

**Technology Development of a Transfer Plate
for Mass Spectrometry Imaging Based on
a Platinum-Coated Glass Bead Porous Structure
and Multi-Perspective Internal Observation
of the Product Development Project Team**

Doctoral Thesis

by

Takamasa Ikeda



The Graduate School for the Creation of New Photonics Industries

March 2026

Copyright © 2026 Takamasa Ikeda



This thesis is licensed under a Creative Commons Attribution–NonCommercial 4.0 International License (CC BY-NC 4.0). To view a copy of this license, visit <https://creativecommons.org/licenses/by-nc/4.0/>

Except where otherwise noted, you may share and adapt this thesis for noncommercial purposes, provided that appropriate credit is given and any changes are indicated. Third-party material included in this thesis (e.g., figures, images, or other content not created by the author) is used under permission or other applicable terms and is not covered by the Creative Commons license unless explicitly stated. For such material, separate permission from the respective rights holder may be required.

Technology Development of a Transfer Plate for Mass Spectrometry Imaging Based on a Platinum-Coated Glass Bead Porous Structure and Multi-Perspective Internal Observation of the Product Development Project Team

Takamasa Ikeda

Abstract

This doctoral thesis, “Technology Development of a Transfer Plate for Mass Spectrometry Imaging Based on a platinum-coated Glass Bead Porous Structure and Multi-Perspective Internal Observation of the Product Development Project Team addresses two main themes:

- (1) Development and applications of transfer plates for mass spectrometry imaging (MSI)
- (2) Analysis of management practices in the transfer plate product development process

This thesis consists of five chapters: introduction (Chapter 1), fundamental validation of the transfer plate (Chapter 2), proposal of advanced application methods aimed at improving analytical sensitivity (Chapter 3), multi-perspective internal observation of the project team (Chapter 4), and conclusions (Chapter 5).

MSI is an imaging technique that enables the simultaneous visualization of the spatial distribution of multiple molecular species by leveraging the high sensitivity inherent to mass spectrometry. It has produced numerous achievements, particularly in pharmaceutical research, including biomarker identification and the elucidation of pharmacological mechanisms. Currently, the most widely applied MSI technique is matrix-assisted laser desorption/ionization (MALDI). However, MALDI-MSI requires technically demanding sample preparation procedures, notably thin sectioning and matrix applications. In particular, thin sectioning severely restricts the range of samples that can be analyzed. If an alternative approach to thin sectioning becomes available, the barriers to MSI adoption will be significantly reduced, enabling the technique to be applied to its full potential across diverse fields. Of previously reported alternatives, methods based on sample “transfer” have been validated for viability and efficacy. However, few media have been developed specifically for transfer, and none have found commercial applications. “DIUTHAME,” an MSI sample preparation technology commercialized by

the author's affiliated company, is also unsuitable for transfer and does not serve as a replacement for thin sectioning. To address this deficit, the present study initiated the development and commercialization of a transfer-dedicated medium, referred to as a transfer plate.

A porous glass bead substrate coated with a platinum thin film was developed as a transfer plate. The fundamental validation of this technology is presented in Chapter 2. For a transfer medium to find viable application, it must not only exhibit the capability to withstand the physical act of transfer, but also be compatible with time-of-flight mass spectrometer (TOFMS), which is commonly used in MALDI-MSI. The robustness and water-absorption characteristics of the porous glass bead structure enabled the transfer of a wide variety of samples. In addition, the platinum thin film simultaneously imparted the electrical conductivity required for TOFMS measurements and enabled matrix-free laser desorption/ionization. Consequently, the conventional MALDI-MSI sample preparation steps for thin sectioning and matrix application can be consolidated into a single transfer step when using a transfer plate. As proof of principle, additive stains formed on polyethylene terephthalate films and cross sections of strawberries were selected as representative dry and wet samples, respectively. MSI measurements following transfer successfully yielded clear spatial distributions of the additive components and strawberry metabolites, demonstrating that thin-section- and matrix-free MSI sample preparation can be achieved using the transfer plate.

In addition, application methods that combine a transfer plate with matrix deposition techniques are demonstrated experimentally in Chapter 3. The measurement approach described in Chapter 2 may be insufficient for certain objectives such as the direct detection of molecular species that are prone to fragmentation. Conventional MALDI, which presupposes the use of organic matrices, involves a wide range of established measurement protocols. If these protocols can be combined with a transfer plate, the existing knowledge can be extrapolated to thin-section-free MSI. Indeed, when the MSI of strawberry metabolites was performed using a combination of transfer plate and matrix applications, glycosylated molecules that were not detected using the transfer plate alone were successfully observed without fragmentation. Furthermore, the matrix application did not cause analyte delocalization or degradation of spatial resolution. These results demonstrate that the transfer plate can be effectively combined with additional processing steps such as matrix applications.

Following the technological development of the transfer plate, this study also analyzes the practical knowledge and motivational transitions that emerged within the product development project through a novel observational method called multi-

perspective internal observation. This method refers to internal observations made by members of a team and is based on group discussions. When applied to the project team, including the author, the analysis revealed how the team evolved through the acquisition of practical knowledge essential for product development such as communication practices and accelerated trial-and-error cycles. Simultaneously, the act of observation functioned as a motivational management practice that engaged all team members. The multi-perspective internal observation method proposed in this study is applicable not only to the present project but also to qualitative research on a wide range of team-based activities.

The technological developments of transfer plates in Chapters 2 and 3 present new applications for laser-based mass spectrometry techniques and aim to contribute to the further advancement of photonics and their societal impact. In addition, the extraction of practical knowledge and analysis of motivational effects through multi-perspective internal observation presented in Chapter 4 proposes a novel qualitative research model in management studies that can be applied to various product development settings. Furthermore, the process of commercializing transfer plate technology and initiating a business represents a practical endeavor toward the creation of new industries.

(In Japanese)

質量分析イメージング用途の Pt 薄膜ーガラスビーズ多孔体＝転写プレートの技術開拓 およびその製品開発プロジェクトチームの多角的内部観測

池田 貴将

要旨

本博士論文は、質量分析イメージング (mass spectrometry imaging, MSI) の前処理を簡便にする技術「転写プレート」の開拓、その製品開発プロジェクトチームの「多角的内部観測」、以上二つを主題とする。論文は、序論 (第一章)、転写プレートの基礎技術実証 (第二章)、感度向上に向けた応用提案 (第三章)、プロジェクトチームの多角的内部観測 (第四章)、結論 (第五章) から構成される。

MSI は、質量分析法の高感度特性を生かしながら、異なる分子種の局在を一度に得られるイメージング技術で、バイオマーカーの特定や薬理作用の解明など医薬分野を中心に多くの成果をもたらしている。現在の代表的な MSI 手法は、マトリックス支援レーザー脱離イオン化 (matrix-assisted laser desorption/ionization, MALDI) である。ただし、MALDI-MSI の測定には高度な試料前処理、薄切とマトリックス塗布を必要とする。とりわけ薄切作業は、測定可能なサンプルを強く制限している。薄切に代わるアプローチがあれば、MSI の導入障壁は下がり、様々な分野でその威力を発揮することができる。中でも、サンプルを「転写」する手法は、現実的な代替案の一つとしてこれまでも報告されてきた。しかし、転写専用開発された媒体は限られ、また商業的にも確立されていない。筆者の所属する企業が製品化した MSI 用前処理技術である「DIUTHAME」も、転写には適さず薄切を代替するものではなかった。こうした背景から、転写専用の媒体「転写プレート」の新規開発とその製品化に筆者が着手したことが、本研究の経緯である。

本研究では、白金薄膜を最表面に付したガラスビーズ多孔体を転写プレートとして新たに開発した。その基礎的な原理実証について第二章に記載した。実用的な転写媒体であるためには、転写行為に耐えうることに加え、MALDI-MSI で一般的な飛行時間型質量分析計 (time-of-flight mass spectrometer, TOFMS) にも適合する必要がある。ガラスビーズ多孔体のもつ堅牢性および吸水性などの特徴は、様々なサンプルの転写を可能にし、白金薄膜は、TOFMS で求められる試料の導電性付与と、マトリックスを必要としないレーザー脱離イオン化 (matrix-free LDI) を同時に実現する。すなわち、MALDI-MSI での薄切およびマトリックス塗布という前処理は、転写プレートを用いた MSI では転写のみに集約さ

れる。この原理実証において、PET フィルム上に形成した添加剤のシミを乾燥サンプル、イチゴの断面を湿潤サンプルとして選定し、異なるサンプル特性に対する実用性を評価した。その結果、添加剤成分およびイチゴ代謝物いずれの局在情報も明瞭に得ることに成功し、転写プレートがサンプルの湿潤状態を問わず適用できることを実証した。

また、転写プレートとマトリックス塗布技術を組合せた応用手法についても実証し、第三章に記した。第二章に示した測定方法は幅広い目的で利用できるものの、断片化しやすい分子をそのまま検出するなど、特定の目的では不十分な場合もある。有機マトリックス塗布を前提とする従来の MALDI は、様々な測定プロトコルが既に開発されており、これらを転写プレートと組み合わせることができれば、従来の知見を薄切不要の MSI に生かすことができる。実際に、イチゴ代謝物の MSI で転写プレートとマトリックス塗布とを併用したところ、転写プレートのみでは検出されなかった配糖体の分子が断片化することなく検出され、加えて空間分解能にも大きな変化はなかった。したがって、転写プレートはマトリックス塗布のような追加処理と組合せられることが実証された。

上記の技術開発に加え、第四章では「多角的内部観測」という新たな観測手法により、製品開発プロジェクトで生じた実践知や動機づけの変遷を経営学的に分析した。多角的内部観測とは、チームメンバーによる所属チームの内部観測を意味し、グループディスカッションをベースとした分析手法である。この手法を、筆者を含むプロジェクトチームに適用したところ、製品開発に不可欠な実践知（コミュニケーション、試行サイクルの加速）を獲得しチームが変遷する過程が見出された。また、この観測行為自体が、チーム全員を動機づけるマネジメント手法として機能したことも同時に示された。本研究で提案する多角的内部観測とその具体的なアプローチは、本プロジェクトのみならず様々なチーム活動の質的研究に応用可能である。

第二章および第三章での転写プレートの技術開発は、レーザーを用いた質量分析技術に新たな応用を提示するもので、光技術の更なる発展と社会貢献を企図している。加えて、第四章での多角的内部観測による実践知の抽出過程および動機づけ作用は、様々な製品開発現場に応用できる経営学的な質的研究モデルを新たに提示した。なお、転写プレートの技術を製品化してビジネスを開始する過程は新事業創成を目指した実践そのものである。

Acknowledgements

All work presented in this thesis was carried out at the Graduate School for the Creation of New Photonics Industries (GPI) and Hamamatsu Photonics K.K. (HPK), with financial support from HPK's research and development budget. I gratefully acknowledge the Electron Tube Division at HPK for facilitating my research activities at GPI.

I would like to express my sincere gratitude to Associate Professor Yasuhide Naito (GPI) for valuable technical discussions and insightful advice. I also wish to extend my heartfelt appreciation to Professor Yasushi Masuda (GPI) for multifaceted managerial guidance and extensive discussions. I would like to thank all staff members and students at GPI for their support.

My heartfelt thanks go to Yoshihiro Ishigami (Group Leader, HPK), Ryo Kawashima (Section Manager, HPK), and other members of the Applied Analysis Group for giving me the opportunity to pursue my work at GPI. I am also deeply indebted to Takayuki Omura (Chief Expert, HPK) and Masahiro Kotani (Team Leader, HPK) for introducing me to research and development in mass spectrometry and for their generous support. To the members of the transfer plate product development project: this achievement would not have been possible without your dedication. I would like to thank Yuri Yamazaki and Keiko Ikeda for their assistance in preparing the figures.

The transfer plate addressed in this study is marketed under the name Poropare. When I entered the program in 2022, I could not have imagined that this project would be completed and commercialized. Through the development of the transfer plate, I have learned a great deal and built valuable relationships. I would like to recognize everyone involved in Poropare, including our collaborators and users. With your continued support, I will continue striving to advance this technology. I also hope to have opportunities to build on the experience and learning gained through this endeavor and take on new challenges in creating new photonics industries.

Finally, I am especially grateful to my wife, Kazuko, and to my children, Motohiro, Sakiko, and Haruaki. Their presence and the happiness they brought me each day were the driving force that enabled me to complete this thesis. I dedicate this thesis to them.

List of Figures*

Figure 1.1. General overview of MSI (MALDI as an example). A matrix-coated sample on a conductive surface is irradiated with a laser to generate ions. Mass spectra are acquired at each (x, y) coordinate. A peak of interest (m/z) is selected from the spectra, and its intensity distribution is visualized as an ion image.	2
Figure 1.2. Schematic illustrations of representative ionization techniques used in MSI: (A) MALDI, (B) DESI, (C) LA-ICP-MS, and (D) TOF-SIMS.	4
Figure 1.3. Schematic illustration of conventional sample preparation procedures for MALDI-MSI: (A) thin-sectioning of a biological sample using a cryomicrotome and (B) matrix application to the sample surface by spray coating.....	5
Figure 1.4. Schematic illustration of the transfer-based sample introduction process. The sample (e.g., plant tissue or fruit) is pressed onto the transfer plate (left), allowing molecular components to be transferred onto the plate surface. After the removal of the original sample (right), transferred molecules remain on the plate and can be subjected directly to mass spectrometry imaging, while the bulk tissue sample itself is no longer required for analysis.	6
Figure 1.5. Examples of transfer-based approaches in MSI using SALDI substrates. (A) Transfer of tissue sections onto surface-engineered porous silicon substrates, followed by SALDI-MSI analysis after section removal. (<i>Reprinted with permission from Ref. 60. Copyright 2012, American Chemical Society.</i>) (B) Schematic illustration of SALDI-MSI using a hydrophobic polydopamine (hPDA)-modified TiO ₂ nanotube (TDNT) substrate coated with plasmonic gold nanoparticles (AuNP–hPDA–TDNT), demonstrating simultaneous detection of analytes in positive and negative ion modes and the acquisition of ion images from transferred plant samples. (<i>Reprinted with permission from Ref. 85. Copyright 2012, Tsinghua University Press.</i>) (C) Transfer of a fingerprint onto a porous silicon substrate coated with a silver nanolayer, enabling SALDI-MSI of fingerprint components. (<i>Reprinted with permission from Ref. 88. Copyright 2015, American Chemical Society.</i>)	10

* Figures 1.1–1.4 and Figure 5.1 are used with permission. © Hamamatsu Photonics K.K. Figures 2.1–2.9 are reproduced/adapted from Ikeda & Kotani (2024) under the Creative Commons Attribution (CC BY) license. Figures 3.1–3.5 are reprinted with permission. © 2025 John Wiley & Sons Ltd. Copyright notices and permission information for other third-party material are provided in the individual captions.

Figure 1.6. (A) An example of the through hole porous alumina membranes observed by scanning electron microscopy (SEM). The scale bar indicates 1 μm . Schematic view (B), and photograph (C), of the DIUTHAME chip in its final form. (*Reprinted from Ref. 91. Copyright 2018 The Authors Rapid Communications in Mass Spectrometry Published by John Wiley & Sons Ltd.*) 12

Figure 2.1 (A) Optical image of the 75 mm-wide and 50 mm-long transfer plate. The gray area on the plate corresponds to the platinum layer. (B) Magnified image of the active surface on a transfer plate. The image was obtained using the back scattered electron mode of a secondary electron microprobe..... 31

Figure 2.2. (A) Polymer additive stains on the PET film; 1 μL of 1, 0.1, and 0.01% Irganox B225 solutions in acetone were loaded. (B) Stains on the transfer plate after the transfer process. MSI measurements were performed in the area enclosed by the red line. 33

Figure 2.3. (A) Optical image of a strawberry cross-section. (B) Strawberry transfer process. (C, D) Traces of the strawberry cross-section on the transfer plate after removing the strawberry. MSI measurements were performed in the area enclosed by the red line. 34

Figure 2.4. (A, B) Traces of the strawberry cross-section on a transfer plate using the same method as in Figure 2.3C and D. MSI measurements were performed in the area enclosed by the red line. 35

Figure 2.5. Averaged mass spectra of the 1% Irganox B225 solution on a target plate (A) with a DHB matrix and (B) without a matrix. The vertical scale of (A) is consistent with that of (B); therefore, the matrix peaks ($\sim m/z$ 400) were saturated in (A). 37

Figure 2.6. Ion images of the polymer additives on transfer plates. (A) m/z 669.8 (Irgafos 168 $[\text{M} + \text{Na}]^+$), (B) m/z 685.4 (Irgafos 168 (Oxidized) $[\text{M} + \text{Na}]^+$), and (C) m/z 1199.5 (Irganox 1010 $[\text{M} + \text{Na}]^+$). The solutions from top to bottom correspond to the 1, 0.1, and 0.01% solutions. The color gradient in the figures corresponds to the relative intensity scaled by SCiLS. 38

Figure 2.7. Averaged mass spectra of the strawberry cross-section obtained in the (A) positive and (B) negative ion modes. No main peaks were observed in the range above m/z 450..... 39

Figure 2.8. Ion images of strawberry metabolites on the transfer plate. (A) m/z 219.0 (hexose $[\text{M} + \text{K}]^+$), (B) m/z 268.9 (citric acid $[\text{M} - \text{H} + 2\text{K}]^+$), (C) m/z 381.1 (sucrose $[\text{M}$

+ K]⁺), (D) m/z 179.1 (hexose [M – H][–]), (E) m/z 191.1 (citric acid [M – H][–]), and (F) m/z 341.2 (sucrose [M – H][–]). The color gradient in the figures corresponds to the relative intensity scaled by SCiLS..... 41

Figure 2.9. Reproduced ion images of strawberry metabolites on a transfer plate: m/z 219.0 (hexose [M + K]⁺), m/z 268.9 (citric acid [M – H + 2K]⁺), and m/z 381.1 (sucrose [M + K]⁺). (A) and (B) were obtained by using the transfer plate shown in Figure 2.4A and B, respectively. The color gradient in the figures corresponds to the relative intensity scaled by SCiLS..... 42

Figure 3.1. Mass spectra averaged over strawberry cross-sections obtained via (A) SALDI-MSI and (B) MALDI-MSI. (C) Mass spectrum averaged over selected spots of the outer part of the strawberry cross-section..... 55

Figure 3.2. Mass spectrum averaged over the area outside the region where the strawberry sample was transferred, obtained via MALDI-MSI. Peaks at m/z values of 177.0, 193.0, and 230.9, which are also clearly evident in Figure 3.1B, corresponding to the DHB matrix-related ions [M + Na]⁺, [M + K]⁺, and [M – H + 2K]⁺, respectively. 56

Figure 3.3. (A) MALDI mass spectrum of the extract from the edge of the strawberry fruit. MS/MS product ion spectra of the precursor ion at m/z (B) 433.1 and (C) 519.1. 58

Figure 3.4. Ion images of the strawberry metabolites obtained via SALDI-MSI (A–C) and MALDI-MSI (D–H): (A, D) m/z 219.0 (hexose [M + K]⁺), (B, E) m/z 268.9 (citric acid [M – H + 2K]⁺), (C, F) m/z 381.1 (sucrose [M + K]⁺), (G) m/z 433.1 (pelargonidin-3-*O*-glucoside [M]⁺), and (H) m/z 519.1 (pelargonidin-3-*O*-malonylglucoside [M]⁺). The color gradient corresponds to the relative intensity scaled by SCiLS. 60

Figure 3.5. SEM images of the surface of a transfer plate (A) before and (B) after matrix-spraying. (C) is an SEM image of (B) magnified five times. The arrows shown in (C) indicate matrix deposition on the neck between glass beads..... 62

Figure 4.1. Overview of multi-perspective internal observation. “Another stories” that cannot be observed from the viewpoint of a single researcher are captured through multi-perspective internal observation, conducted from the team’s viewpoint. 80

Figure 4.2. Specific research methods for the multi-perspective internal observation conducted in this study. 82

Figure 5.1. (A) Product brochure for the transfer plate. (B) Conference exhibition of the transfer plate. 123

List of Tables*

Table 1.1. SALDI substrates reported as transfer media for MSI and their applications.	9
Table 2.1. Assigned polymer additives detected from an Irganox B225 solution on a transfer plate.	37
Table 2.2. Assigned metabolites detected from a strawberry cross-section on a transfer plate.	40
Table 3.1. Assigned strawberry metabolites detected in the SALDI and MALDI mass spectra shown in Figure 3.1.	59
Table 4.1. Modes of <i>katari</i> identified in prior research.	74
Table 4.2. Details of project members. Age and company tenure are fixed based on the date of the group discussion.	83
Table 4.3. Topics discussed in the first group discussion.	86
Table 4.4. Topics discussed in the second group discussion.	98

* Tables 2.1–2.2 are reproduced/adapted from Ikeda & Kotani (2024) under the Creative Commons Attribution (CC BY) license. Table 3.1 is reprinted with permission. © 2025 John Wiley & Sons Ltd.

