Mini Review

Picosecond Infrared Laser Mass Spectrometry for the Analysis of Biological Materials

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Abstract The 49th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry (JSBMS) featured the 3rd International Joint Symposium with Mass Spectrometry: Applications to the Clinical Lab (MSACL), marking the first in-person collaboration. Dr. Arash Zarrine-Afsar, Department of Medical Biophysics, University of Toronto, presented "10-Second Cancer Diagnosis with Picosecond Infrared Laser Mass Spectrometry (PIRL-MS)," highlighting PIRL-MS technology. This method combines two innovations: PIRL-DIVE (**p**icosecond **i**nfrared **l**aser ablation/**d**esorption by **i**mpulsive **v**ibrational **e**xcitation) for precise biomolecule extraction and ion detection without conventional sources for minimally invasive diagnostics. The PIRL-MS enables intact biomolecule analysis, tissue classification, and cancer biomarker detection with minimal tissue damage. These advancements make the PIRL-MS a promising intraoperative diagnostics and biomedical research tool. This mini-review focuses on the evolution of his method and briefly summarizes the discoveries and applications that led to this method.

Key words: picosecond infrared laser (PIRL), mass spectrometry (MS), picosecond infrared laser ablation/desorption by impulsive vibrational excitation (PIRL-DIVE)

Abbreviations: PIRL, picosecond infrared laser; DIVE, desorption by impulsive vibrational excitation; PIRL(A), picosecond infrared laser ablation; MS, mass spectrometry

Introduction

The 49th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry (JSBMS) was held at the Shimadzu Corporation Hall in Kyoto on September 13−14, 2024. As part of this event, the 3rd International Joint Symposium of the JSBMS and Mass Spectrometry: Applications to the Clinical Lab (MSACL) was convened. This symposium represented the first opportunity for an in-person international joint symposium, as the first and second editions were conducted online due to the global COVID-19 pandemic. On this occasion, MSACL nominated Dr. Arash Zarrine-Afsar as their representative speaker, and the JSBMS International Committee invited him to participate. Dr. Zarrine-Afsar delivered an outstanding presentation entitled "10-Second Cancer Diagnosis with Picosecond Infrared Laser Mass Spectrometry[1)]."

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Dr. Zarrine-Afsar presented his work on intraoperative molecular diagnosis using picosecond infrared laser mass spectrometry (PIRL-MS)[2], a novel application of mass spectrometry that in a manner similar to rapid evaporative ionization mass spectrometry (REIMS)[3] has shown potential in biomedical research. REIMS, along with other ambient mass spectrometry analysis methods, represents a significant advancement in clinical mass spectrometry by enabling real-time, direct analysis of biological tissues without the need for complex sample preparation or chromatographic separation. This approach is particularly valuable in clinical settings where rapid and accurate results are essential. By analyzing the molecular composition of surgical aerosols, REIMS facilitates rapid tissue classification, disease detection, and intraoperative diagnostics[2–4]. Furthermore, its integration with machine learning algorithms enhances diagnostic accuracy and tissue classification. As clinical mass spectrometry advances toward point-of-care applications, REIMS demonstrates remarkable potential by bridging the gap between laboratory analysis and real-time clinical decision-making[2–5].

Following his presentation, the symposium organizers acknowledged the significance of his methodology and expressed their intention to explore and introduce his innovative technique in this journal. With his kind cooperation, this initiative aims to enhance the understanding of Japanese medical mass spectrometrists and promote the adoption of this advanced approach in clinical practice.

This mini-review, prepared with the kind cooperation of Dr. Zarrine-Afsar, aims to introduce his picosecond infrared laser mass spectrometry (PIRL-MS) to the members of JSBMS. The mini-review is structured into three sections: (1) the initial discovery of PIRL(A)-DIVE (**p**icosecond **i**nfrared **l**aser **a**blation/**d**esorption by **i**mpulsive **v**ibrational **e**xcitation), (2) the attractive integration of PIRL(A) technology with electrospray ionization (ESI), and (3) the breakthrough detection of ionized biomolecules without conventional ion sources. For clarity and accessibility, this mini-review adopts the term "PIRL(A)" instead of the original PIRL, with kind permission from Dr. Zarrine-Afsar.

