, the system detected low insulin levels in aerosol particles. Its sensitivity and efficiency indicate wide-ranging applications, including human health monitoring and identifying environmental hazards associated with aerosol particles.

## ACKNOWLEDGEMENT

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