Table of Contents

Abstract	i
Acknowledgements	vi
List of Figures	vii
List of Tables.....	x
Table of Contents.....	xi
 Chapter 1. General Introduction	 1
1.1 Mass Spectrometry Imaging.....	2
1.2 Previous Studies on Transfer Media for MSI.....	6
1.3 DIUTHAME (Desorption Ionization Using Through-Hole Alumina Membrane)	11
1.4 Background to Transfer Plate Development and Doctoral Research	13
1.5 Objectives of This Thesis	15
1.6 Scope of Chapters	15
References for Chapter 1	17
 Chapter 2. Thin-Section- and Matrix-Free Mass Spectrometry Imaging: Reproducible Sample Transfer Using Novel Platinum-Coated Porous Plate Formed of Glass Beads.....	 29
2.1. Introduction	30
2.2. Methods	31
2.2.1. Fabrication of transfer plates.....	31
2.2.2. Sample preparation of polymer additives.....	32
2.2.3. Sample preparation of strawberries.....	33
2.2.4. Instrumentation	35
2.3. Results and Discussion	36

2.3.1.	Polymer additives.....	36
2.3.2.	Strawberry	38
2.3.3.	Spatial resolution and reproducibility of MSI measurements using a transfer plate...	42
2.4.	Conclusions	44
	References for Chapter 2	45

Chapter 3. Transfer-Based Thin-Section-Free MALDI Mass Spectrometry Imaging Using a Platinum-Coated Porous Plate Formed of Glass Beads..... 50

3.1	Introduction	51
3.2	Methods	52
3.2.1	Preparation of samples for SALDI experiment.....	52
3.2.2	Matrix application for MALDI experiment.....	52
3.2.3	Instrumentation	53
3.3	Results and Discussion	54
3.3.1	Mass spectra of SALDI-MSI and MALDI-MSI	54
3.3.2	Ion images obtained from thin-section-free SALDI-MSI and MALDI-MSI	59
3.3.3	Characteristics of thin-section-free MSI using a transfer plate	61
3.4	Conclusion.....	62
	References for Chapter 3.	64

Chapter 4. Multi-Perspective Internal Observation in Product Development Teams: Practical Knowledge and Motivation Dynamics..... 67

4.1	Introduction	68
4.2	Theoretical Framework.....	72
4.2.1	Internal observation and the theory of <i>katari</i>	72
4.2.2	Previous studies using internal observation and the theory of <i>katari</i>	75
4.2.3	Theoretical framework of motivation.....	77
4.2.3.1	Deci's theory of intrinsic motivation and Csikszentmihalyi's flow theory	77
4.2.3.2	Bakhtin's dialogic theory and carnival theory	77
4.3	Multi-Perspective Internal Observation and Specific Research Methods	78
4.3.1	Multi-perspective internal observation.....	78
4.3.2	Specific research methods.....	81

4.4	Observation Results in Multi-Perspective Internal Observation	83
4.4.1	Research participants.....	83
4.4.2	First group discussion (D1)	84
4.4.2.1	Overview of the D1 investigation	84
4.4.2.2	[D1-1] Project participation history	86
4.4.2.3	[D1-2] Development challenge i.....	88
4.4.2.4	[D1-3] Development challenge ii.....	89
4.4.2.5	[D1-4] Development challenge iii	90
4.4.2.6	[D1-5] Sales challenge.....	91
4.4.2.7	[D1-6] Motivation.....	92
4.4.2.8	[D1-7] Communication.....	94
4.4.2.9	[D1-8] Future prospects	97
4.4.3	Second group discussion (D2).....	98
4.4.3.1	Overview of the D2 investigation	98
4.4.3.2	[D2-1] Reviews of D1 and development challenge iv	98
4.4.3.3	[D2-2] Team cohesion	100
4.4.3.4	[D2-3] Changes in motivation	102
4.5	Discussion.....	103
4.5.1	Extraction and integration of “another stories”	103
4.5.1.1	Extraction of “another stories”	103
4.5.1.2	Integration of “another stories”.....	106
4.5.2	Practical knowledge identified from the product development project.....	107
4.5.2.1	Characteristics of the project and analytical perspectives.....	107
4.5.2.2	From individual exchanges to a multilayered team communication system	107
4.5.2.3	Acceleration of trial cycles through task decomposition and rapid feedback.....	109
4.5.2.4	Managerial significance of the practical knowledge identified through multi- perspective internal observation	110
4.5.3	Motivational management effects of multi-perspective internal observation	111
4.5.3.1	Motivation moving toward a future orientation in D1	111
4.5.3.2	Motivation through active recognition change in D2	114
4.6	Conclusion.....	115
	References for Chapter 4.	117

Chapter 5.	Concluding Remarks	120
5.1.	General Conclusion	121
5.1.1.	Technological contribution	121
5.1.2.	Managerial contribution	122
5.1.3.	Business practices	123
5.1.4.	Overall conclusion.....	124
5.2.	Future Perspectives.....	125

Chapter 1.

General Introduction

1.1 Mass Spectrometry Imaging

Mass spectrometry (MS) has evolved as a foundational analytical technology that combines exceptional selectivity based on the mass-to-charge ratio (m/z) of molecular species with high detection sensitivity, enabling the simultaneous analysis of numerous components within complex samples.¹ Its application areas are extensive, encompassing proteomics and metabolomics, which aim to comprehensively characterize endogenous proteins and metabolites, as well as pharmacokinetics, pathology, materials science, and environmental sciences. Over the past decade, systematic advancements in instrumentation, measurement methodologies, and data analysis have resulted in higher speeds, greater resolving power, and improved specificity across the MS ecosystem.

Mass spectrometry imaging (MSI) has emerged as a technique that preserves the intrinsic advantages of MS, while enabling label-free and multiplexed visualization of molecular distributions through the spatial reconstruction of spectra acquired on a pixel-by-pixel basis (Figure 1.1).^{2,3} MSI provides localization information that is typically lost during conventional sample extraction processes, thereby offering unique analytical value. MSI has found application in a wide range of fields, including clinical pathology, drug research, plant biology, food science, and materials science.⁴⁻¹² By enabling the spatial mapping of metabolites, lipids, peptides, and proteins, and characterization of their microenvironmental heterogeneity, MSI has increasingly positioned itself as a key platform for spatial multi-omics.⁴

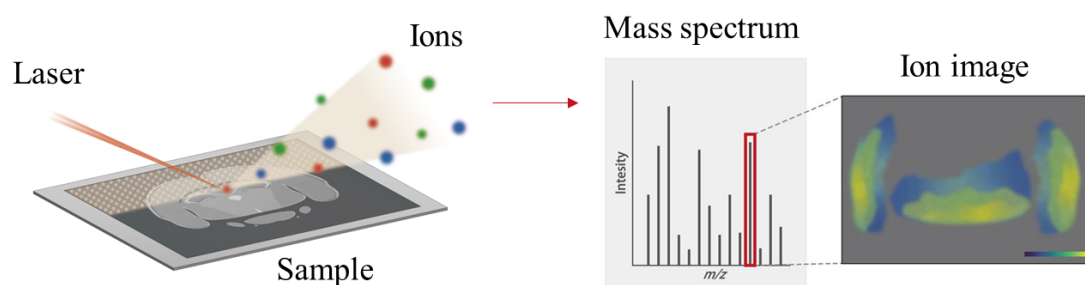


Figure 1.1. General overview of MSI (MALDI as an example). A matrix-coated sample on a conductive surface is irradiated with a laser to generate ions. Mass spectra are acquired at each (x, y) coordinate. A peak of interest (m/z) is selected from the spectra, and its intensity distribution is visualized as an ion image.

Matrix-assisted laser desorption/ionization (MALDI) remains the predominant ionization method for MSI (Figure 1.2A), and its combination with time-of-flight mass

spectrometer (TOFMS) offers practical advantages, including robust instrumentation, broad m/z coverage, high spatial resolution, and fast pixel throughput. MALDI-MSI, which emerged at least as early as 1997, is one of the earliest MSI modalities applicable to organic molecules.¹³ Nearly three decades of refinement efforts account for the evolution of the technique into its present form. Regarding throughput, continuous scanning, in which the sample is moved continuously while being irradiated by the laser, has enabled acquisition rates exceeding 100 pixels per second.¹⁴ The recent development of laser post-ionization—commonly referred to as MALDI-2—has greatly improved sensitivity and molecular coverage, particularly for lipids, small molecules, and pharmaceuticals, with reports of up to two orders of magnitude signal enhancement.^{15,16} MALDI-2 also helps overcome the typical trade-off between spatial resolution and molecular coverage by improving the signal-to-noise ratio at increasingly finer pixel sizes. The practical spatial resolution of MALDI-TOFMS is determined by both the laser spot size and the obtainable ion yield, and typical laser diameters of 10–50 μm remain widely used. Because many MSI applications require comparison with histologically stained images, achieving subcellular resolution has become a major technological objective, prompting efforts such as transmission-mode MALDI that achieves focal diameters on the order of 1 μm .^{17,18} Moreover, integration with trapped ion mobility spectrometry (TIMS) has introduced an additional modality capable of separating isomeric species and improving identification confidence.¹⁹

In parallel, the rapid progress in other ionization and detection platforms has expanded the methodological landscape of MSI. Desorption electrospray ionization (DESI), an ambient-pressure technique based on electrospray ionization, has emerged as a widely used modality (Figure 1.2B).²⁰ Coupling DESI with triple-quadrupole mass spectrometry enables targeted imaging in multiple reaction monitoring (MRM) mode, offering high specificity and sensitivity for drug molecules and low-abundance metabolites.^{21,22} In the field of elemental and isotopic imaging, laser ablation–inductively coupled plasma mass spectrometry (LA-ICP-MS) has advanced significantly, offering exceptionally high sensitivity for the spatial mapping of trace metals in biological tissues (Figure 1.2C).²³ Time-of-flight secondary ion mass spectrometry (TOF-SIMS), originally developed for surface analysis, offers submicron spatial resolution and high surface sensitivity (Figure 1.2D). Recent developments in gas-cluster primary ion beams have enabled softer ionization, expanding the detectable chemical classes beyond elemental and small-molecule fragments.²⁴

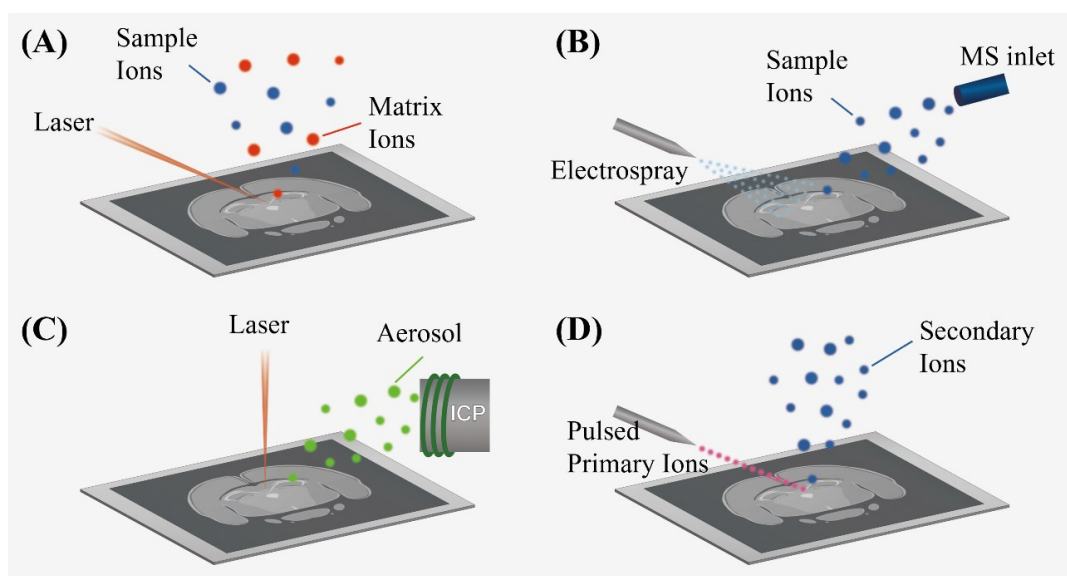


Figure 1.2. Schematic illustrations of representative ionization techniques used in MSI: (A) MALDI, (B) DESI, (C) LA-ICP-MS, and (D) TOF-SIMS.

In addition to ionization methods and instrumental technologies, sample preparation for MSI is a critical factor that strongly influences data quality, including signal intensity and spatial resolution. For MALDI-MSI, the two essential preparation steps are thin sectioning and matrix application (Figure 1.3).^{25–28} Thin sectioning involves cutting a sample into slices that are typically several to tens of micrometers thick. Paraffin embedding and formalin fixation are commonly used for histological tissue staining. However, in MSI, these treatments are avoided to preserve the native molecular composition, and the samples are cryosectioned in a frozen state. The ease of sectioning frozen samples depends on the hardness and fragility of the materials. Mammalian tissues, which are relatively easy to section, have been widely investigated for MSI applications. In contrast, plant tissues and polymeric materials are often fragile or mechanically hard, making them difficult to thin-section. Therefore, MSI studies on these samples remain limited despite increasing recent interest. Several approaches have been proposed to overcome these limitations. One strategy involves reducing the difficulty of thin sectioning by using supporting materials such as adhesive tapes during sectioning.²⁹ In another strategy, thin sectioning is eliminated entirely. Among these approaches, transfer sampling has been used in applications where sectioning is particularly challenging. In this method, the sample is brought in direct contact with an appropriate transfer medium, allowing the analyte to migrate onto the medium. Transfer sampling and the selection of suitable transfer media are central to this study, and relevant prior technologies are discussed in detail in the following section.

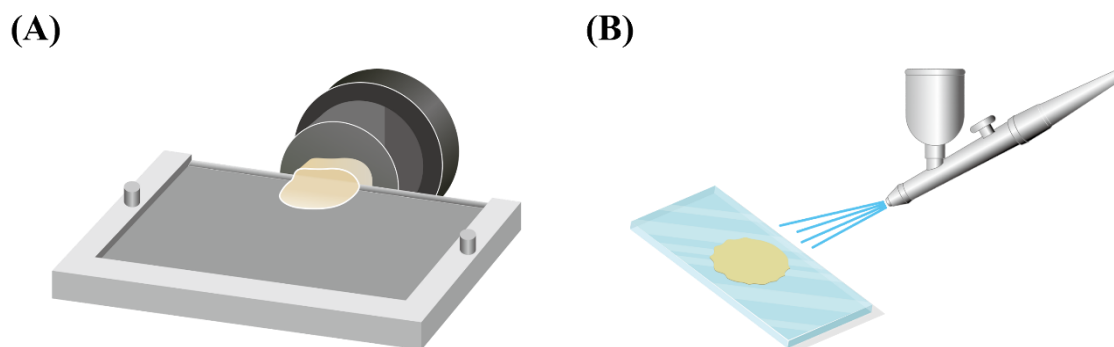


Figure 1.3. Schematic illustration of conventional sample preparation procedures for MALDI-MSI: (A) thin-sectioning of a biological sample using a cryomicrotome and (B) matrix application to the sample surface by spray coating.

Matrix application is an essential step when MALDI is used as the ionization method, involving the two-dimensional deposition of an appropriate matrix compound onto a thin-sectioned sample. Various approaches have been developed for this purpose, including the spray and vacuum deposition methods. Similar to thin sectioning, matrix applications require specialized knowledge and technical expertise with numerous considerations, such as selecting a suitable matrix and optimizing the conditions for uniform deposition. Recently, dedicated instruments for matrix applications have become widely available. Guidelines for controlling particle size, crystallinity, and film thickness have also been established. These parameters are important when choosing between spray-based and deposition approaches.^{30,31} Advances in reactive matrices and on-tissue derivatization for small-molecule analysis have further improved isomer differentiation and the detection of acidic functional groups.^{32,33} In addition, combining MALDI with immunohistochemistry (MALDI-IHC) allows protein-level imaging by detecting mass-tag-labeled antibodies bound to specific targets.³⁴ In parallel, matrix-free ionization strategies, such as surface-assisted laser desorption/ionization (SALDI), have also been explored, employing silicon nanostructures, metal nanoparticles, and other substrates.^{35–37} SALDI and MALDI generally deliver different modalities, and their optimal fields of analysis vary.³⁵ Because SALDI, like MALDI, uses laser-based desorption/ionization, it can be implemented on MALDI-TOFMS instruments, which provides an advantage over other MSI approaches, such as DESI or TOF-SIMS, both of which require dedicated instrumentation.

This section provides an overview of the technical aspects and recent trends related to MSI. Since its emergence in the late twentieth century, MSI has remained a

relatively nascent analytical technique within the broader field of mass spectrometry and continues to undergo rapid technological innovation. Moreover, applications once considered distant from MSI are now increasingly exploring its implementation. Both technological advances and the expansion of application areas are expected to continue driving the growth of MSI in the years ahead.

1.2 Previous Studies on Transfer Media for MSI

This section focuses on the transfer method as an alternative to conventional thin-sectioning sample preparation for MSI. Here, the term “transfer” refers broadly to approaches that have also been described in the literature as imprinting, blotting, or stamping. These processes entail transferring the target chemical constituents from the sample surface to another medium, indicating that high-quality thin sections are not required (Figure 1.4). Consequently, transfer-based approaches are particularly useful for samples that are difficult to section and have therefore been explored mainly in applications involving plant samples (e.g. leaves), as well as human skin. Transfer media can broadly be classified into two categories. One category consists of media intended primarily for sampling and not designed to contribute to the ionization process, whereas the other comprises media that serve dual functions, enabling both sampling and ionization. Representative examples from each category are presented below.

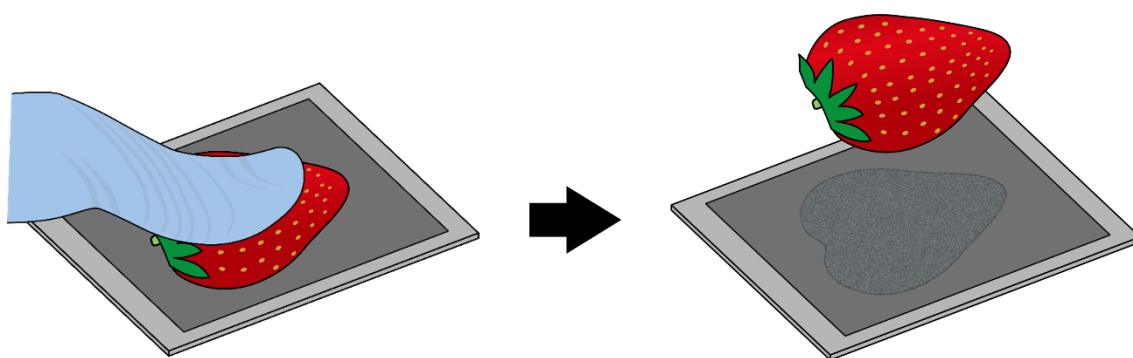


Figure 1.4. Schematic illustration of the transfer-based sample introduction process. The sample (e.g., plant tissue or fruit) is pressed onto the transfer plate (left), allowing molecular components to be transferred onto the plate surface. After the removal of the original sample (right), transferred molecules remain on the plate and can be subjected directly to mass spectrometry imaging, while the bulk tissue sample itself is no longer required for analysis.

Transfer media, whose primary function is sampling, do not possess an intrinsic ionization capability, and therefore must be used in conjunction with an external ionization technique, such as MALDI or DESI. To enable effective sampling, various porous materials capable of absorbing and retaining analytes have been investigated. Porous polytetrafluoroethylene (PTFE) is a typical example and its use in combination with MALDI^{38,39} and DESI^{40–43} has been reported. TLC plates have also been used as sampling and transfer media, in combination with MALDI^{44–46} and DESI^{47,48}. These materials are chemically stable and readily available, making them convenient candidates for exploratory use as transfer media. In MALDI-based approaches, transfer to other media, such as cellulose membranes^{13,49,50}, resin particles¹³, and porous alumina⁵¹, has also been reported. In addition, a method known as TLC-Blot has been proposed, in which components separated on a TLC plate are transferred onto a polyvinylidene difluoride (PVDF) membrane for measuring the TLC-separated components with higher sensitivity using MSI^{52–54}. When MALDI is employed, particularly for introduction into MALDI-TOFMS, the electrical conductivity of the sample surface must be considered. Accordingly, practical measures such as using sufficiently thin media or applying conductive coatings are required to avoid issues during measurement. In contrast, in DESI, non-conductive media are often more suitable; thus, conductivity-related considerations required for MALDI measurements are unnecessary. Accordingly, DESI-based approaches have explored other transfer media, such as tape strips⁵⁵ and oil-absorbing films^{56,57}. Most of the transfer media described above were not originally designed for MSI but have been applied as transfer media for the technique.