1. The Initial Discovery of PIRL(A)-DIVE (Picosecond Infrared Laser Ablation/Desorption by Impulsive Vibrational Excitation) Phenomenon

Picosecond infrared laser (PIRL) technology was developed and proposed to overcome the limitations of conventional medical lasers in achieving single-cell precision cutting, essential for minimally invasive procedures and tissue restructuring by Dr. R. J. Dwayne Miller[6, 7]. Traditional lasers failed to reach this precision due to collateral damage caused by thermal and shock wave effects on surrounding tissue. The PIRL ablation offered a groundbreaking solution by selectively energizing water molecules in the tissue much faster than heat transfer from superheated water molecules to their surroundings[8–10]. This process drove ablation or cutting faster than thermal energy could spread, thus avoiding the collateral damage inherent in other laser technologies[7–11]. Unlike conventional lasers, PIRL did not produce plasma formation or harmful ionizing radiation effects[7, 9–11]. The advantage of PIRL technology lies in its minimal energy deposition in the surrounding tissue, eliminating photochemical and photothermal side effects. This precision-made PIRL uniquely suited for delicate medical applications, representing a significant advancement in laser-based surgical techniques[7, 9, 11, 12].

In 2015, Ren et al., from Dr. Miller's group, introduced an innovative method known as PIRL ablation, based on the desorption by impulsive vibrational excitation (DIVE) principle[13]. This technique enabled the efficient extraction of biological entities from aqueous and natural environments into aerosols (Fig. 1), preserving their biological integrity and activity. Key findings were demonstrated that extracted enzymes retained enzymatic activity without oxidation, denaturation, or inactivation; metabolic molecules remained intact without oxidation or fragmentation; nucleic acids, including DNAs and RNAs, were retrieved in their native forms; and infectious viruses were recovered with preserved infectivity. In addition, of particular interest, the rapid extraction of proteins has promised novelties in future use for proteomic applications[14]. Unlike conventional methods that often involve harsh chemicals or heat, the DIVE-driven PIRL (hereafter referred to as PIRL(A)-DIVE) method precisely targeted water molecules, minimizing thermal and mechanical stress. This ensured the structural and functional preservation of biological materials, establishing the PIRL(A)-DIVE as a valuable tool for sensitive biological extractions in research and medical applications. Not limited to its potential as an instantaneous tool with micrometer precision for biopsy, the PIRL(A)-DIVE method brought promising benefits to chemical analysis, biological diagnosis, and disease control[13, 15]. Further-

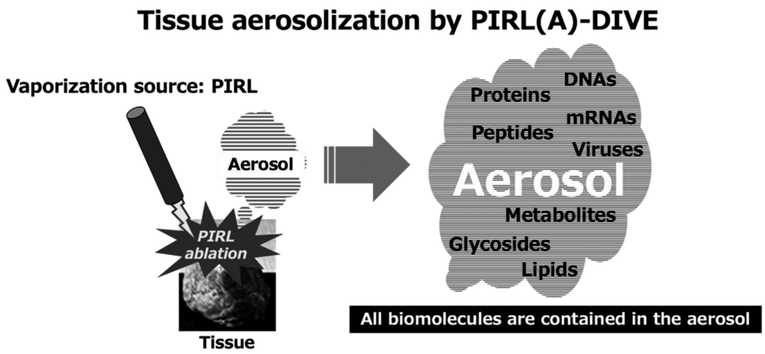

Fig. 1. The initial discovery of PIRL(A)-DIVE (Picosecond Infrared Laser Ablation/Desorption by Impulsive Vibrational Excitation) phenomenon.