The other category, defined as media that possess both sampling and ionization functions, is intended to refer to cases in which SALDI substrates are employed as transfer media. Some of these substrates were specifically designed for SALDI, and in certain cases were developed with their potential use in transfer-based MSI explicitly in mind. Both transfer-based methods and SALDI typically require dedicated media (e.g., transfer substrates or engineered surfaces). Accordingly, several MSI approaches combining transfer methods with SALDI substrates have been reported. In these approaches, the transfer media induces a SALDI effect, thereby eliminating the need for matrix applications and allowing direct introduction into MALDI-TOFMS. Although transfer-based approaches are often discussed as alternatives to thin sectioning, there are also reports in which thin tissue sections are intentionally transferred onto SALDI substrates to exploit the ionization characteristics inherent to SALDI that differ from those of MALDI. Representative SALDI substrates based on silicon nanostructures, such as porous silicon^{58–63} and silicon nanowires^{64–69}, have been widely used as transfer media

for MSI. Because these silicon-based substrates possess fine nanostructures and are relatively fragile, they have mainly been used in gentle transfer approaches for soft biological samples such as tissue sections or fingerprints. By contrast, there have also been reports of flower petals being transferred onto titanium oxide (TiO₂) nanowire-structured SALDI substrates⁷⁰. In addition, transfer onto SALDI substrates loaded with metal (e.g., Au and Ag) nanoparticles (NPs) has also been reported. The supporting media for such nanoparticles vary widely, including MALDI target plates^{71–80}, filter paper^{81,82}, and TiO₂ nanostructures^{83–87}, and can be designed to withstand the transfer of plant samples such as leaves. Compared with silicon nanostructured SALDI substrates, the metal nanoparticle-loaded media described above are more practical as transfer-based SALDI media capable of replacing thin sectioning. Furthermore, attempts have been made to perform transfers using SALDI media coated with metal nanolayers. Representative examples include porous silicon coated with Ag⁸⁸, porous alumina coated with Pt⁸⁹ (DIUTHAME), and black silicon coated with Au⁹⁰. Table 1.1 provides an overview of the SALDI substrates reported as transfer media for MSI, while Figure 1.5 illustrates representative examples of transfer-based approaches in MSI employing SALDI substrates for ionization.

Table 1.1. SALDI substrates reported as transfer media for MSI and their applications.

Category	SALDI substrates	Application	Ref.
Nanostructure-based substrates	Porous Si	Tissue / Fingerprint	58-63
	Si nanowires	Tissue	64–69
	TiO ₂ nanowires	Plant	70
Metal nanoparticles (NPs)-loaded substrates	Au/AgNPs on MALDI target plate	Tissue / Fingerprint/ Plant	71-80
	AuNP-immersed filter paper	Plant	81, 82
	AuNPs in TiO ₂ nanostructures	Tissue / Plant	83–87
Metal nanolayer-coated substrates	Ag nanolayer on porous Si	Tissue / Fingerprint	88
	Pt nanolayer on porous alumina	Plant	89
	Au nanolayer on black Si	Tissue / Fingerprint	90

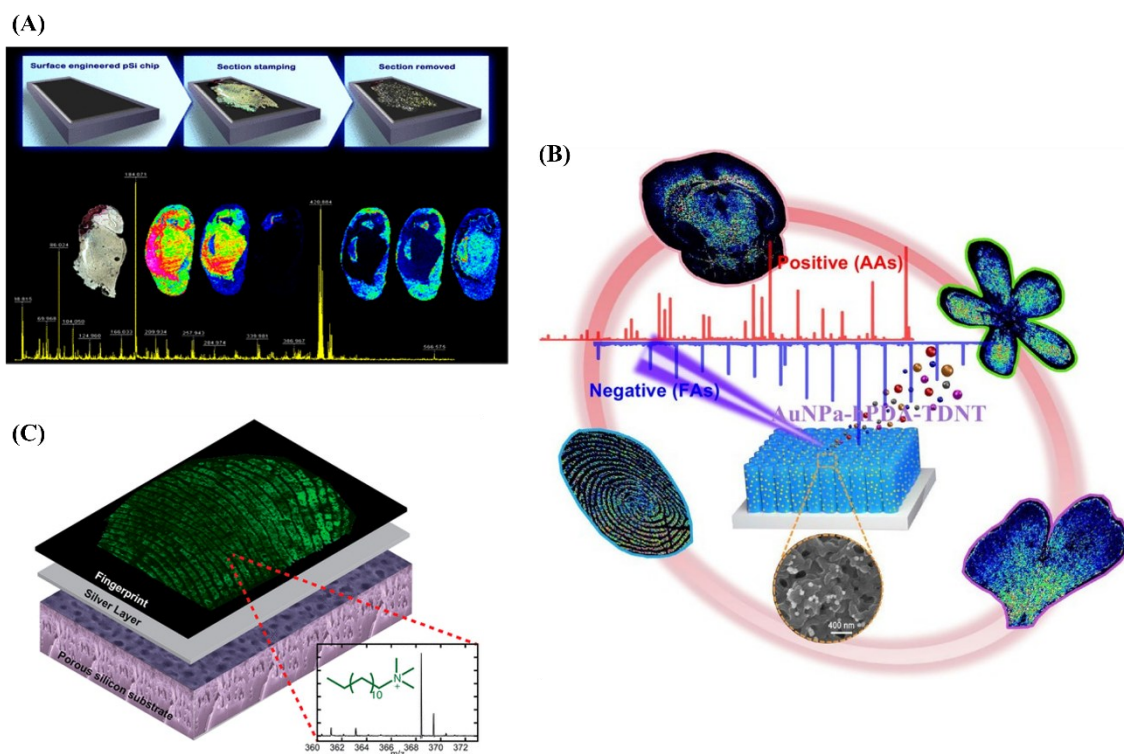


Figure 1.5. Examples of transfer-based approaches in MSI using SALDI substrates. (A) Transfer of tissue sections onto surface-engineered porous silicon substrates, followed by SALDI-MSI analysis after section removal. (*Reprinted with permission from Ref. 60. Copyright 2012, American Chemical Society.*) (B) Schematic illustration of SALDI-MSI using a hydrophobic polydopamine (hPDA)-modified TiO₂ nanotube (TDNT) substrate coated with plasmonic gold nanoparticles (AuNP-hPDA-TDNT), demonstrating simultaneous detection of analytes in positive and negative ion modes and the acquisition of ion images from transferred plant samples. (*Reprinted with permission from Ref. 85. Copyright 2012, Tsinghua University Press.*) (C) Transfer of a fingerprint onto a porous silicon substrate coated with a silver nanolayer, enabling SALDI-MSI of fingerprint components. (*Reprinted with permission from Ref. 88. Copyright 2015, American Chemical Society.*)

As described above, transfer-based approaches to MSI have been extensively developed as alternative sample preparation methods for conventional thin sectioning. In particular, approaches employing SALDI substrates that combine sampling and ionization functions have demonstrated effectiveness in simplifying sample preparation and enabling the detection of low-molecular-weight regions ($m/z < 500$). However, most existing transfer media were not originally designed for transfer in MSI; rather, they were developed either for purposes unrelated to MSI or primarily to exploit SALDI effects, and only a limited number were designed with transfer in mind. In addition, many of these media are not commercially available, and their use often requires fabrication of the media themselves and sample preparation on the media side. Furthermore, when factors such as the fragility of media materials and fabrication costs are considered, significant challenges remain from the perspective of practical implementation of MSI using transfer-based approaches. In light of these considerations, there is strong motivation to develop and generalize transfer media specifically designed for MSI that are based on transfer as a fundamental concept and are capable of exploiting SALDI characteristics. This doctoral study was conducted to address these challenges. In the following section, an important technology underlying this work, Desorption Ionization Using Through-Hole Alumina Membrane (DIUTHAME), is described in detail.

1.3 DIUTHAME (Desorption Ionization Using Through-Hole Alumina Membrane)

Here, Desorption Ionization Using Through-Hole Alumina Membrane (DIUTHAME), one of SALDI technologies that is particularly important for the background and development of this doctoral research, is described. DIUTHAME was conceived at the Graduate School for the Creation of New Photonics Industries (GPI) in the cooperation of Hamamatsu Photonics K.K. (HPK).⁹¹ It was subsequently validated through collaborative studies conducted by these two organizations together with the National Institute of Advanced Industrial Science and Technology (AIST) and Nagoya University.^{92,93} DIUTHAME is a matrix-free ionization method that employs an anodic porous alumina membrane (APAM) featuring a highly ordered array of straight, submicron through-holes. APAMs fabricated via electrochemical anodization can be produced uniformly over a large area, typically exhibiting a pore diameter of

approximately 200 nm, a porosity of around 50%, and a membrane thickness of roughly 5 μm (Figure 1.6).

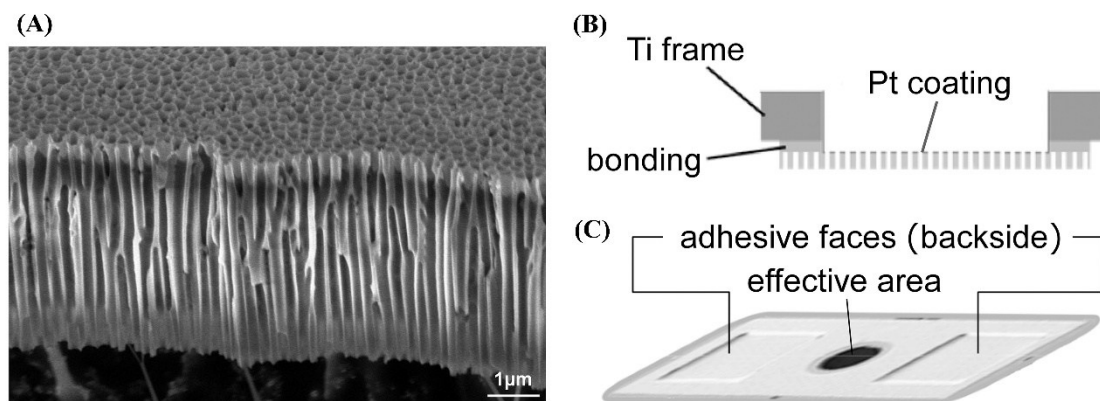


Figure 1.6. (A) An example of the through hole porous alumina membranes observed by scanning electron microscopy (SEM). The scale bar indicates 1 μm . Schematic view (B), and photograph (C), of the DIUTHAME chip in its final form. (*Reprinted from Ref. 91. Copyright 2018 The Authors Rapid Communications in Mass Spectrometry Published by John Wiley & Sons Ltd.*)

The subsequent deposition of platinum onto the laser-irradiated (front) surface generated platinum-coated APAM (Pt-APAM), which functioned as an effective SALDI substrate. Although the precise mechanism of ionization enhancement remains unclear, it is presumed that the synergistic interaction between the regular nanostructure of APAM and the platinum film promotes efficient desorption/ionization through photothermal conversion and charge-transfer processes. Pt-APAM inherently eliminates matrix-derived background peaks in the low- m/z region and avoids the heterogeneous co-crystallization processes that give rise to the so-called “sweet spot” effect. When applied to MSI, DIUTHAME facilitates the visualization of low-mass analytes and reduces pixel-to-pixel variability in terms of both signal intensity and mass accuracy. Several applications of DIUTHAME have been reported, particularly those highlighting its stability as an ionization method and utility in MSI.^{89,94–97}

The DIUTHAME protocol for MSI and its underlying mechanisms are described herein. The tissue sections were prepared in a cryostat and mounted onto a conductive glass slide. A Pt-APAM was then placed on top of the section with its back in contact with the tissue. When the assembly was removed from the cryostat and allowed to return to room temperature, the sample components migrated from the back to the front of the membrane via through-holes via capillary action. Once the sample was introduced into

the MALDI-TOFMS, the laser irradiated the sample components that had permeated the front surface of the membrane, enabling their detection as ions. As a result, DIUTHAME can intrinsically achieve a high spatial resolution, which is one of its principal advantages. Additionally, an initial report demonstrated that DIUTHAME is fully compatible with commercial MALDI-TOFMS instruments and requires no hardware modifications. Sample preparation is also straightforward, consisting simply of placing a prefabricated Pt-APAM onto the section, and the method inherently avoids fluctuations associated with matrix deposition, thereby improving reproducibility. The effects of APAM nanostructural parameters such as pore diameter, pitch, porosity, and membrane thickness on the sensitivity and spatial response have been clarified through systematic investigations in recent years.^{98,99}

Pt-APAM was subsequently commercialized by HPK and marketed under the product name “DIUTHAME.” This development eventually led to the mass spectrometry imaging “transfer plate,” which is the central subject of this thesis. The following section explains how the challenges associated with DIUTHAME motivated the development of the transfer plate and ultimately led to this doctoral research. (hereafter, DIUTHAME as a SALDI method and “DIUTHAME” as a commercial product are distinguished by the presence or absence of quotation marks.)

1.4 Background to Transfer Plate Development and Doctoral Research

“DIUTHAME” was launched in 2018 by HPK following evaluations of the manufacturing framework and mass-production feasibility. At the time of its release, the MSI user community sought both a higher spatial resolution and simplified sample preparation protocols. “DIUTHAME” was recognized as one approach that could address these needs and therefore saw rapid uptake for evaluation, particularly among research institutions.

During this period, extensive feedback was obtained from users who employed this method in practice. While the anticipated benefits were largely realized, several issues were identified. The fragility of the APAM substrate and the resulting limitations on how and where it can be used are among the most frequently raised concerns. The APAM used in DIUTHAME is a fragile material with a thickness of only about 5 μm , making it highly susceptible to breakage if mishandled. Although a thicker, mechanically reinforced version was released later, this inherent fragility could not be fully eliminated.

In addition, some researchers have attempted to apply DIUTHAME as a medium for transfer-based sampling, although it was originally designed for frozen tissue sections. Several attempts resulted in membrane breakage. Based on user feedback, the DIUTHAME business team identified these issues as customer challenges, leading to the emergence of a new development objective: the creation of an ionization-assisting substrate with the rigidity and durability required for transfer sampling.

As an HPK employee, the author had been involved in improving the performance of “DIUTHAME” since its initial commercialization and began working on the above development objective around 2020. The first step was to examine materials traditionally used for transfer-based sampling. However, most of these materials are unsuitable for commercialization, leading to the decision to design a more practical transfer medium from the ground up. After prototyping various candidates, a porous glass bead substrate was proposed as a medium offering both sufficient functionality and high scalability for mass production. Consequently, the author became the primary lead of a product development project in 2021 to create an ionization-assisted substrate based on this porous glass bead structure. Notably, this is the author’s first experience leading a product development project.

The design concept of the transfer plate fundamentally differs from that of DIUTHAME because the transfer plate was conceived specifically for transfer-based sampling. DIUTHAME was designed for thin-sectioned samples, which is the standard sample preparation method for MSI; therefore, it targeted users already experienced with MSI workflows. In contrast, the transfer plate was expected to serve not only users unfamiliar with MSI sample preparation, but also applications that had been avoided because of the difficulties associated with cryosectioning. Therefore, the transfer plate required a business strategy distinct from that of DIUTHAME. However, the DIUTHAME business team did not have sufficient knowledge or experience in this area, and the author recognized the need to strengthen his expertise in the areas of project leadership and business strategy development. Accordingly, in 2022, the author enrolled in GPI—an institution known for supporting business creation—on a temporary transfer from the affiliated company, enabling full-time engagement with the project. It was under these circumstances that the present doctoral research was undertaken.

1.5 Objectives of This Thesis

➤ Major Theme

To acquire practical academic knowledge in both technology and management, through the practical product development process of the transfer plate, for *the creation of new photonics industries*.

➤ Technical Research Objective

To demonstrate the technological principles of the transfer plate for MSI and to verify its application methods.

➤ Managerial Research Objective

To analyze the management of the product development team and thereby elucidate the practical methodologies and motivational processes involved.

As described in Section 1.1, MSI is a photonics-based technology that relies on laser desorption/ionization and contributes to a wide range of industrial fields. With recent technological advances such as MALDI-2 and LA-ICP-MS, the importance of photonics-based technologies in MSI has continued to increase. Transfer plates aimed at expanding MSI applications have the potential to further broaden the scope of photonics applications while simultaneously revealing new challenges in MSI. Such challenges are expected to be addressed through advances in mass spectrometry instrumentation and sample preparation technologies, thereby providing to new opportunities for the application of photonics technologies. In this context, the development of a transfer plate as a new product for MSI represents an instance of photonics-driven industrial creation, that is, *the creation of new photonics industries*. Both the technological development of the transfer plate and the author's leadership of the product development team constitute practical new-business development activities, and the outcomes of these activities form the core subjects of this study. Thus, the objectives and outcomes of this study align with the diploma policy for Ph.D. degree at GPI.

1.6 Scope of Chapters

In accordance with the objectives described above, this thesis is organized into three major components of practical business development activities. (1) proof of concept for the transfer plate, (2) demonstration of its applications through the combination of the transfer plate with MALDI, and (3) a management analysis of the product development project using multi-perspective internal observation framework.

Chapter 2 demonstrates the fundamental technological concepts of the transfer plate, which enable thin-section-free sampling and matrix-free ionization for MSI. Using model samples such as polymer additives on surfaces and cross sections of strawberries, this chapter shows that transfer sampling is feasible regardless of the sample's moisture state. This also demonstrates that matrix-free ionization minimizes interfering peaks and yields high image reproducibility. A portion of the content in this chapter has been published as a peer-reviewed article titled "Thin-section- and matrix-free mass spectrometry imaging: Reproducible sample transfer using novel platinum-coated porous plate formed of glass beads" in the international journal *Rapid Communications in Mass Spectrometry*.¹⁰⁰

Chapter 3 demonstrates that the integration of a transfer plate with MALDI expands its application potential. By combining the thin-section-free characteristics of the transfer plate with the soft-ionization advantages of matrix-assisted techniques, this chapter proposes a thin-section-free MALDI-MSI approach. This demonstrates that, in realistic cases where matrix-free ionization provides insufficient sensitivity, this limitation can be overcome by integrating the transfer plate with conventional MALDI methods. A portion of the content in this chapter has also been published as a peer-reviewed article titled "Transfer-Based Thin-Section-Free MALDI Mass Spectrometry Imaging Using a Platinum-Coated Porous Plate Formed of Glass Beads" in the international journal *The Journal of Mass Spectrometry*.¹⁰¹

Chapter 4 analyzes the practical activities of the product development project team through a new theoretical framework, multi-perspective internal observation. By reviewing project activities from multiple perspectives rather than from a single viewpoint, the chapter elucidates the nature of practical knowledge in the field, the process through which it is formed, and how members are motivated throughout the project. A portion of the content in this chapter was submitted as a peer-reviewed article in the international journal *The Journal of Organization and Discourse*.

References for Chapter 1

1. Gross HJ. *Mass Spectrometry: A Textbook*. 3rd ed. Springer; 2017.
2. Shimma S. Mass Spectrometry Imaging. *Mass Spectrom*. 2022;11(1):A0102-A0102. doi:10.5702/massspectrometry.A0102
3. Körber A, Anthony IGM, Heeren RMA. Mass Spectrometry Imaging. *Anal Chem*. 2025;97(29):15517-15549. doi:10.1021/acs.analchem.4c05249
4. Zhang H, Lu KH, Ebbini M, Huang P, Lu H, Li L. Mass spectrometry imaging for spatially resolved multi-omics molecular mapping. *npj Imaging*. 2024;2(1):20. doi:10.1038/s44303-024-00025-3
5. Sun Z, Wang F, Liu Y, et al. Recent strategies for improving MALDI mass spectrometry imaging performance towards low molecular weight compounds. *TrAC Trends in Anal Chem*. 2024;175:117727. doi:10.1016/j.trac.2024.117727
6. De Raad M, Northen TR, Bowen BP. Analysis and Interpretation of Mass Spectrometry Imaging Datasets. In: *Comprehensive Anal Chem*. Vol 82. Elsevier; 2018:369-386. doi:10.1016/bs.coac.2018.06.006
7. Granborg JR, Handler AM, Janfelt C. Mass spectrometry imaging in drug distribution and drug metabolism studies – Principles, applications and perspectives. *TrAC Trends in Anal Chem*. 2022;146:116482. doi:10.1016/j.trac.2021.116482
8. Horn PJ, Chapman KD. Imaging plant metabolism *in situ*. Li-Beisson Y, ed. *J Exp Bot*. 2024;75(6):1654-1670. doi:10.1093/jxb/erad423
9. Cao X, Cong P, Song Y, Liu Y, Xue C, Xu J. Promising mass spectrometry imaging: exploring microscale insights in food. *Crit Rev Food Sci Nutr*. 2025;65(30):1-32. doi:10.1080/10408398.2025.2451189
10. Vats M, Cillero-Pastor B, Cuypers E, Heeren RMA. Mass spectrometry imaging in plants, microbes, and food: a review. *Analyst*. 2024;149(18):4553-4582. doi:10.1039/D4AN00644E

11. Tortorella S, Bartels B, Suman M, Heeren RMA, Righetti L. Mass spectrometry imaging in food safety and authenticity: Overcoming challenges and exploring opportunities. *Trends Food Sci Technol*. 2025;155:104803. doi:10.1016/j.tifs.2024.104803
12. Gruendling T, Weidner S, Falkenhagen J, Barner-Kowollik C. Mass spectrometry in polymer chemistry: a state-of-the-art up-date. *Polym Chem*. 2010;1(5):599-617. doi:10.1039/b9py00347a
13. Caprioli RM, Farmer TB, Gile J. Molecular Imaging of Biological Samples: Localization of Peptides and Proteins Using MALDI-TOF MS. *Anal Chem*. 1997;69(23):4751-4760. doi:10.1021/ac970888i
14. Bednařík A, Macháľková M, Moskovets E, et al. MALDI MS Imaging at Acquisition Rates Exceeding 100 Pixels per Second. *J Am Soc Mass Spectrom*. 2019;30(2):289-298. doi:10.1007/s13361-018-2078-8
15. Soltwisch J, Ketting H, Vens-Cappell S, Wiegelmann M, Müthing J, Dreisewerd K. Mass spectrometry imaging with laser-induced postionization. *Science*. 2015;348(6231):211-215. doi:10.1126/science.aaa1051
16. Barré FPY, Paine MRL, Flinders B, et al. Enhanced Sensitivity Using MALDI Imaging Coupled with Laser Postionization (MALDI-2) for Pharmaceutical Research. *Anal Chem*. 2019;91(16):10840-10848. doi:10.1021/acs.analchem.9b02495
17. Niehaus M, Soltwisch J, Belov ME, Dreisewerd K. Transmission-mode MALDI-2 mass spectrometry imaging of cells and tissues at subcellular resolution. *Nat Methods*. 2019;16(9):925-931. doi:10.1038/s41592-019-0536-2
18. Young RSE, Piper AK, McAlary L, et al. Subcellular mass spectrometry imaging of lipids and nucleotides using transmission geometry ambient laser desorption and plasma ionisation. *Nat Commun*. 2025;16(1):9130. doi:10.1038/s41467-025-64604-7
19. Spraggins JM, Djambazova KV, Rivera ES, et al. High-Performance Molecular Imaging with MALDI Trapped Ion-Mobility Time-of-Flight (timsTOF) Mass Spectrometry. *Anal Chem*. 2019;91(22):14552-14560. doi:10.1021/acs.analchem.9b03612

20. Takáts Z, Wiseman JM, Gologan B, Cooks RG. Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. *Science*. 2004;306(5695):471-473. doi:10.1126/science.1104404
21. Lamont L, Eijkel GB, Jones EA, et al. Targeted Drug and Metabolite Imaging: Desorption Electrospray Ionization Combined with Triple Quadrupole Mass Spectrometry. *Anal Chem*. 2018;90(22):13229-13235. doi:10.1021/acs.analchem.8b03857
22. Qian Y, Ma X. Advances in Tandem Mass Spectrometry Imaging for Next-Generation Spatial Metabolomics. *Anal Chem*. 2025;97(14):7589-7599. doi:10.1021/acs.analchem.5c00157
23. Doble PA, De Vega RG, Bishop DP, Hare DJ, Clases D. Laser Ablation–Inductively Coupled Plasma–Mass Spectrometry Imaging in Biology. *Chem Rev*. 2021;121(19):11769-11822. doi:10.1021/acs.chemrev.0c01219
24. Jia F, Zhao X, Zhao Y. Advancements in ToF-SIMS imaging for life sciences. *Front Chem*. 2023;11:1237408. doi:10.3389/fchem.2023.1237408
25. Schwartz SA, Reyzer ML, Caprioli RM. Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation. *J Mass Spectrom*. 2003;38(7):699-708. doi:10.1002/jms.505
26. Kaletaş BK, Van Der Wiel IM, Stauber J, et al. Sample preparation issues for tissue imaging by imaging MS. *Proteomics*. 2009;9(10):2622-2633. doi:10.1002/pmic.200800364
27. Dong Y, Li B, Malitsky S, et al. Sample Preparation for Mass Spectrometry Imaging of Plant Tissues: A Review. *Front Plant Sci*. 2016;7:60. doi:10.3389/fpls.2016.00060
28. Mahamdi T, Serna CG, Giné R, et al. Improving MALDI Mass Spectrometry Imaging Performance: Low-Temperature Thermal Evaporation for Controlled Matrix Deposition and Improved Image Quality. *J Am Soc Mass Spectrom*. 2025;36(5):1100-1110. doi:10.1021/jasms.5c00015
29. Nakabayashi R, Hashimoto K, Toyooka K, Saito K. Keeping the shape of plant tissue for visualizing metabolite features in segmentation and correlation analysis

- of imaging mass spectrometry in *Asparagus officinalis*. *Metabolomics*. 2019;15(2):24. doi:10.1007/s11306-019-1486-5
30. Lee PY, Yeoh Y, Omar N, Pung YF, Lim LC, Low TY. Molecular tissue profiling by MALDI imaging: recent progress and applications in cancer research. *Crit Rev Clin Lab Sci*. 2021;58(7):513-529. doi:10.1080/10408363.2021.1942781
 31. Unsihuay D, Mesa Sanchez D, Laskin J. Quantitative Mass Spectrometry Imaging of Biological Systems. *Annu Rev Phys Chem*. 2021;72(1):307-329. doi:10.1146/annurev-physchem-061020-053416
 32. Qiao Z, Lissel F. MALDI Matrices for the Analysis of Low Molecular Weight Compounds: Rational Design, Challenges and Perspectives. *Chem. Asian J*. 2021;16(8):868-878. doi:10.1002/asia.202100044
 33. Harkin C, Smith KW, Cruickshank FL, et al. On-tissue chemical derivatization in mass spectrometry imaging. *Mass Spectrom Rev*. 2022;41(5):662-694. doi:10.1002/mas.21680
 34. Lim MJ, Yagnik G, Henkel C, Frost SF, Bien T, Rothschild KJ. MALDI HiPLEX-IHC: multiomic and multimodal imaging of targeted intact proteins in tissues. *Front Chem*. 2023;11:1182404. doi:10.3389/fchem.2023.1182404
 35. Samarah LZ, Vertes A. Mass spectrometry imaging based on laser desorption ionization from inorganic and nanophotonic platforms. *VIEW*. 2020;1(4):20200063. doi:10.1002/VIW.20200063
 36. Müller WH, Verdin A, De Pauw E, Malherbe C, Eppe G. Surface-assisted laser desorption/ionization mass spectrometry imaging: A review. *Mass Spectrom Rev*. 2022;41(3):373-420. doi:10.1002/mas.21670
 37. Nizioł J, Krupa S, Szuberla W, Ruman T. Advances in metallic nanostructures-assisted laser desorption/ionization mass spectrometry imaging of biological samples: A review. *Anal Chim Acta*. 2025;1366:344256. doi:10.1016/j.aca.2025.344256
 38. Klein AT, Yagnik GB, Hohenstein JD, et al. Investigation of the Chemical Interface in the Soybean–Aphid and Rice–Bacteria Interactions Using MALDI-

- Mass Spectrometry Imaging. *Anal Chem.* 2015;87(10):5294-5301.
doi:10.1021/acs.analchem.5b00459
39. Hapuarachchige PNP, Tat VT, Lee YJ. Advanced Tissue Imprinting With Pneumatic Press for Mass Spectrometry Imaging of Plant Tissues. *J Mass Spectrom.* 2026;61(1):e70007. doi:10.1002/jms.70007
 40. Thunig J, Hansen SH, Janfelt C. Analysis of Secondary Plant Metabolites by Indirect Desorption Electrospray Ionization Imaging Mass Spectrometry. *Anal Chem.* 2011;83(9):3256-3259. doi:10.1021/ac2004967
 41. Müller T, Oradu S, Ifa DR, Cooks RG, Kräutler B. Direct Plant Tissue Analysis and Imprint Imaging by Desorption Electrospray Ionization Mass Spectrometry. *Anal Chem.* 2011;83(14):5754-5761. doi:10.1021/ac201123t
 42. Wu L, Qi K, Liu C, Hu Y, Xu M, Pan Y. Enhanced Coverage and Sensitivity of Imprint DESI Mass Spectrometry Imaging for Plant Leaf Metabolites by Post-photoionization. *Anal Chem.* 2022;94(43):15108-15116.
doi:10.1021/acs.analchem.2c03329
 43. Lorensen MDBB, Hayat SY, Wellner N, Bjarnholt N, Janfelt C. Leaves of *Cannabis sativa* and their trichomes studied by DESI and MALDI mass spectrometry imaging for their contents of cannabinoids and flavonoids. *Phytochem Anal.* 2023;34(3):269-279. doi:10.1002/pca.3202
 44. De Oliveira DN, Siqueira M, Sartor S, Catharino R. Direct analysis of lipsticks by Sorptive tape-like extraction laser desorption/ionization mass spectrometry imaging. *Int J Cosmet Sci.* 2013;35(5):467-471. doi:10.1111/ics.12066
 45. De Oliveira DN, Ferreira MS, Catharino RR. Rapid and Simultaneous In Situ Assessment of Aflatoxins and Stilbenes Using Silica Plate Imprinting Mass Spectrometry Imaging. Koomen JM, ed. *PLoS One.* 2014;9(3):e90901.
doi:10.1371/journal.pone.0090901
 46. Ferreira MS, de Oliveira DN, Gonçalves RF, Catharino RR. Lipid characterization of embryo zones by silica plate laser desorption ionization mass spectrometry imaging (SP-LDI-MSI). *Anal Chim Acta.* 2014;807:96-102.
doi:10.1016/j.aca.2013.11.033

47. Cabral EC, Mirabelli MF, Perez CJ, Ifa DR. Blotting Assisted by Heating and Solvent Extraction for DESI-MS Imaging. *J Am Soc Mass Spectrom.* 2013;24(6):956-965. doi:10.1007/s13361-013-0616-y
48. Hemalatha RG, Pradeep T. Understanding the Molecular Signatures in Leaves and Flowers by Desorption Electrospray Ionization Mass Spectrometry (DESI MS) Imaging. *J Agric Food Chem.* 2013;61(31):7477-7487. doi:10.1021/jf4011998
49. Bunch J, Clench MR, Richards DS. Determination of pharmaceutical compounds in skin by imaging matrix-assisted laser desorption/ionisation mass spectrometry. *Rapid Commun Mass Spectrom.* 2004;18(24):3051-3060. doi:10.1002/rcm.1725
50. Mullen AK, Clench MR, Crosland S, Sharples KR. Determination of agrochemical compounds in soya plants by imaging matrix-assisted laser desorption/ionisation mass spectrometry. *Rapid Commun Mass Spectrom.* 2005;19(18):2507-2516. doi:10.1002/rcm.2078
51. Fournelle F, Yang E, Dufresne M, Chaurand P. Minimizing Visceral Fat Delocalization on Tissue Sections with Porous Aluminum Oxide Slides for Imaging Mass Spectrometry. *Anal Chem.* 2020;92(7):5158-5167. doi:10.1021/acs.analchem.9b05665
52. Goto-Inoue N, Hayasaka T, Taki T, Gonzalez TV, Setou M. A new lipidomics approach by thin-layer chromatography-blot-matrix-assisted laser desorption/ionization imaging mass spectrometry for analyzing detailed patterns of phospholipid molecular species. *J Chromatogr A.* 2009;1216(42):7096-7101. doi:10.1016/j.chroma.2009.08.056
53. Valdes-Gonzalez T, Goto-Inoue N, Hirano W, et al. New approach for glyco- and lipidomics - Molecular scanning of human brain gangliosides by TLC-Blot and MALDI-QIT-TOF MS: New approach for glyco- and lipidomics. *J Neurochem.* 2011;116(5):678-683. doi:10.1111/j.1471-4159.2010.07152.x
54. Zaima N, Goto-Inoue N, Adachi K, Setou M. Selective Analysis of Lipids by Thin-Layer Chromatography Blot Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry. *J Oleo Sci.* 2011;60(2):93-98. doi:10.5650/jos.60.93

55. Meng Y, Chiou AS, Aasi SZ, See NA, Song X, Zare RN. Noninvasive Detection of Skin Cancer by Imprint Desorption Electrospray Ionization Mass Spectrometry Imaging. *Anal Chem.* 2024;96(1):28-32. doi:10.1021/acs.analchem.3c04918
56. Li J, Wei R, Meng Y, Zare RN. Evaluation of Oil-Absorbing Film for Imprint Desorption Electrospray Ionization Mass Spectrometry Imaging (IDESI-MSI) on Biological Samples. *Metabolites.* 2024;14(3):160. doi:10.3390/metabo14030160
57. Liu H, Tian Y, Wei R, Meng Y, Zare RN. Imprint Desorption Electrospray Ionization Mass Spectrometry Imaging (IDESI-MSI) Reveals Absorption of Triclopyr-Based Herbicide in Plants and Mouse Organs. *Metabolites.* 2025;15(7):437. doi:10.3390/metabo15070437
58. Liu Q, Guo Z, He L. Mass Spectrometry Imaging of Small Molecules Using Desorption/Ionization on Silicon. *Anal Chem.* 2007;79(10):3535-3541. doi:10.1021/ac0611465
59. Greving MP, Patti GJ, Siuzdak G. Nanostructure-Initiator Mass Spectrometry Metabolite Analysis and Imaging. *Anal Chem.* 2011;83(1):2-7. doi:10.1021/ac101565f
60. Ronci M, Rudd D, Guinan T, Benkendorff K, Voelcker NH. Mass Spectrometry Imaging on Porous Silicon: Investigating the Distribution of Bioactives in Marine Mollusc Tissues. *Anal Chem.* 2012;84(21):8996-9001. doi:10.1021/ac3027433
61. Louie KB, Bowen BP, Cheng X, et al. "Replica-Extraction-Transfer" Nanostructure-Initiator Mass Spectrometry Imaging of Acoustically Printed Bacteria. *Anal Chem.* 2013;85(22):10856-10862. doi:10.1021/ac402240q
62. Rudd D, Ronci M, Johnston MR, Guinan T, Voelcker NH, Benkendorff K. Mass spectrometry imaging reveals new biological roles for choline esters and Tyrian purple precursors in muricid molluscs. *Sci Rep.* 2015;5(1):13408. doi:10.1038/srep13408
63. Rudd DA, Benkendorff K, Chahal C, et al. Mapping insoluble indole metabolites in the gastrointestinal environment of a murine colorectal cancer model using desorption/ionisation on porous silicon imaging. *Sci Rep.* 2019;9(1):12342. doi:10.1038/s41598-019-48533-2

64. Vidová V, Novák P, Strohalm M, Pól J, Havlíček V, Volný M. Laser Desorption-Ionization of Lipid Transfers: Tissue Mass Spectrometry Imaging without MALDI Matrix. *Anal Chem.* 2010;82(12):4994-4997. doi:10.1021/ac100661h
65. Tata A, Fernandes AMAP, Santos VG, et al. Nanoassisted Laser Desorption-Ionization-MS Imaging of Tumors. *Anal Chem.* 2012;84(15):6341-6345. doi:10.1021/ac301202q
66. Chen PY, Hsieh CY, Shih CJ, Lin YJ, Tsao CW, Yang YL. Exploration of Fungal Metabolic Interactions Using Imaging Mass Spectrometry on Nanostructured Silicon. *J Nat Prod.* 2018;81(7):1527-1533. doi:10.1021/acs.jnatprod.7b00866
67. Chen X, Gao J, Wang T, et al. Hepatocarcinoma Discrimination by Ratiometric Lipid Profiles Using Tip-Contact Sampling/Ionization Mass Spectrometry. *Anal Chem.* 2019;91(16):10376-10380. doi:10.1021/acs.analchem.9b02623
68. Liu X, Chen Z, Wang T, et al. Tissue Imprinting on 2D Nanoflakes-Capped Silicon Nanowires for Lipidomic Mass Spectrometry Imaging and Cancer Diagnosis. *ACS Nano.* 2022;16(4):6916-6928. doi:10.1021/acsnano.2c02616
69. Liu X, Tao L, Jiang X, et al. Isoporous Membrane Mediated Imprinting Mass Spectrometry Imaging for Spatially-Resolved Metabolomics and Rapid Histopathological Diagnosis. *Small Methods.* 2024;8(10):2301644. doi:10.1002/smtd.202301644
70. Dutkiewicz EP, Su C, Lee H, Hsu C, Yang Y. Visualizing vinca alkaloids in the petal of *Catharanthus roseus* using functionalized titanium oxide nanowire substrate for surface-assisted laser desorption/ionization imaging mass spectrometry. *Plant J.* 2021;105(4):1123-1133. doi:10.1111/tpj.15092
71. Nizioł J, Ruman T. Surface-Transfer Mass Spectrometry Imaging on a Monoisotopic Silver Nanoparticle Enhanced Target. *Anal Chem.* 2013;85(24):12070-12076. doi:10.1021/ac4031658
72. Sekuła J, Nizioł J, Rode W, Ruman T. Gold nanoparticle-enhanced target (AuNPET) as universal solution for laser desorption/ionization mass spectrometry analysis and imaging of low molecular weight compounds. *Anal Chim Acta.* 2015;875:61-72. doi:10.1016/j.aca.2015.01.046

73. Sekuła J, Nizioł J, Misiorek M, et al. Gold nanoparticle-enhanced target for MS analysis and imaging of harmful compounds in plant, animal tissue and on fingerprint. *Anal Chim Acta*. 2015;895:45-53. doi:10.1016/j.aca.2015.09.003
74. Nizioł J, Ossoliński K, Ossoliński T, et al. Surface-Transfer Mass Spectrometry Imaging of Renal Tissue on Gold Nanoparticle Enhanced Target. *Anal Chem*. 2016;88(14):7365-7371. doi:10.1021/acs.analchem.6b01859
75. Misiorek M, Sekuła J, Ruman T. Mass Spectrometry Imaging of low Molecular Weight Compounds in Garlic (*Allium sativum* L.) with Gold Nanoparticle Enhanced Target. *Phytochem Anal*. 2017;28(6):479-486. doi:10.1002/pca.2696
76. Arendowski A, Ruman T. Laser Desorption/Ionisation Mass Spectrometry Imaging of European Yew (*Taxus baccata*) on Gold Nanoparticle-enhanced Target. *Phytochem Anal*. 2017;28(5):448-453. doi:10.1002/pca.2693
77. Nizioł J, Sekuła J, Ruman T. Visualizing spatial distribution of small molecules in the rhubarb stalk (*Rheum rhabarbarum*) by surface-transfer mass spectrometry imaging. *Phytochemistry*. 2017;139:72-80. doi:10.1016/j.phytochem.2017.04.006
78. Nizioł J, Misiorek M, Ruman T. Mass spectrometry imaging of low molecular weight metabolites in strawberry fruit (*Fragaria x ananassa* Duch.) cv. Primoris with 109Ag nanoparticle enhanced target. *Phytochemistry*. 2019;159:11-19. doi:10.1016/j.phytochem.2018.11.014
79. Nizioł J, Sunner J, Beech I, et al. Localization of Metabolites of Human Kidney Tissue with Infrared Laser-Based Selected Reaction Monitoring Mass Spectrometry Imaging and Silver-109 Nanoparticle-Based Surface Assisted Laser Desorption/Ionization Mass Spectrometry Imaging. *Anal Chem*. 2020;92(6):4251-4258. doi:10.1021/acs.analchem.9b04580
80. Ossoliński K, Ruman T, Ossoliński T, et al. Monoisotopic silver nanoparticles-based mass spectrometry imaging of human bladder cancer tissue: Biomarker discovery. *Adv Med Sci*. 2023;68(1):38-45. doi:10.1016/j.advms.2022.12.002
81. Wu X, Qin R, Wu H, et al. Nanoparticle-immersed paper imprinting mass spectrometry imaging reveals uptake and translocation mechanism of pesticides in plants. *Nano Res*. 2020;13(3):611-620. doi:10.1007/s12274-020-2700-5