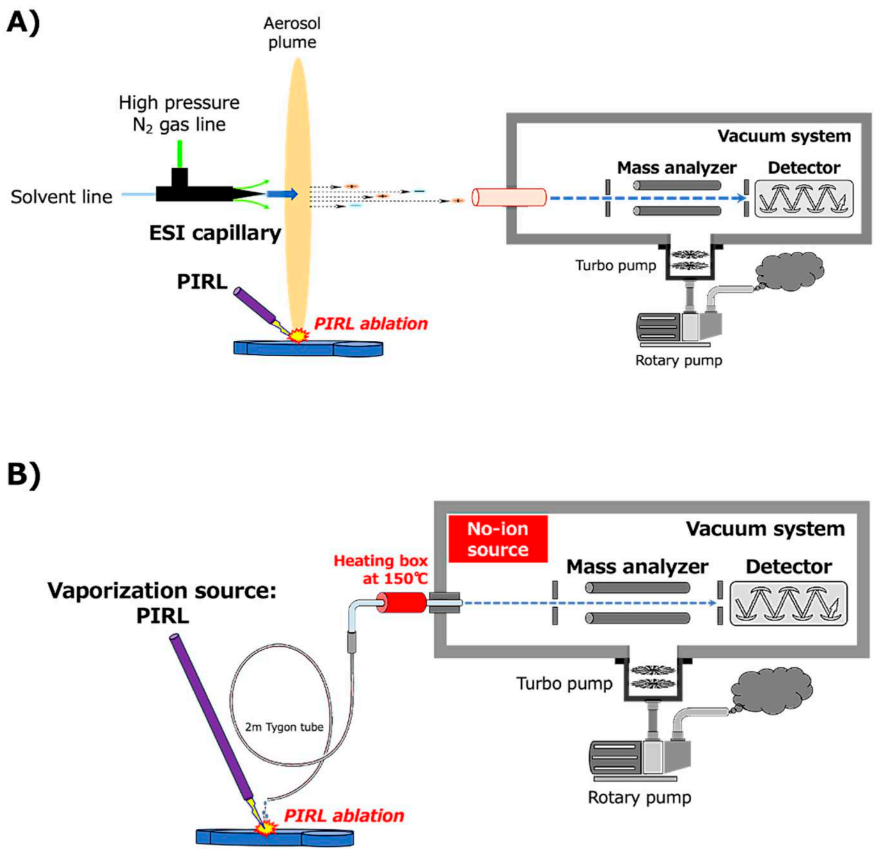

Fig. 2. Analytical technologies of PIRL(A)-MS combining two groundbreaking discoveries.
A) The attractive integration of the PIRL(A)-DIVE and electrospray ionization (ESI) technologies. B) The breakthrough detection of ionized biomolecules through the heating box at 150°C without conventional ion sources.

more, applications of PIRL(A)-DIVE conducted under atmospheric conditions, demonstrated its exceptional effectiveness in extracting molecules from water-rich biological materials and its suitability for characterizing biological tissues with high water content. Subsequent studies utilizing PIRL(A)-DIVE have revealed that the technique induces minimal tissue damage beyond the ablation zone, emphasizing its significant potential for a wide range of biomedical applications[2, 13].

2. The Attractive Integration of the PIRL(A)-DIVE and Electrospray Ionization (ESI) Technologies

In 2015, the attractive integration of the PIRL(A)-DIVE and ESI technologies was attempted and reported by Dr. Arash Zarrine-Afsar's group in collaboration with Dr. R. J.

Dwayne Miller[16]. As shown in Fig. 2, both PIRL(A)-DIVE and ESI were integrated to develop a novel analytical system for imaging mass spectrometry, which has been successfully reported[16]. PIRL(A) was employed to generate a plume directly from the target bio-material. This plume contained all intact biomolecules, preserved in an aerosol-like state. The plume was subsequently intercepted by an electrospray, which efficiently ionized the biomolecules. The resulting ions were then transferred to a mass spectrometry (MS) detector for analysis. This process enabled the direct and gentle ionization of biomolecules, preserving their structural integrity. This PIRL(A)-ESI-MS system was utilized to image the distribution of gadoteridol, an exogenous magnetic resonance contrast agent that was administered via intravenous injection into the tail vein of the mice, within mouse kidneys[16]. This study also demonstrated that PIRL(A)-ESI is capable of performing desorption ionization of small molecules, proteins as well as phospholipids[16] and concluded that PIRL(A)-ESI is a suitable ion source for potential future applications in ambient mass spectrometry. Notably, a PIRL probe combined with secondary ionization techniques, such as ESI, and mass spectrometry holds significant potential for providing molecular feedback to guide laser surgery. Since this initial implementation, additional studies utilizing PIRL(A)-DIVE have suggested that the technique induces minimal tissue damage outside the ablation zone, highlighting its promise for further biomedical applications[2, 16].