82. Qin R, Li P, Du M, et al. Spatiotemporal Visualization of Insecticides and Fungicides within Fruits and Vegetables Using Gold Nanoparticle-Immersed Paper Imprinting Mass Spectrometry Imaging. *Nanomaterials*. 2021;11(5):1327. doi:10.3390/nano11051327
83. Wang XN, Tang W, Gordon A, et al. Porous TiO₂ Film Immobilized with Gold Nanoparticles for Dual-Polarity SALDI MS Detection and Imaging. *ACS Appl Mater Interfaces*. 2020;12(38):42567-42575. doi:10.1021/acsami.0c12949
84. Wang XN, Li B. Monolithic Gold Nanoparticles/Thiol- β -cyclodextrin-Functionalized TiO₂ Nanowires for Enhanced SALDI MS Detection and Imaging of Natural Products. *Anal Chem*. 2022;94(2):952-959. doi:10.1021/acs.analchem.1c03764
85. Chen D, Du M, Huang Y, et al. Plasmonic polydopamine-modified TiO₂ nanotube substrates for surface-assisted laser desorption/ionization mass spectrometry imaging. *Nano Res*. 2023;16(2):3028-3039. doi:10.1007/s12274-022-4924-z
86. Chen D, Xu Y, Huang Y, et al. Mapping Molecular Signatures in Plant Leaves, Flowers, and Fruits by a TiO₂ Nanotube-Based Plasmonic Chip for Imprint Mass Spectrometry Imaging. *ACS Agric Sci Technol*. 2023;3(1):119-130. doi:10.1021/acsagscitech.2c00254
87. Sun Y, Chen D, Chen X, Wu X. Stress Response of Citrus Leaves under Mechanical Damage and Huanglongbing Disease Infection Using Plasmonic TiO₂ Nanotube Substrate-Based Imprinting Mass Spectrometry Imaging. *Agronomy*. 2024;14(8):1797. doi:10.3390/agronomy14081797
88. Guinan TM, Gustafsson OJR, McPhee G, Kobus H, Voelcker NH. Silver Coating for High-Mass-Accuracy Imaging Mass Spectrometry of Fingerprints on Nanostructured Silicon. *Anal Chem*. 2015;87(22):11195-11202. doi:10.1021/acs.analchem.5b02567
89. Enomoto H, Kotani M, Ohmura T. Novel Blotting Method for Mass Spectrometry Imaging of Metabolites in Strawberry Fruit by Desorption/Ionization Using Through Hole Alumina Membrane. *Foods*. 2020;9(4):408. doi:10.3390/foods9040408

90. Iakab SA, Ràfols P, Tajés M, Correig-Blanchar X, García-Altares M. Gold Nanoparticle-Assisted Black Silicon Substrates for Mass Spectrometry Imaging Applications. *ACS Nano*. 2020;14(6):6785-6794. doi:10.1021/acsnano.0c00201
91. Naito Y, Kotani M, Ohmura T. A novel laser desorption/ionization method using through hole porous alumina membranes. *Rapid Commun Mass Spectrom*. 2018;32(21):1851-1858. doi:10.1002/rcm.8252
92. Kuwata K, Itou K, Kotani M, Ohmura T, Naito Y. DIUTHAME enables matrix-free mass spectrometry imaging of frozen tissue sections. *Rapid Commun Mass Spectrom*. 2020;34(9):e8729. doi:10.1002/rcm.8729
93. Sato H, Nakamura S, Fouquet TNJ, Ohmura T, Kotani M, Naito Y. Simple Pretreatment for the Analysis of Additives and Polymers by Surface-Assisted Laser Desorption/Ionization Mass Spectrometry Using a Through-Hole Alumina Membrane as a Functional Substrate. *J Am Soc Mass Spectrom*. 2020;31(2):298-307. doi:10.1021/jasms.9b00048
94. Naito Y, Kotani M, Ohmura T. Feasibility of Acetylcholinesterase Reaction Assay Monitoring in DIUTHAME-MS. *J Am Soc Mass Spectrom*. 2020;31(10):2154-2160. doi:10.1021/jasms.0c00255
95. Müller MA, Bhandari DR, Spengler B. Matrix-Free High-Resolution Atmospheric-Pressure SALDI Mass Spectrometry Imaging of Biological Samples Using Nanostructured DIUTHAME Membranes. *Metabolites*. 2021;11(9):624. doi:10.3390/metabo11090624
96. Hasan MdM, Eto F, Mamun MdA, et al. Desorption ionization using through-hole alumina membrane offers higher reproducibility than 2,5-dihydroxybenzoic acid, a widely used matrix in Fourier transform ion cyclotron resonance mass spectrometry imaging analysis. *Rapid Commun Mass Spectrom*. 2021;35(10):e9076. doi:10.1002/rcm.9076
97. Naito Y. Quantitative analysis capability of DIUTHAME mass spectrometry verified by acetylcholinesterase enzyme-catalyzed reaction assays. *J Mass Spectrom*. 2022;57(12):e4895. doi:10.1002/jms.4895

98. Kotani M, Yanagishita T. Effects of Heat Treatment on Anodic Porous Alumina Membranes for Mass Spectrometry. *ECS J Solid State Sci Technol*. 2024;13(9):094003. doi:10.1149/2162-8777/ad7b75
99. Kotani M, Yanagishita T. Nanostructure-Dependent Signal Intensity in Through-Hole Porous Alumina Membranes for Mass Spectrometry Imaging. *Rapid Commun Mass Spectrom*. 2026;40(1):e10149. doi:10.1002/rcm.10149
100. Ikeda T, Kotani M. Thin-section- and matrix-free mass spectrometry imaging: Reproducible sample transfer using novel platinum-coated porous plate formed of glass beads. *Rapid Commun Mass Spectrom*. 2024;38(6):e9697. doi:10.1002/rcm.9697
101. Ikeda T. Transfer-Based Thin-Section-Free MALDI Mass Spectrometry Imaging Using a Platinum-Coated Porous Plate Formed of Glass Beads. *J Mass Spectrom*. 2025;60(9):e5162. doi:10.1002/jms.5162

Chapter 2.

Thin-Section- and Matrix-Free Mass Spectrometry Imaging: Reproducible Sample Transfer Using Novel Platinum-Coated Porous Plate Formed of Glass Beads.

This chapter is a reconstructed version of the article by Ikeda & Kotani (2024), whose reuse and adaptation are permitted under the terms of the Creative Commons CC BY license.

Ikeda, T., & Kotani, M. (2024). Thin-section- and matrix-free mass spectrometry imaging: Reproducible sample transfer using novel platinum-coated porous plate formed of glass beads. *Rapid Communications in Mass Spectrometry*, 38(6), e9697. <https://doi.org/10.1002/rcm.9697>

2.1. Introduction

As described in Chapter 1, the practical application of mass MSI strongly depends on sample preparation procedures, such as thin sectioning and matrix application. To address these challenges, various approaches that combine transfer methods with SALDI substrates have been proposed.^{1–32} However, SALDI substrates that are specifically designed with transfer as the primary consideration are limited. DIUTHAME, a product previously developed by the authors' affiliated company, is also a SALDI substrate; however, it was fundamentally designed for use in combination with thin tissue sections.^{33–35} Although its application in transfer-based approaches has been explored, its intrinsic fragility makes it unsuitable for transfer-oriented applications. Meanwhile, as DIUTHAME became more widely adopted, practical user requirements increasingly highlighted the need for media that enable transfer-based approaches. This trend simultaneously indicates that fundamental MSI preparation steps such as thin sectioning and matrix applications continue to represent significant barriers for many users.

Nevertheless, transfer media possessing SALDI functionality continue to face substantial limitations within the scope of existing technologies. Media based on nanostructures require the preservation of their fine surface features, which tends to restrict their application to relatively soft samples. In addition, although SALDI substrates suitable for transfer-based approaches in MSI have been technically reported, most have not been commercialized and are not generally accessible. In practice, users are often required to fabricate such media themselves, which conflicts with the goal of simplifying MSI sample preparation. Furthermore, even if commercialization was pursued, these technologies were found—at least within the technical evaluations conducted at the author's affiliated company—to be challenging to scale up for mass production from the perspectives of fabrication complexity and cost. Therefore, there is a clear need to develop a commercially available and broadly accessible transfer medium. After evaluating various candidate materials, this study focused on a platinum-coated porous plate formed from glass beads (the transfer plate), and proceeded with its technical validation and product development.

This chapter investigates the fundamental characteristics of the transfer plate. For product realization, it is essential to first conduct rigorous technical validation and to demonstrate results that are persuasive to the target user base. In feedback from DIUTHAME users, questions regarding the influence of sample moisture on sampling performance were particularly frequent. Therefore, the present fundamental study specifically pursued this line of inquiry. Specifically, stains of the polymer additives were

selected as representative dry samples, whereas cross sections of strawberries were used as representative wet samples. MSI measurements using the transfer plate were performed on these samples to evaluate the applicability of thin-section- and matrix-free MSI.

2.2. Methods

2.2.1. Fabrication of transfer plates

The porous plate was formed by sintering commercially available spherical glass beads in an electric furnace such that the gaps between the glass beads were not filled. The temperature of the electric furnace was set to 600°C or higher. The approximately 50 μm -diameter glass beads were made of soda glasses. The size and shape of the porous plate could be changed arbitrarily depending on the sintering mold. The approximate sizes of the prepared porous plates were $75 \times 50 \times 1$ mm (width, length, and height) (Figure 2.1A). The platinum layer was deposited on a single side of the porous plate using electron beam evaporation, with its platinum-coated side acting as the active surface for ionization. The average thickness of the platinum layer was 50 nm. Figure 2.1B shows an example of an active surface on the fabricated transfer plate obtained using scanning electron microscopy (JCM-6000, JEOL Ltd., Akishima, Japan), indicating that there were many pores between the glass beads and their fusion sites. The pores extended from the active surface to the opposite side, forming random network structures. The deposited platinum layer was macroscopically uniform.

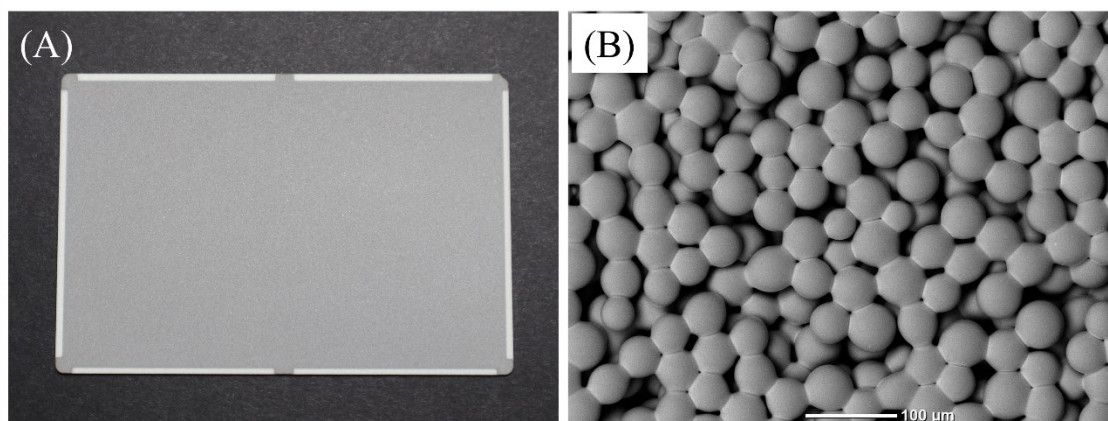


Figure 2.1 (A) Optical image of the 75 mm-wide and 50 mm-long transfer plate. The gray area on the plate corresponds to the platinum layer. (B) Magnified image of the active surface on a transfer plate. The image was obtained using the back scattered electron mode of a secondary electron microprobe.

2.2.2. Sample preparation of polymer additives

Irganox B225 (Ciba Inc., Basel, Switzerland), a blend of 50% Irgafos 168 and 50% Irganox 1010, was used as the example polymer additive. Ultrapure water (LC/MS grade), acetone (min. 99.5%), acetonitrile (min. 99.5%), sodium trifluoroacetate, and 2,5-dihydroxybenzoic acid (DHB) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Lumirror™ Polyester Film #250-T60 (Toray Industries, Inc., Tokyo, Japan) was used as the polyethylene terephthalate (PET) film. Irganox B225 solutions in acetone at different concentrations (1, 0.1, and 0.01% weight ratios) were prepared. A 20 mg/ml DHB solution in 70% aqueous acetonitrile containing 0.1% TFA was used.

To compare the mass spectra using MALDI and using the transfer plate, an equal volume of 1% Irganox B225 and DHB solution were mixed, and 1 μ L of the mixture was loaded on the target plate (MTP 384 target plate ground steel BC, Bruker, Bremen, Germany). Alternatively, 1 μ L of a 1% Irganox B225 solution without the matrix was loaded on the transfer plate. The so-called “sweet spot” did not emerge on the transfer plate because of its solution-absorption properties. To verify that dried constituents can be transferred onto a transfer plate, stains of an Irganox B225 solution residual were prepared by spotting and completely drying 1 μ L of 1, 0.1, and 0.01% Irganox B225 solutions on a PET film (Figure 2.2A). The diameter of each stain on the PET film was 5–7 mm. These stains were placed on the active surface of the transfer plate and moderately thumbbed for 10 s before removing the film. All stains were transferred to the transfer plate (Figure 2.2B), although they were barely visible to the naked eye. The transfer plate attached to these stains was installed into the MALDI-TOFMS instrument without any additional processes, such as matrix spraying. All mass spectra and ion images of the polymer additives were obtained in the positive ion mode.

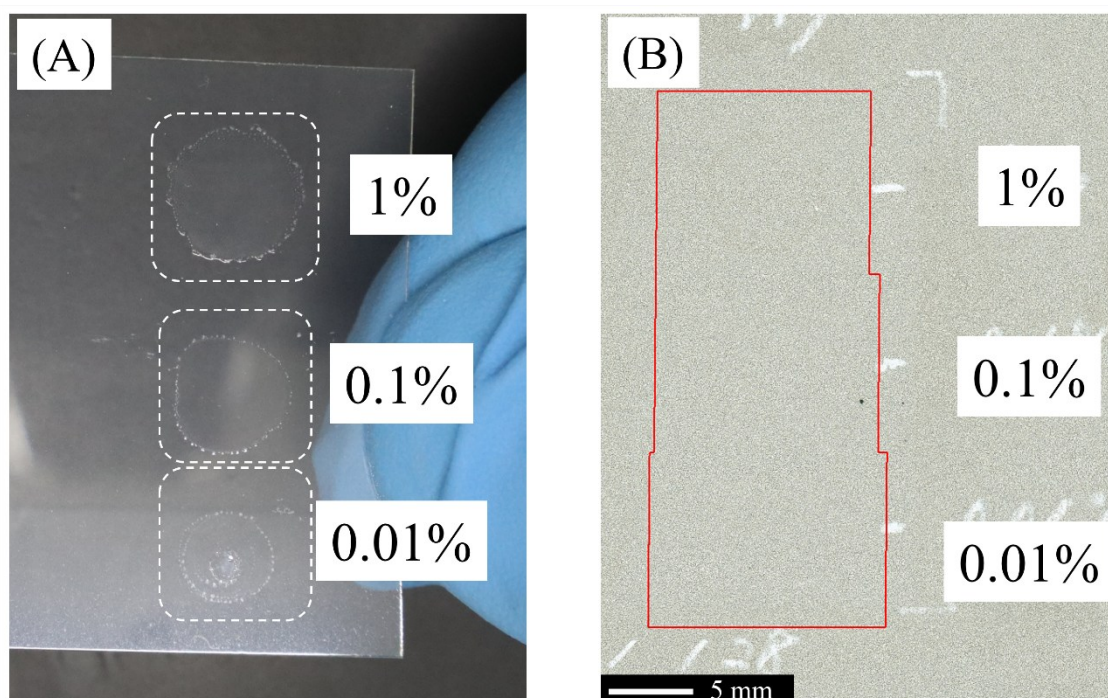


Figure 2.2. (A) Polymer additive stains on the PET film; 1 μL of 1, 0.1, and 0.01% Irganox B225 solutions in acetone were loaded. (B) Stains on the transfer plate after the transfer process. MSI measurements were performed in the area enclosed by the red line.

2.2.3. Sample preparation of strawberries

For evaluating the application of a transfer plate to MSI, strawberries were chosen as the samples because there are many previous MSI studies of strawberries that are suitable for qualitative validation.^{35–39} “Benihoppe” strawberries cultivated in a local strawberry farm (Shizuoka, Japan) were used in this study. The strawberries were sliced in half using a commercial cutting knife. Figure 2.3A shows an optical image of the cut-surface of the strawberry cross-section. The strawberry cross-section was placed on the active surface of the transfer plate and moderately thumbed for 10 s (Figure 2.3B). The strawberry was removed from the transfer plate. Figure 2.3C shows a trace of the strawberry cross-section on the transfer plate after its removal. Figure 2.3D shows a duplication of Figure 2.3C obtained by transferring the same cross-section onto another transfer plate. Multiple similar samples were duplicated in the same manner (Figure 2.4). MSI was performed in positive- and negative-ion modes using different transfer plates (positive: Figures 2.3C, 2.4A, and 2.4B; negative: Figure 2.3D).

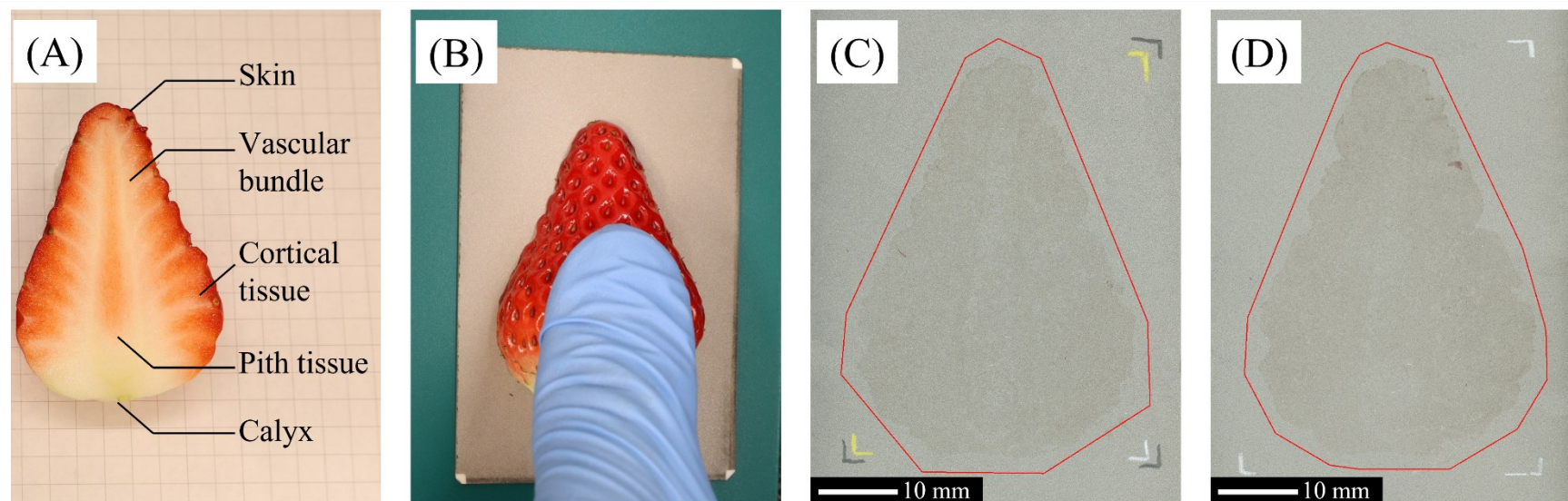


Figure 2.3. (A) Optical image of a strawberry cross-section. (B) Strawberry transfer process. (C, D) Traces of the strawberry cross-section on the transfer plate after removing the strawberry. MSI measurements were performed in the area enclosed by the red line.

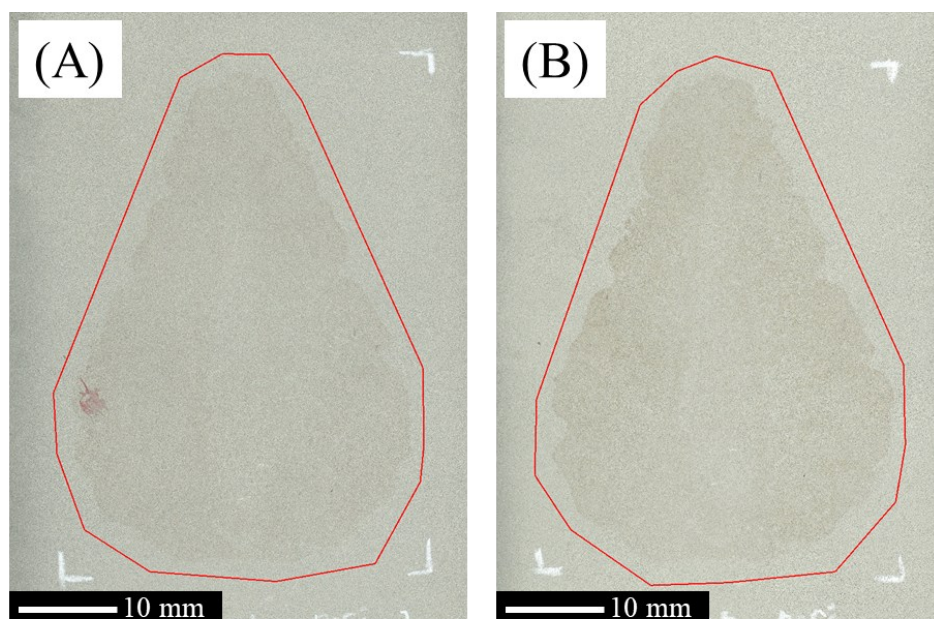


Figure 2.4. (A, B) Traces of the strawberry cross-section on a transfer plate using the same method as in Figure 2.3C and D. MSI measurements were performed in the area enclosed by the red line.

2.2.4. Instrumentation

All the mass spectra and ion images in the present study were obtained using a MALDI TOF/TOF instrument (Autoflex maX; Bruker, Billerica, MA, U.S.). All results in the positive and negative ion modes were acquired using reflector mode, and the pulsed ion extraction time was 200 ns. The measured m/z ranges were 60 to 2000 for polymer additives and 60 to 800 for strawberry metabolites, respectively. However, only m/z regions of interest are shown in the following mass spectra. After transferring the samples, the transfer plates were installed into the MALDI TOF/TOF instrument without any additional processes, such as matrix-spraying. MSI measurements were performed using a step size of 200 μm , 100 shots per spot, and a large laser diameter mode ($\sim 80\ \mu\text{m}$). The acquired MSI data were analyzed using SCiLSTM Lab (Bruker, Billerica, MA, U.S.). The automatic hotspot removal function was performed using the 99% quantile for the cutoff value. No further normalization was performed.

2.3. Results and Discussion

2.3.1. Polymer additives

The mass spectra of the polymer additives (Irgafos 168 and Irganox 1010) obtained using the DHB matrix and transfer plate were compared under the same equipment conditions. Figure 2.5A and B show the mass spectra of the polymer additives obtained using MALDI and the transfer plate (without the matrix), respectively. The target ions derived from Irgafos 168 and Irganox 1010 were clearly observed in the mass spectra. Therefore, matrix-free ionization was implemented on the transfer plate. The intensity ranges (y-axis) shown in Figure 2.5A and B were tuned to the peaks of the polymer additives. Therefore, the intensities of the matrix peaks below m/z 400 in Figure 2.5A were saturated. The main ions detected in Figure 2.5A and B are summarized in Table 2.1. The mass spectrum pattern below m/z 600 in Figure 2.5A is complicated by the presence of many matrix peaks; however, no complicated pattern is present in Figure 2.5B. Irgafos 168 was detected as the $[M + H]^+$ ion in Figure 2.5A; however, $[M + Na]^+$ ions were detected in Figure 2.5B because the Na ions contained in the transfer plate were added instead of protons. Irganox 1010 was detected as $[M + Na]^+$ ions regardless of the presence of a matrix, which is consistent with previous studies on MALDI.^{40,41} The detection of oxidized Irgafos 168 in Figure 2.5B may be due to temporal changes in the polymer additive solution.⁴¹ The absence of Irgafos 168 oxide in Figure 2.5A is likely due to the insufficient peak intensity.

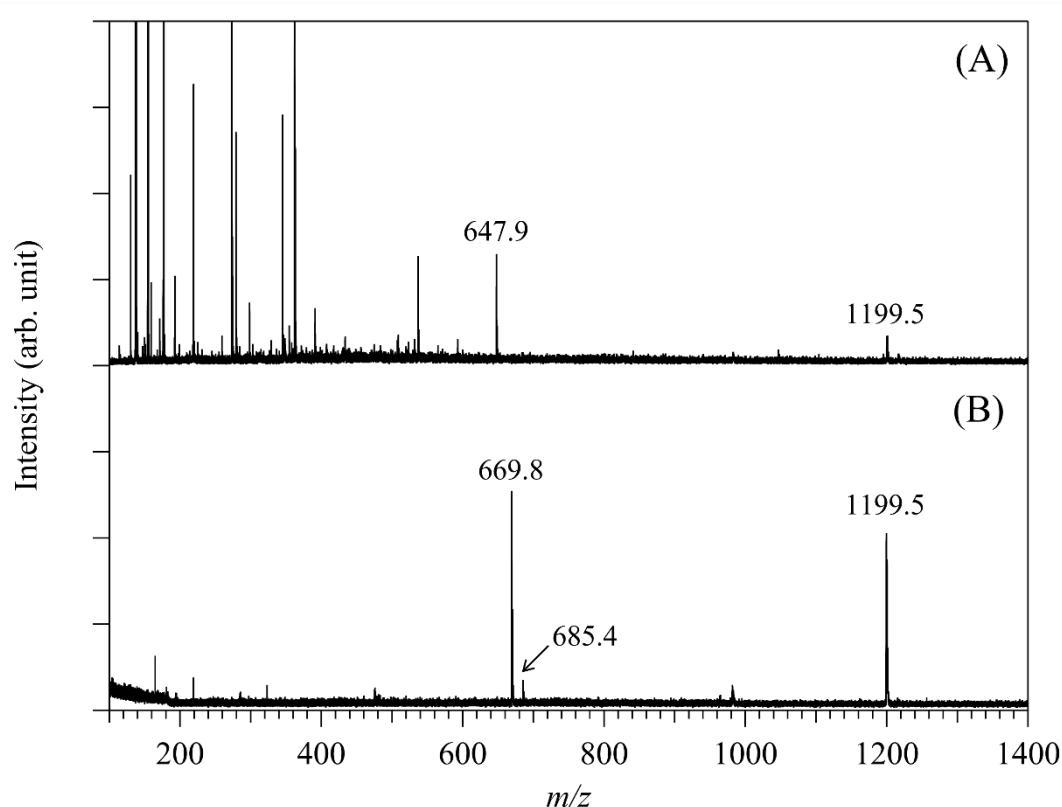


Figure 2.5. Averaged mass spectra of the 1% Irganox B225 solution on a target plate (A) with a DHB matrix and (B) without a matrix. The vertical scale of (A) is consistent with that of (B); therefore, the matrix peaks ($\sim m/z$ 400) were saturated in (A).

Table 2.1. Assigned polymer additives detected from an Irganox B225 solution on a transfer plate.

Polymer additive	Ion type	Detected m/z	Corresponding Ion image
Irgafos 168	$[M + H]^+$	647.9	
	$[M + Na]^+$	669.8	Figure 2.6A
Irgafos 168 (oxidized)	$[M + Na]^+$	685.4	Figure 2.6B
Irganox 1010	$[M + Na]^+$	1199.5	Figure 2.6C

Figure 2.6A–C show the ion images of Irgafos 168, oxidized Irgafos 168, and Irganox 1010, respectively, obtained by MSI measurements of the polymer additive stains transferred from the PET film to the transfer plate. All ion images corresponding to the 1% sample solution (top in Figure 2.6A–C) were clearly visible, which demonstrates that

it is possible to transfer the completely dried stain. All ion images corresponding to the 0.1% solution (middle of Figure 2.6A–C) were faintly visible around the edges of the stains. Only the ion image of the 0.01% oxidized Irgafos 168 (bottom of Figure 2.6B) was barely obtained. The polymer additive stains for MSI measurements were exposed to air for long enough to dry; therefore, oxidized Irgafos 168 was predominantly present on the film. These results shown in Figure 2.6A–C indicates a dry sample on the film can be transferred onto a transfer plate. MSI measurements of thin samples, such as a stain on a film, are difficult to perform in terms of not only thin-sectioning but also charge-up when the sample is loaded without thin-sectioning into a MALDI-TOFMS instrument. MSI measurements using a transfer plate can overcome these restrictions.

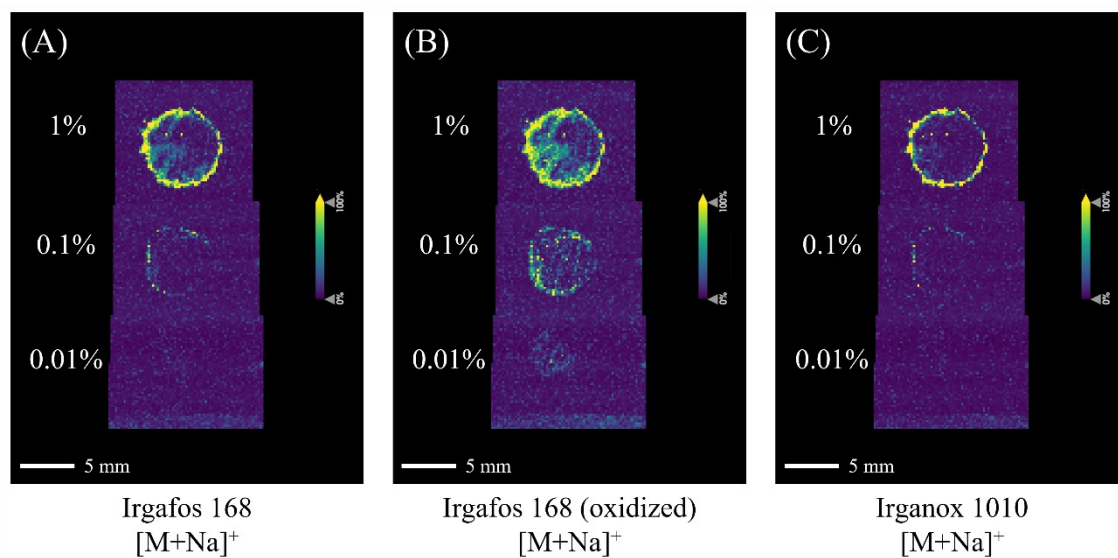


Figure 2.6. Ion images of the polymer additives on transfer plates. (A) m/z 669.8 (Irgafos 168 $[M + Na]^+$), (B) m/z 685.4 (Irgafos 168 (Oxidized) $[M + Na]^+$), and (C) m/z 1199.5 (Irganox 1010 $[M + Na]^+$). The solutions from top to bottom correspond to the 1, 0.1, and 0.01% solutions. The color gradient in the figures corresponds to the relative intensity scaled by SCiLS.

2.3.2. Strawberry

MSI measurement of the strawberry cross-section, which has a high water content, was performed using a transfer plate. Figure 2.7A and B show the average mass spectra of the strawberry cross-section obtained in the positive and negative ion modes, respectively. The peaks in the mass spectra were considered to correspond to strawberry metabolites because a matrix for MALDI was not used in this measurement. The positive and negative

ions detected in Figure 2.7A and B, respectively, are summarized in Table 2.2. These ions were assigned by referencing previous studies.^{35–39} The positive ions detected in Figure 2.7A were obtained as adduct ions of not only K but also Na, such as hexose $[M + Na]^+$ and sucrose $[M + Na]^+$, for the same reason as in the case of the polymer additives. Furthermore, the intensity of the citric acid $[M - H + 2K]^+$ peak was higher than that of the $[M + K]^+$ peak. The apparent patterns of the adduct ions depend on the alkali ion concentrations in the samples and transfer plate, which complicated the mass spectrum shown in Figure 2.7A. However, it was considered that the detected negative ions were mostly proton-desorption ions; therefore, the mass spectrum shown in Figure 2.6B was simpler.

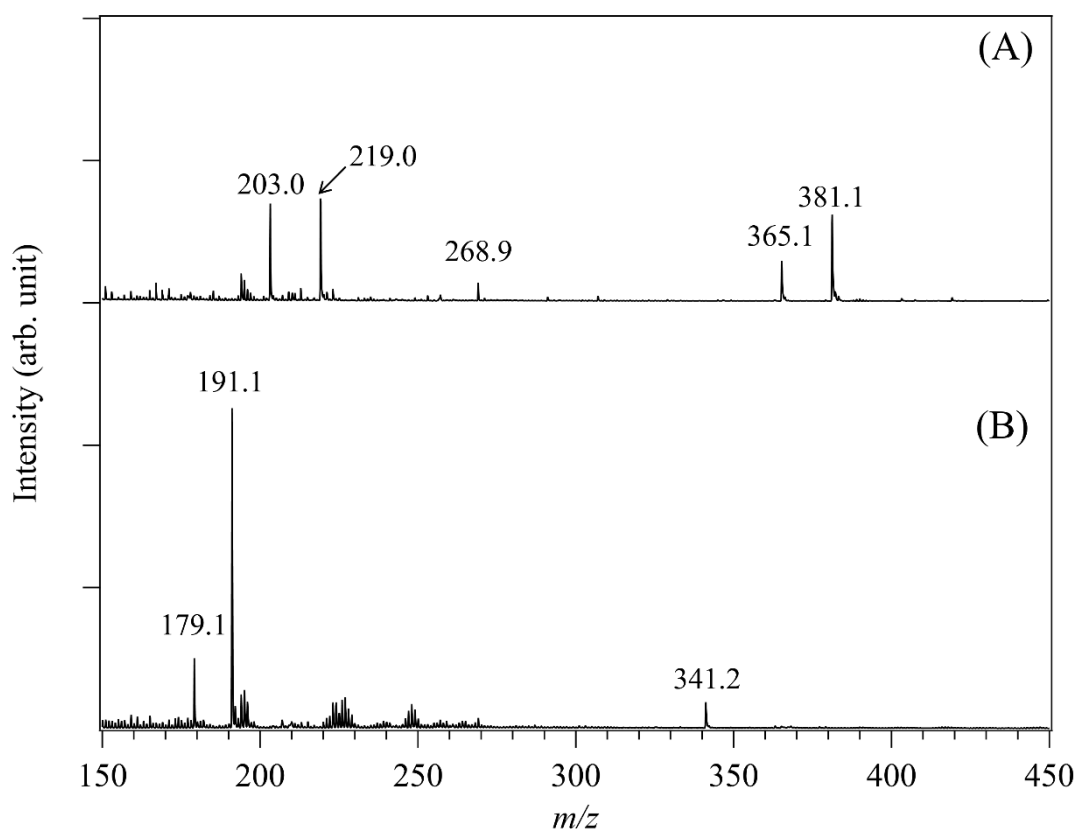


Figure 2.7. Averaged mass spectra of the strawberry cross-section obtained in the (A) positive and (B) negative ion modes. No main peaks were observed in the range above m/z 450.

Table 2.2. Assigned metabolites detected from a strawberry cross-section on a transfer plate.

Metabolite	Ion type	Detected m/z	Corresponding Ion image
Hexose	$[M + Na]^+$	203.0	
	$[M + K]^+$	219.0	Figure 2.8A
	$[M - H]^-$	179.1	Figure 2.8D
Citric acid	$[M + K]^+$	230.9	
	$[M - H + 2K]^+$	268.9	Figure 2.8B
	$[M - H]^-$	191.1	Figure 2.8E
Sucrose	$[M + Na]^+$	365.1	
	$[M + K]^+$	381.1	Figure 2.8C
	$[M - H]^-$	341.2	Figure 2.8F

Figure 2.8 shows the ion images of the metabolites listed in Table 2.2. MSI of strawberries has previously been using various preparation methods.^{35–39} Hexose (Figure 2.8A and D) was distributed throughout the fruits, particular in the vascular bundles. Although different distributions of hexose have been reported in each of the previous studies, the signals from the entire strawberry are common. Citric acid (Figure 2.8B and E) was mostly localized in the cortical tissue. Citric acid has previously been found to be distributed on the outside of strawberries (cortical tissue), which is qualitatively consistent with the present results. The distributions of hexose and citric acid were almost consistent in both the positive and negative ion modes. Sucrose in the positive ion mode (Figure 2.8C) was distributed over the entire area of the cortical tissue and vascular bundle, whereas sucrose in the negative ion mode (Figure 2.8F) was mainly distributed in the vascular bundle and pith tissue. Previous studies have reported a similar phenomenon, in which the ion image of a strawberry was different in the positive and negative ion modes.^{35,39} $[M + K]^+$ ions were detected in the positive ion mode, indicating that the distribution of K^+ ions may affect the ion image of the metabolites. Significant delocalization of these metabolites was not observed in Figure 2.8, indicating that MSI measurement of wet samples is also possible using the transfer plate.

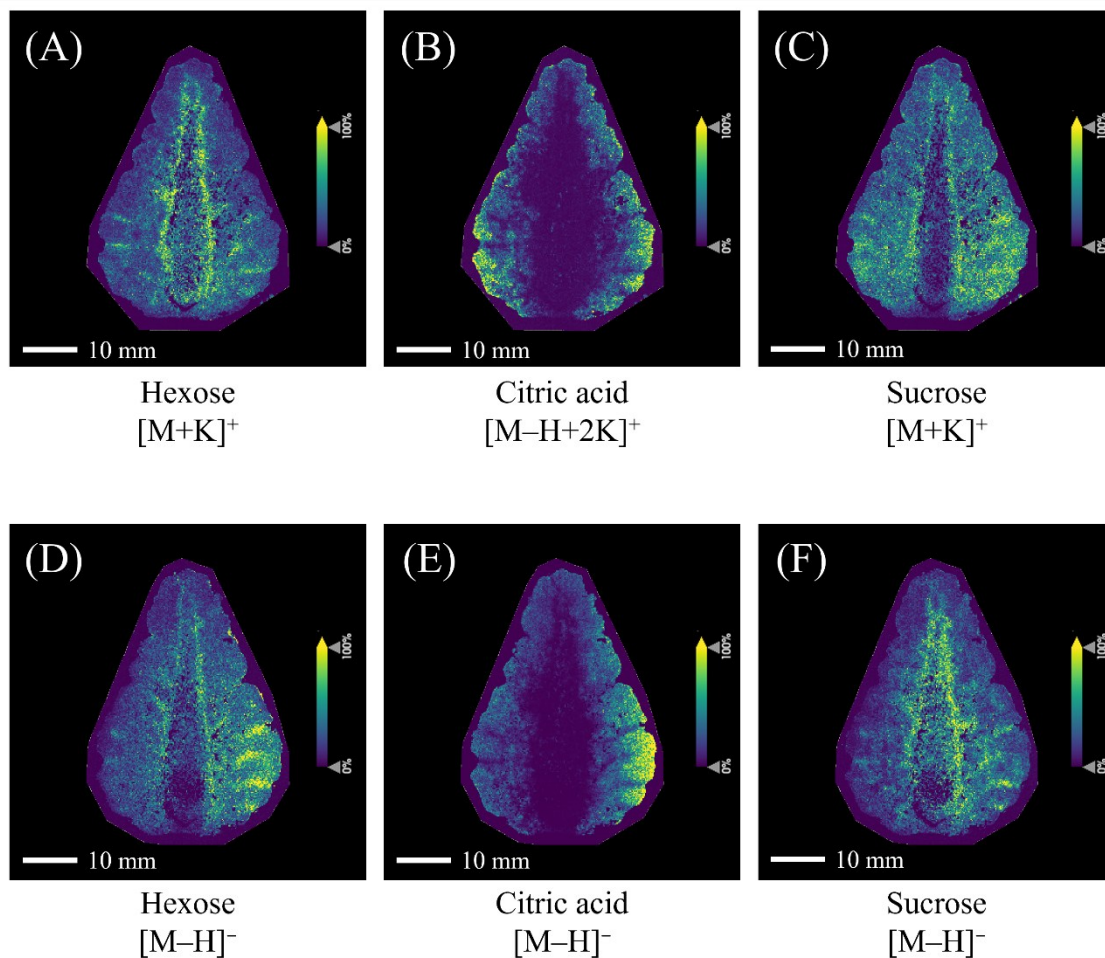


Figure 2.8. Ion images of strawberry metabolites on the transfer plate. (A) m/z 219.0 (hexose $[M + K]^+$), (B) m/z 268.9 (citric acid $[M - H + 2K]^+$), (C) m/z 381.1 (sucrose $[M + K]^+$), (D) m/z 179.1 (hexose $[M - H]^-$), (E) m/z 191.1 (citric acid $[M - H]^-$), and (F) m/z 341.2 (sucrose $[M - H]^-$). The color gradient in the figures corresponds to the relative intensity scaled by SCiLS.