3. The Breakthrough Detection of Ionized Biomolecules without Conventional Ion Sources

In 2017, Woolman et al., from Dr. Zarrine-Afsar's group, utilized the efficient and rapid extraction capabilities of PIRL, combined with the plume's rapid cooling upon expansion, to investigate the potential of coupling PIRL(A) with soft ionization techniques like thermal desolvation[17]. This innovative approach enabled the analysis of pre-charged lipid molecules carrying negative charges, leading to the development of a novel analytical platform termed PIRL-MS which is referred to herein as PIRL(A)-MS to highlight the ablative mechanism behind the laser action. Notably, this discovery and implementation were built on the previous creation of PIRL desorption, and only added a suitable means of ionization, as illustrated in Fig. 2B. Woolman et al. demonstrated that negatively charged lipids could be detected in an MS system without using an external ion source per se such as ESI, requiring only biomolecule heating at 150°C before introducing into the MS device to prevent resolvation[17]. Furthermore, this method successfully classified mouse organs based on extracted and desolvated lipid molecules analyzed in the negative ion mode. An international patent subsequently protected this groundbreaking technology[18].

Independent subcutaneous murine xenograft tumors—derived from six distinct, well-established human medulloblastoma cell lines representing the Sonic Hedgehog and Group 3 subgroups—were analyzed using the PIRL(A)-MS system[19, 20]. Following the efficient classification of mouse organs, tumor analytical data were further evaluated through multivariate statistical analysis, with robustness validated via an iterative 5%-leave-out-and-remodel approach. Additionally, the potential of integrating PIRL(A)-MS with imaging modalities, such as wide-field tissue polarimetry for cancer region guidance, was explored. Polarimetric imaging was used to identify tissue heterogeneities, effectively directing the PIRL desorption source. The PIRL(A)-MS plume was transported to the mass spectrometer through a 2-meter-long Tygon tube, facilitating the development of a handheld MS point sampling device for analyzing thick tissue sections *in situ*[2, 17]. Specifically, PIRL(A)-MS point sampling, guided by polarimetry, successfully identified necrotic regions in metastatic breast tumors and efficiently detected the necrotic cancer marker ion at *m/z* 572.48 ([ceramide (d34:1)+Cl]$^-$)[2, 17]. Polarimetry-directed sampling precisely targeted necrotic areas, while PIRL(A)-MS spectra revealed the presence of common fatty acids and phospholipids, consistent with findings from other MS techniques used for breast cancer analysis. These results underscored the potential of combining polarimetric imaging with PIRL(A)-MS for accurate and targeted tissue analysis in complex biological samples. Subsequently, the hand-held PIRL(A)-MS probe has been used in several patient specimen explorations to determine various tumor pathologies faster than currently possible. These have included rapid exploratory subgrouping of human medulloblastoma[20] and other pediatric brain cancer types[21], human skin cancer[22] and 10-second identification of actionable BRAF-V600E mutation[23]. Integration of PIRL(A)-MS probe with surgical positioning and navigation technologies has enabled on-the-spot delivery of MS-based pathology classifications via virtual reality augmentation[24].

This mini-review considered a potential mechanism to explain why PIRL(A)-MS can detect negatively ionized lipid molecules. The two hypotheses were offered: (1) the detectable ionization may occur solely through the application of heat, depending on the specific type of biomolecule, and (2) a proportion of molecules in living organisms naturally exist in a negatively charged state such as anionic phospholipids[25, 26], which the PIRL(A)-MS system can effectively detect and analyze as in vivo ionized biomolecules, especially anionic phospholipids.

Conclusions

The PIRL(A)-MS is a technology that has advanced significantly through the successful integration of two groundbreaking discoveries, as mentioned earlier, and has emerged as a highly promising analytical tool. The keynote lecture titled "10-Second Cancer Diagnosis with Picosecond Infrared Laser Mass Spectrometry[1)]" presented a revolutionary and pioneering research topic that leveraged these technological advancements to develop a novel intraoperative molecular diagnostic method. In Japan, the medical application of mass spectrometry has not yet advanced to the level of intraoperative molecular diagnosis. Therefore, the active contributions of young JSBMS members are eagerly anticipated to drive future progress in this field.

Declaration of Competing Interest

AZA is an inventor of the soft ionization method for PIRL-MS and is a consultant with Point Surgical Inc. with a financial interest.

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