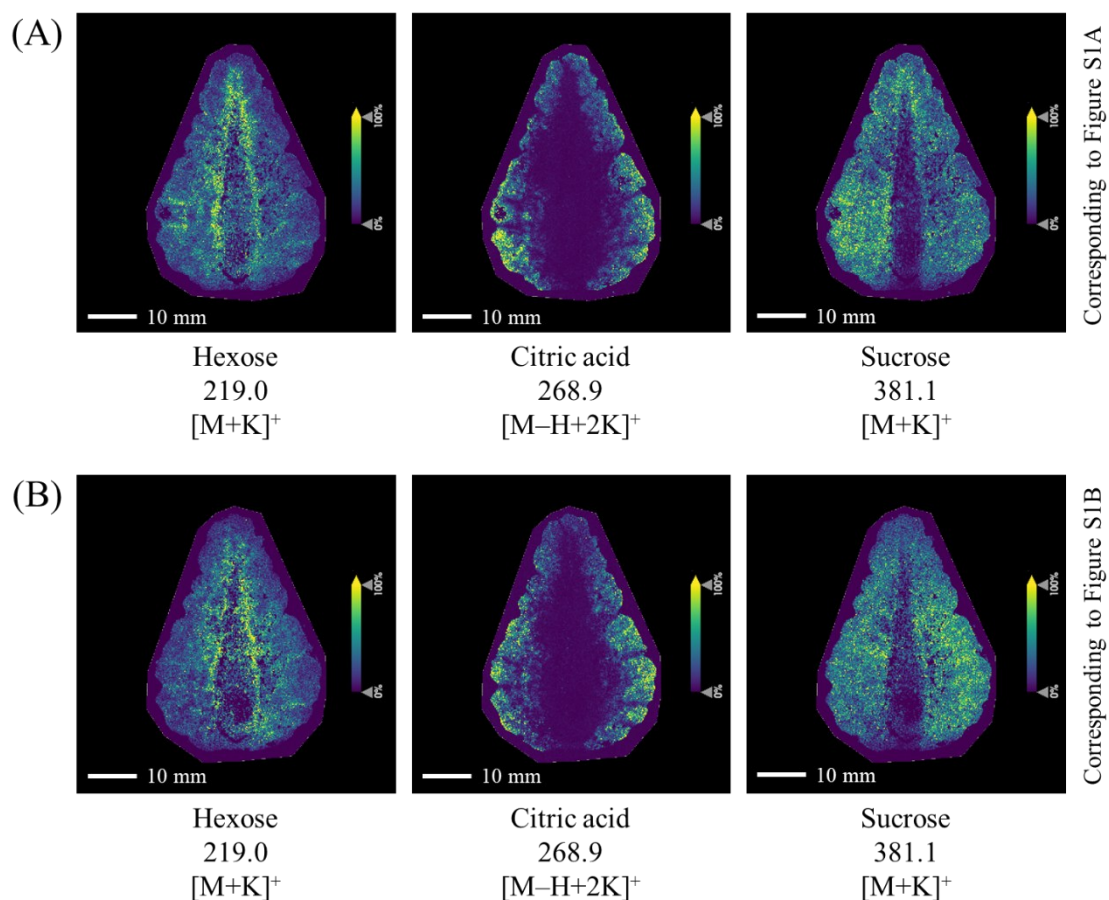


Figure 2.9. Reproduced ion images of strawberry metabolites on a transfer plate: m/z 219.0 (hexose [M + K]⁺), m/z 268.9 (citric acid [M – H + 2K]⁺), and m/z 381.1 (sucrose [M + K]⁺). (A) and (B) were obtained by using the transfer plate shown in Figure 2.4A and B, respectively. The color gradient in the figures corresponds to the relative intensity scaled by SCiLS.

2.3.3. Spatial resolution and reproducibility of MSI measurements using a transfer plate

The shapes of the ion images shown in Figure 2.8A–F qualitatively reflected the cross sectional anatomy of the strawberry fruit shown in Figure 2.3A and the metabolite distribution of previous studies. This indicates that delocalization of the transferred constituents was insignificant. The structure of the transfer plate is an aggregate of glass beads, as shown in Figure 2.1; therefore, the diameter of the glass beads should be the spatial resolution limit. The glass bead diameter used in this study was approximately 50 μm , which is sufficiently smaller than the step size of MSI (200 μm). However, hand

shaking during the transfer process may deteriorate spatial resolution. For higher spatial resolution, the MALDI- or SALDI-MSI method using thin sections is considered advantageous.

Reproducibility of MSI measurements is expected when using a transfer plate because of the relatively simple and easy preparation process. Figure 2.9A and B show the positive ion images of the strawberry obtained using the transfer plate shown in Figure 2.9A and B, respectively. Figure 2.9A and B qualitatively reproduce the ion images of hexose, citric acid, and sucrose shown in Figure 2.8A–C. Reproducibility was achieved owing to (i) the strength of the porous structure, (ii) absorbability of the solution, and (iii) ionization on the pre-coated platinum layer, which are characteristic functions of the transfer plate. A strong structure obtained by sintering enables the samples to be thumbed on the transfer plate, decreasing non-sample transferred areas. The amount of sample is constantly retained on the active surface of the transfer plate because the residual sample is absorbed into the porous structure interior, and furthermore, the delocalization of the sample is limited. Ionization on the uniformly pre-coated platinum layer is probably stable without the fluctuations from matrix-spraying.

Sample preparation using a transfer plate has excellent potential for the development of new applications in which MSI using the traditional preparation process for MALDI-MSI cannot be realized. A statistical application that requires many measurement results or an application that acquires rapid treatment are ideal in terms of the ease of processing and lower dependence on the operator skill. A large sample, whose thin-sectioning is relatively difficult, is also a suitable application because the transfer plate can be manufactured to be as large as the size that can be introduced into the mass spectrometer, for example, the microtiter plate size. Moreover, there was no limitation on the moisture content of the samples when the target constituents were transferred, as explained above. However, highly viscous or brittle samples may cause excess sample to adhere onto the active surface. In such cases, desorption/ionization may not occur because the laser does not reach the platinum layer. The use of a transfer plate may expand MSI applications if these characteristics can overcome the difficulty or complexity of MSI measurements.

2.4. Conclusions

In this chapter, the fundamental feasibility of thin-section- and matrix-free MSI using a platinum-coated porous plate formed of glass beads was examined as a transfer-based sample preparation approach. MSI measurements were conducted using representative dry samples (polymer additive stains) and wet samples (strawberry cross-sections) to evaluate the applicability of this transfer plate under different sample conditions. Despite the simplicity of the preparation process, the macroscopic delocalization during transfer was minimal for both sample types. In addition, the resulting ion images showed qualitative reproducibility across independently prepared transfer plates. These results indicate that the transfer process using the transfer plate provides stable sampling and ionization performance suitable for MSI measurements. The observed performance can be attributed to the characteristic properties of the transfer plate, including the mechanical robustness of the sintered porous structure, the ability of the porous network to absorb excess sample moisture, and the stable matrix-free ionization enabled by the uniformly coated platinum layer. This fundamental validation provides a technical basis for further investigation of application-oriented performance and expanded use cases, which are addressed in subsequent chapters.

References for Chapter 2

1. Liu Q, Guo Z, He L. Mass Spectrometry Imaging of Small Molecules Using Desorption/Ionization on Silicon. *Anal Chem.* 2007;79(10):3535-3541. doi:10.1021/ac0611465
2. Vidová V, Novák P, Strohalm M, Pól J, Havlíček V, Volný M. Laser Desorption-Ionization of Lipid Transfers: Tissue Mass Spectrometry Imaging without MALDI Matrix. *Anal Chem.* 2010;82(12):4994-4997. doi:10.1021/ac100661h
3. Greving MP, Patti GJ, Siuzdak G. Nanostructure-Initiator Mass Spectrometry Metabolite Analysis and Imaging. *Anal Chem.* 2011;83(1):2-7. doi:10.1021/ac101565f
4. Ronci M, Rudd D, Guinan T, Benkendorff K, Voelcker NH. Mass Spectrometry Imaging on Porous Silicon: Investigating the Distribution of Bioactives in Marine Mollusc Tissues. *Anal Chem.* 2012;84(21):8996-9001. doi:10.1021/ac3027433
5. Tata A, Fernandes AMAP, Santos VG, et al. Nanoassisted Laser Desorption-Ionization-MS Imaging of Tumors. *Anal Chem.* 2012;84(15):6341-6345. doi:10.1021/ac301202q
6. Louie KB, Bowen BP, Cheng X, et al. "Replica-Extraction-Transfer" Nanostructure-Initiator Mass Spectrometry Imaging of Acoustically Printed Bacteria. *Anal Chem.* 2013;85(22):10856-10862. doi:10.1021/ac402240q
7. Nizioł J, Ruman T. Surface-Transfer Mass Spectrometry Imaging on a Monoisotopic Silver Nanoparticle Enhanced Target. *Anal Chem.* 2013;85(24):12070-12076. doi:10.1021/ac4031658
8. Guinan TM, Gustafsson OJR, McPhee G, Kobus H, Voelcker NH. Silver Coating for High-Mass-Accuracy Imaging Mass Spectrometry of Fingerprints on Nanostructured Silicon. *Anal Chem.* 2015;87(22):11195-11202. doi:10.1021/acs.analchem.5b02567
9. Rudd D, Ronci M, Johnston MR, Guinan T, Voelcker NH, Benkendorff K. Mass spectrometry imaging reveals new biological roles for choline esters and Tyrian purple precursors in muricid molluscs. *Sci Rep.* 2015;5(1):13408. doi:10.1038/srep13408

10. Sekuła J, Nizioł J, Misiorek M, et al. Gold nanoparticle-enhanced target for MS analysis and imaging of harmful compounds in plant, animal tissue and on fingerprint. *Anal Chim Acta*. 2015;895:45-53. doi:10.1016/j.aca.2015.09.003
11. Sekuła J, Nizioł J, Rode W, Ruman T. Gold nanoparticle-enhanced target (AuNPET) as universal solution for laser desorption/ionization mass spectrometry analysis and imaging of low molecular weight compounds. *Anal Chim Acta*. 2015;875:61-72. doi:10.1016/j.aca.2015.01.046
12. Nizioł J, Ossoliński K, Ossoliński T, et al. Surface-Transfer Mass Spectrometry Imaging of Renal Tissue on Gold Nanoparticle Enhanced Target. *Anal Chem*. 2016;88(14):7365-7371. doi:10.1021/acs.analchem.6b01859
13. Arendowski A, Ruman T. Laser Desorption/Ionisation Mass Spectrometry Imaging of European Yew (*Taxus baccata*) on Gold Nanoparticle-enhanced Target. *Phytochem Anal*. 2017;28(5):448-453. doi:10.1002/pca.2693
14. Misiorek M, Sekuła J, Ruman T. Mass Spectrometry Imaging of low Molecular Weight Compounds in Garlic (*Allium sativum* L.) with Gold Nanoparticle Enhanced Target. *Phytochem Anal*. 2017;28(6):479-486. doi:10.1002/pca.2696
15. Nizioł J, Sekuła J, Ruman T. Visualizing spatial distribution of small molecules in the rhubarb stalk (*Rheum rhabarbarum*) by surface-transfer mass spectrometry imaging. *Phytochemistry*. 2017;139:72-80. doi:10.1016/j.phytochem.2017.04.006
16. Chen PY, Hsieh CY, Shih CJ, Lin YJ, Tsao CW, Yang YL. Exploration of Fungal Metabolic Interactions Using Imaging Mass Spectrometry on Nanostructured Silicon. *J Nat Prod*. 2018;81(7):1527-1533. doi:10.1021/acs.jnatprod.7b00866
17. Chen X, Gao J, Wang T, et al. Hepatocarcinoma Discrimination by Ratiometric Lipid Profiles Using Tip-Contact Sampling/Ionization Mass Spectrometry. *Anal Chem*. 2019;91(16):10376-10380. doi:10.1021/acs.analchem.9b02623
18. Nizioł J, Misiorek M, Ruman T. Mass spectrometry imaging of low molecular weight metabolites in strawberry fruit (*Fragaria x ananassa* Duch.) cv. Primoris with 109Ag nanoparticle enhanced target. *Phytochemistry*. 2019;159:11-19. doi:10.1016/j.phytochem.2018.11.014

19. Rudd DA, Benkendorff K, Chahal C, et al. Mapping insoluble indole metabolites in the gastrointestinal environment of a murine colorectal cancer model using desorption/ionisation on porous silicon imaging. *Sci Rep.* 2019;9(1):12342. doi:10.1038/s41598-019-48533-2
20. Iakab SA, Ràfols P, Tajés M, Correig-Blanchar X, García-Altares M. Gold Nanoparticle-Assisted Black Silicon Substrates for Mass Spectrometry Imaging Applications. *ACS Nano.* 2020;14(6):6785-6794. doi:10.1021/acsnano.0c00201
21. Nizioł J, Sunner J, Beech I, et al. Localization of Metabolites of Human Kidney Tissue with Infrared Laser-Based Selected Reaction Monitoring Mass Spectrometry Imaging and Silver-109 Nanoparticle-Based Surface Assisted Laser Desorption/Ionization Mass Spectrometry Imaging. *Anal Chem.* 2020;92(6):4251-4258. doi:10.1021/acs.analchem.9b04580
22. Wang XN, Tang W, Gordon A, et al. Porous TiO₂ Film Immobilized with Gold Nanoparticles for Dual-Polarity SALDI MS Detection and Imaging. *ACS Appl Mater Interfaces.* 2020;12(38):42567-42575. doi:10.1021/acsaami.0c12949
23. Wu X, Qin R, Wu H, et al. Nanoparticle-immersed paper imprinting mass spectrometry imaging reveals uptake and translocation mechanism of pesticides in plants. *Nano Res.* 2020;13(3):611-620. doi:10.1007/s12274-020-2700-5
24. Dutkiewicz EP, Su C, Lee H, Hsu C, Yang Y. Visualizing vinca alkaloids in the petal of *Catharanthus roseus* using functionalized titanium oxide nanowire substrate for surface-assisted laser desorption/ionization imaging mass spectrometry. *Plant J.* 2021;105(4):1123-1133. doi:10.1111/tpj.15092
25. Qin R, Li P, Du M, et al. Spatiotemporal Visualization of Insecticides and Fungicides within Fruits and Vegetables Using Gold Nanoparticle-Immersed Paper Imprinting Mass Spectrometry Imaging. *Nanomaterials.* 2021;11(5):1327. doi:10.3390/nano11051327
26. Liu X, Chen Z, Wang T, et al. Tissue Imprinting on 2D Nanoflakes-Capped Silicon Nanowires for Lipidomic Mass Spectrometry Imaging and Cancer Diagnosis. *ACS Nano.* 2022;16(4):6916-6928. doi:10.1021/acsnano.2c02616
27. Wang XN, Li B. Monolithic Gold Nanoparticles/Thiol- β -cyclodextrin-Functionalized TiO₂ Nanowires for Enhanced SALDI MS Detection and Imaging

- of Natural Products. *Anal Chem.* 2022;94(2):952-959.
doi:10.1021/acs.analchem.1c03764
28. Chen D, Du M, Huang Y, et al. Plasmonic polydopamine-modified TiO₂ nanotube substrates for surface-assisted laser desorption/ionization mass spectrometry imaging. *Nano Res.* 2023;16(2):3028-3039. doi:10.1007/s12274-022-4924-z
 29. Chen D, Xu Y, Huang Y, et al. Mapping Molecular Signatures in Plant Leaves, Flowers, and Fruits by a TiO₂ Nanotube-Based Plasmonic Chip for Imprint Mass Spectrometry Imaging. *ACS Agric Sci Technol.* 2023;3(1):119-130.
doi:10.1021/acsagscitech.2c00254
 30. Ossoliński K, Ruman T, Ossoliński T, et al. Monoisotopic silver nanoparticles-based mass spectrometry imaging of human bladder cancer tissue: Biomarker discovery. *Adv Med Sci.* 2023;68(1):38-45. doi:10.1016/j.advms.2022.12.002
 31. Sun Y, Chen D, Chen X, Wu X. Stress Response of Citrus Leaves under Mechanical Damage and Huanglongbing Disease Infection Using Plasmonic TiO₂ Nanotube Substrate-Based Imprinting Mass Spectrometry Imaging. *Agronomy.* 2024;14(8):1797. doi:10.3390/agronomy14081797
 32. Liu X, Tao L, Jiang X, et al. Isoporous Membrane Mediated Imprinting Mass Spectrometry Imaging for Spatially-Resolved Metabolomics and Rapid Histopathological Diagnosis. *Small Methods.* 2024;8(10):2301644.
doi:10.1002/smt.202301644
 33. Naito Y, Kotani M, Ohmura T. A novel laser desorption/ionization method using through hole porous alumina membranes. *Rapid Commun Mass Spectrom.* 2018;32(21):1851-1858. doi:10.1002/rcm.8252
 34. Kuwata K, Itou K, Kotani M, Ohmura T, Naito Y. DIUTHAME enables matrix-free mass spectrometry imaging of frozen tissue sections. *Rapid Commun Mass Spectrom.* 2020;34(9):e8729. doi:10.1002/rcm.8729
 35. Enomoto H, Kotani M, Ohmura T. Novel Blotting Method for Mass Spectrometry Imaging of Metabolites in Strawberry Fruit by Desorption/Ionization Using Through Hole Alumina Membrane. *Foods.* 2020;9(4):408.
doi:10.3390/foods9040408

36. Cabral EC, Mirabelli MF, Perez CJ, Ifa DR. Blotting Assisted by Heating and Solvent Extraction for DESI-MS Imaging. *J Am Soc Mass Spectrom.* 2013;24(6):956-965. doi:10.1007/s13361-013-0616-y
37. Enomoto H, Sato K, Miyamoto K, Ohtsuka A, Yamane H. Distribution Analysis of Anthocyanins, Sugars, and Organic Acids in Strawberry Fruits Using Matrix-Assisted Laser Desorption/Ionization-Imaging Mass Spectrometry. *J Agric Food Chem.* 2018;66(19):4958-4965. doi:10.1021/acs.jafc.8b00853
38. Enomoto H, Takahashi S, Takeda S, Hatta H. Distribution of Flavan-3-ol Species in Ripe Strawberry Fruit Revealed by Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry Imaging. *Molecules.* 2020;25(1):103. doi:10.3390/molecules25010103
39. Enomoto H. Adhesive film applications help to prepare strawberry fruit sections for desorption electrospray ionization-mass spectrometry imaging. *Biosci Biotechnol Biochem.* 2021;85(6):1341-1347. doi:10.1093/bbb/zbab033
40. Schnöller J, Pittenauer E, Hutter H, Allmaier G. Analysis of antioxidants in insulation cladding of copper wire: a comparison of different mass spectrometric techniques (ESI-IT, MALDI-TOF and TOF-SIMS). *J Mass Spectrom* 2009;44(12):1724-1732. doi:10.1002/jms.1681
41. Schwarzingner C, Gabriel S, Beißmann S, Buchberger W. Quantitative Analysis of Polymer Additives with MALDI-TOF MS Using an Internal Standard Approach. *J Am Soc Mass Spectrom.* 2012;23(6):1120-1125. doi:10.1007/s13361-012-0367-1

Chapter 3.

Transfer-Based Thin-Section-Free MALDI Mass Spectrometry Imaging Using a Platinum-Coated Porous Plate Formed of Glass Beads.

This chapter is a reconstructed version of the article by Ikeda (2025). Permission to reproduce the content of the article in this thesis has been granted by *John Wiley & Sons Ltd*.

Ikeda, T. (2025). Transfer-based thin-section-free MALDI mass spectrometry imaging using a platinum-coated porous plate formed of glass beads. *Journal of Mass Spectrometry*, 60(9), e5162.
<https://doi.org/10.1002/jms.5162>

3.1 Introduction

Chapter 2 discusses the fundamental technology and effectiveness of a thin-section- and matrix-free MSI approach using a transfer plate. In this chapter, the potential for extending this transfer plate to MALDI-MSI is investigated. The transfer plate introduced in Chapter 2 consists of a porous plate formed of glass beads and a uniform platinum layer deposited on one side.¹ The porous plate provides sufficient mechanical strength to withstand transfer pressure and functions as a base material to suppress analyte delocalization during transfer by absorbing sample-derived solvents. The platinum layer enables SALDI and simultaneously serves as an electrically conductive layer, allowing the transfer plate to be used directly with commercially available MALDI-TOFMS. Using stains of polymer additives and cross-sections of strawberries as representative examples, it was shown that stable transfer can be achieved regardless of the moisture content of the sample. Thus, the transfer plate functions as a platform for MSI preparations that enables thin-section-free sampling while remaining compatible with commercial MALDI-TOFMS instruments. In addition, because the transfer plate itself possesses SALDI functionality, MSI measurements can be performed without the use of a matrix. This feature eliminates the need for matrix selection and application procedures, and avoids matrix-derived background peaks in the low-mass region.

In contrast, in MSI experiments of strawberry cross-sections, molecules that are prone to fragmentation, such as anthocyanins, which have been detected in previous MALDI-MSI studies^{2,3}, were not detected with sufficient sensitivity using SALDI-MSI on the transfer plate. A similar phenomenon was also observed in the SALDI-MSI of strawberry cross-sections using DIUTHAME, which features a platinum layer similar to that of the transfer plate⁴. In other words, the SALDI functionality of the transfer plate alone was insufficient for all analytical objectives. MALDI provides a different ionization modality from SALDI and offers a wide range of options in terms of matrix selection and application methods, enabling optimization for diverse analytical purposes by appropriately adjusting the sample preparation conditions.⁵⁻⁹ If the sample preparation techniques developed for MALDI can be applied to the transfer plates, the transfer plate may serve as a new platform for thin-section-free MALDI-MSI,¹⁰⁻¹⁸ thereby further expanding its range of applications. Such an expansion of versatility is important not only from a technical perspective but also because broader applicability facilitates user acceptance of the transfer plate as a platform for MSI preparation, thereby enhancing its significance in terms of product development and commercialization.

For the practical implementation of thin-section-free MALDI-MSI using the transfer plate, it is necessary to clarify both the effectiveness of the matrix application to samples transferred onto the plate and the influence of the matrix application on spatial resolution, particularly analyte delocalization. In this chapter, these issues are addressed using strawberry cross-sections as a model sample, and both SALDI-MSI and MALDI-MSI measurements are performed on the transfer plate. First, strawberry cross-sections were transferred onto a transfer plate and analyzed by SALDI-MSI following the procedure described in Chapter 2. Subsequently, matrix spraying is applied to the same transferred sample to obtain ion images of anthocyanins. Next, metabolites detectable by both SALDI and MALDI, such as sucrose, are examined by comparing ion images acquired from the same sample before and after matrix application, thereby evaluating the extent to which matrix spraying affects spatial resolution at a step size of 200 μm . Through these investigations, the influence of the matrix application on spatial information was assessed by a qualitative comparison of SALDI-MSI and MALDI-MSI results, and the feasibility of thin-section-free MALDI-MSI using the transfer plate was clarified.

3.2 Methods

3.2.1 Preparation of samples for SALDI experiment

Sampling of strawberry cross-sections for the SALDI experiment was performed as described in Section 2.2.3. “Benihoppe” strawberries cultivated at a local strawberry farm (Shizuoka, Japan), with a long axis of approximately 50 mm, were used. A platinum-coated porous plate formed of glass beads with a dimension of 75 mm $\times$ 50 mm, was used as the transfer plate. The strawberry cross-section was placed on the active surface of the transfer plate and moderately thumbed for 10 s. SALDI-MSI analysis was performed on the strawberries without further preparation after sampling.

3.2.2 Matrix application for MALDI experiment

For MALDI measurements, ultrapure water (LC-MS grade), methanol (min. 99.5 %), 2,5-dihydroxybenzoic acid (DHB), citric acid (min. 98 %), and diammonium hydrogen citrate (min. 99 %) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

After SALDI-MSI, a MALDI-MSI sample was prepared from the sample that was measured by SALDI. DHB solution (25 mg/ml) in 50 % aqueous methanol was applied to the active surface of the sample, as measured by SALDI. Matrix spraying was

performed manually using an airbrush (PS-270, GSI Creos, Tokyo, Japan) with a distance between the spray nozzle and sample of approximately 10 cm. Five hundred microliters of DHB solution was sprayed over an area of 50 mm × 50 mm by scanning the area more than 10 times in both X and Y directions.

MALDI-tandem mass spectrometry (MS/MS) was performed using the metabolites extracted from the strawberries to identify the chemical components detected through MALDI-MSI. The transferred fresh strawberry was immediately frozen and stored under −80 °C. The outer parts of the strawberry, including the skin, were selectively sliced under −20 °C and dissolved in 50 % aqueous methanol to maintain the concentration of the mixture at 50 mg/mL. The mixture was shaken and centrifuged at 6800 rpm for 30 min. The supernatants were then collected as metabolite extracts. The metabolite extracts (10 µL) were mixed with DHB (30 mg/mL, 10 µL) in an aqueous solution and an aqueous solution of citric acid (0.2 mol/mL, 1 µL) and diammonium hydrogen citrate (0.2 mol/mL, 1 µL). A matrix-mixed solution (1 µL) was applied to the MALDI target (MTP 384 target plate ground steel BC, Bruker, Billerica, MA, U.S.) and naturally dried under standard atmospheric conditions.

3.2.3 Instrumentation

All the mass spectra and ion images in this study were obtained using a MALDI-TOF/TOF instrument (autoflex® maX, Bruker). The SALDI- and MALDI-MSI measurements were conducted in the positive-ion reflector mode. The m/z values ranged from 150 to 650. The frequency of the desorption laser was 500 Hz, and the laser power was set to 60 %. MSI measurements were performed using 100 shots per spot with a beam diameter of approximately 80-µm. A larger MSI step size of 200 µm compared to the beam diameter was deliberately set so that the area without laser irradiation remained on the transfer plate, enabling both SALDI- and MALDI-MSI measurements to be performed on the same sample. In addition, previous MALDI-MSI studies of the entire strawberry cross-section were also reproduced using the step size of the same order^{2,3}. MS/MS spectra were obtained using autoflex maX in the LIFT mode. All spectral analyses, including baseline subtraction processing, peak pickings, and signal-to-noise ratio (S/N) calculations were conducted using flexAnalysis (Bruker). MSI data were analyzed using SCiLS™ Lab (Bruker). The automatic hotspot removal function was performed using the 99 % quantile as the cutoff value. The acquired ion images were normalized to the total ion current (TIC). Magnified images of the active surfaces of the matrix-sprayed transfer plates were obtained using a scanning electron microscope (SEM; JCM-6000, JEOL, Akishima, Japan).

3.3 Results and Discussion

3.3.1 Mass spectra of SALDI-MSI and MALDI-MSI

SALDI-MSI was performed on the strawberry cross-section after it was transferred onto the transfer plate, to reproduce the results reported in Chapter 2 and compare them with the subsequent MALDI-MSI results. Figure 3.1A shows the SALDI-MSI mass spectrum averaged over the strawberry cross-section. No matrix-derived intense peak was observed, as shown in Figure 3.1A. In addition, the peaks corresponding to hexose $[M + K]^+$ (m/z 219.0, S/N 562), citric acid $[M - H + 2K]^+$ (m/z 268.9, S/N 117), and sucrose $[M + K]^+$ (m/z 381.1, S/N 927) were observed. The appearance of these peaks was consistent with the results reported in Chapter 2.

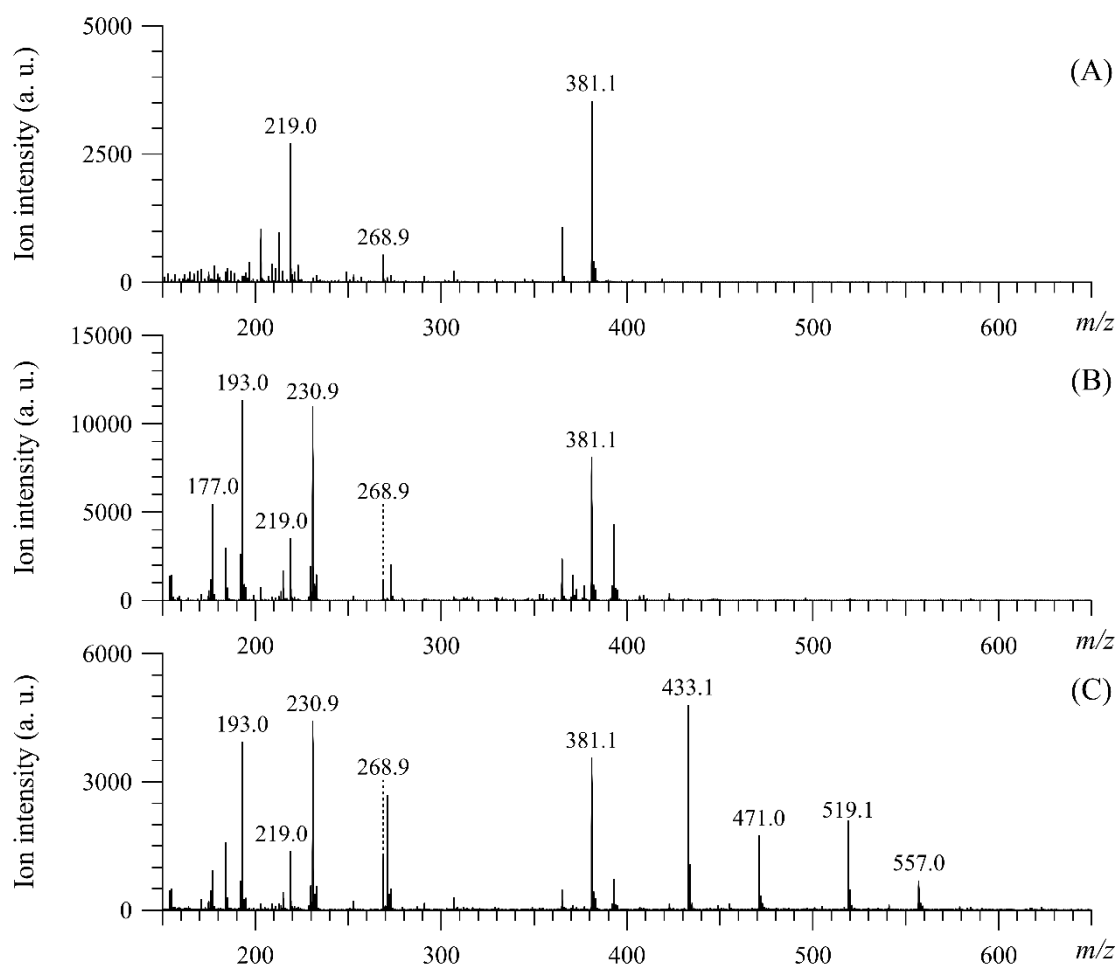


Figure 3.1. Mass spectra averaged over strawberry cross-sections obtained via (A) SALDI-MSI and (B) MALDI-MSI. (C) Mass spectrum averaged over selected spots of the outer part of the strawberry cross-section.

As the second step, after the SALDI-MSI measurements, MALDI-MSI was performed on the same transfer plate after spraying with a DHB matrix solution. Figure 3.1B shows the mass spectrum averaged over the strawberry cross-section obtained via MALDI-MSI. In Figure 3.1B, the ion peaks of hexose $[M + K]^+$ (S/N 529), citric acid $[M - H + 2K]^+$ (S/N 179), and sucrose $[M + K]^+$ (S/N 821) were also detected, corresponding to the same m/z values observed in Figure 3.1A. The S/N values of these peaks in Figure 3.1B were comparable to those in Figure 3.1A, suggesting that the prior SALDI-MSI measurement had no substantial effect the S/N values observed in the MALDI-MSI measurement. A potential explanation is that the MSI step size of 200 μm was larger than the laser beam diameter of 80 μm , allowing some areas which were not irradiated during

the SALDI-MSI measurement to be subsequently irradiated during the MALDI-MSI measurement. The mass spectrum pattern in Figure 3.1B is more complex than that in Figure 3.1A, owing to the appearance of peaks arising from the DHB matrix. Peaks at m/z values of 177.0, 193.0, and 230.9 were also detected in Figure 3.2, with the spectrum averaged over the area outside the region where the strawberry sample was transferred. Therefore, these peaks were considered to originate from the DHB matrix, corresponding to $[M + Na]^+$, $[M + K]^+$, and $[M - H + 2K]^+$ respectively. Although the m/z value of 230.9 is comparable to that of citric acid $[M + K]^+$, in this study, the peak at m/z 230.9 was evidently detected in the region lacking the strawberry sample. Determining whether the peak at m/z 230.9 (Figure 3.1B) originated from the DHB matrix or from the citric acid is challenging.

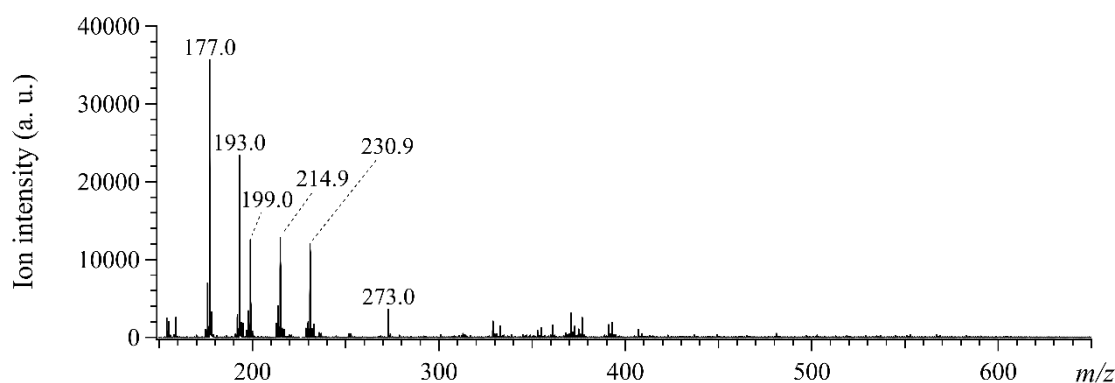


Figure 3.2. Mass spectrum averaged over the area outside the region where the strawberry sample was transferred, obtained via MALDI-MSI. Peaks at m/z values of 177.0, 193.0, and 230.9, which are also clearly evident in Figure 3.1B, corresponding to the DHB matrix-related ions $[M + Na]^+$, $[M + K]^+$, and $[M - H + 2K]^+$, respectively.

Anthocyanins localized in strawberry skin were detected at m/z values greater than or equal to 400 in previous MSI studies^{2,3}. Figure 3.1C shows the MALDI-MSI mass spectrum averaged over different regions along the edges of the strawberry cross-section. The four ion peaks (m/z 433.1, 471.0, 519.1, and 557.0), which are unclear in Figure 3.1B, are clearly evident in Figure 3.1C. The ion peaks at m/z values of 433.1 and 519.1 (Figure 3.1C) are consistent with peaks that have previously been assigned to pelargonidin-3-*O*-glucoside and pelargonidin-3-*O*-malonylglucoside, respectively, whereas the ion peaks at m/z 471.0 and 557.0 (Figure 3.1C) were not detected in the previous study^{2,3}. However, a mass difference of 37.9 is observed between m/z 471.0 and 433.1 as well as between m/z 557.0 and 519.1, which could be attributed to hydrogen substitution by potassium.

This assumption was supported by the MS/MS analysis (Figure 3.3) obtained using an extraction solution that only included the outer layer of the strawberry fruit used for transfer. Figure 3.3A shows the MALDI mass spectrum of the strawberry extraction solution that selectively included only the outer layer of the strawberry fruit used for transfer. The measured extraction solution was mixed with the DHB matrix, citric acid, and diammonium hydrogen citrate, which indicates that the peak intensity of the ion whose hydrogen was substituted for potassium would be expected to have decreased in Figure 3.3A. Ion peaks were detected at m/z 433.1 and 519.1 in Figure 3.3A. In contrast, almost no ion peaks at m/z 471.0 and 557.0 were detected, indicating that these ions in Figure 3.1C were potassium substitutes for the ions detected at m/z 433.1 and 519.1. Figure 3.3B and 3C show the product ion spectra of the precursor ions at m/z 433.1 and 519.1. In both spectra, an intense fragment ion was detected at m/z 271.1, regarded as pelargonidin aglycone $[M]^+$. Neutral losses of 162 and 248 Da correspond to the loss of glucose and malonylglucose moieties, respectively. Therefore, the ion peaks detected at m/z 433.1 and 519.1 in Figure 3.3A are assigned as pelargonidin-3-*O*-glucoside and pelargonidin-3-*O*-malonylglucoside, respectively. The corresponding peaks detected at m/z 433.1 and 519.1 in Figure 3.1C can also be assigned in a similar manner.

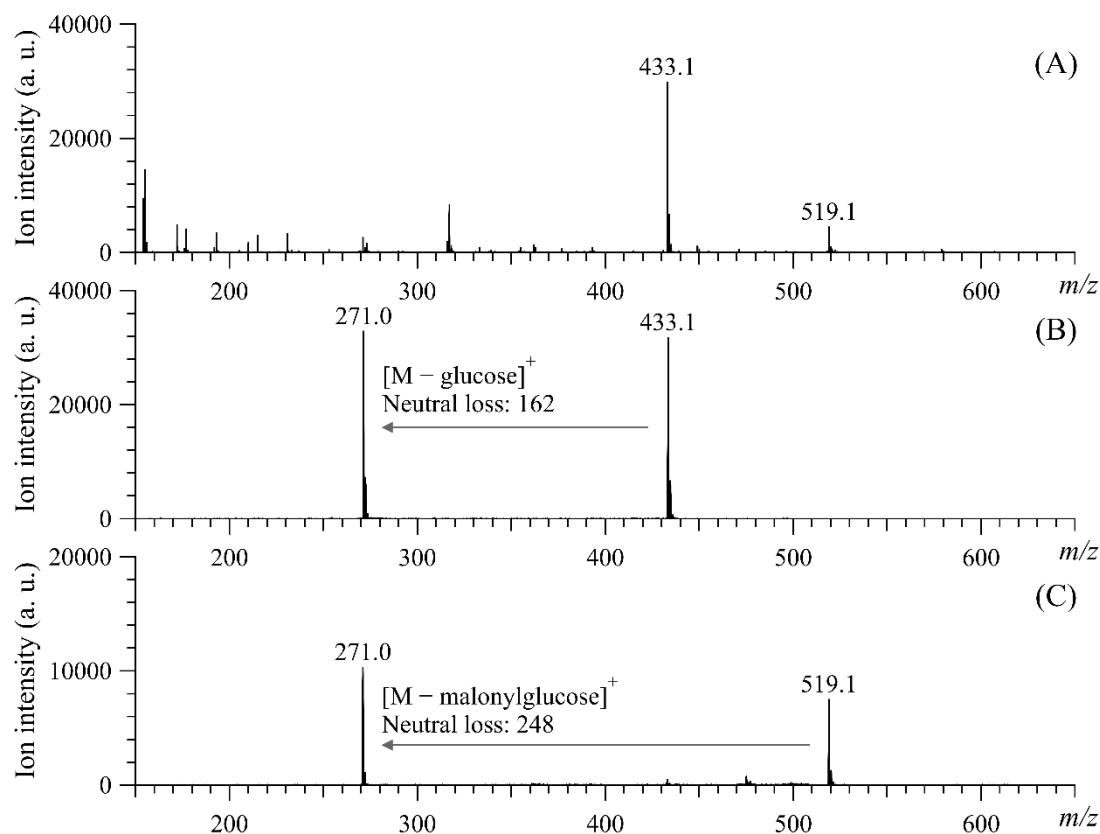


Figure 3.3. (A) MALDI mass spectrum of the extract from the edge of the strawberry fruit. MS/MS product ion spectra of the precursor ion at m/z (B) 433.1 and (C) 519.1.

The primary ions detected in Figure 3.1A–C are summarized in Table 3.1. Therefore, SALDI-MSI was found to be more suitable for analysis in the low-mass region than MALDI-MSI because intense matrix peaks and their overlap with some target ion peaks were eliminated, in spite of the S/N values observed in SALDI-MSI being not significantly different from those in MALDI-MSI. However, the ions corresponding to anthocyanins, which were not detected in SALDI-MSI, were clearly evident in MALDI-MSI.

Table 3.1. Assigned strawberry metabolites detected in the SALDI and MALDI mass spectra shown in Figure 3.1.

Metabolite	Ion type	Detected m/z	SALDI ion image	MALDI ion image
Hexose	$[M + K]^+$	219.0	Figure 3.4A	Figure 3.4D
Citric acid	$[M - H + 2K]^+$	268.9	Figure 3.4B	Figure 3.4E
Sucrose	$[M + K]^+$	381.1	Figure 3.4C	Figure 3.4F
Pelargonidin-3- <i>O</i> -glucoside	$[M]^+$	433.1	–	Figure 3.4G
	$[M - H + K]^+$	471.0	–	–
Pelargonidin-3- <i>O</i> -malonylglucoside	$[M]^+$	519.1	–	Figure 3.4H
	$[M - H + K]^+$	557.0	–	–

3.3.2 Ion images obtained from thin-section-free SALDI-MSI and MALDI-MSI

The ion images of hexose, citric acid, and sucrose obtained via SALDI-MSI are shown in Figure 3.4A–C. Hexose (Figure 3.4A) was slightly localized at the center of the fruit (vascular bundle) although it was generally distributed throughout the fruit. Citric acid (Figure 3.4B) was localized to the cortical tissue and skin. Sucrose (Figure 3.4C) was distributed throughout the fruit area; however, its concentration was slightly lower in the vascular bundle. According to the previous studies on MSI, these metabolites vary in distribution depending on the variety and maturity stages of strawberries^{2,3}. The characteristics observed in Figure 3.4A–C qualitatively correspond to Figure 2.8, further confirming the qualitative reproducibility of SALDI-MSI method using the transfer plate.

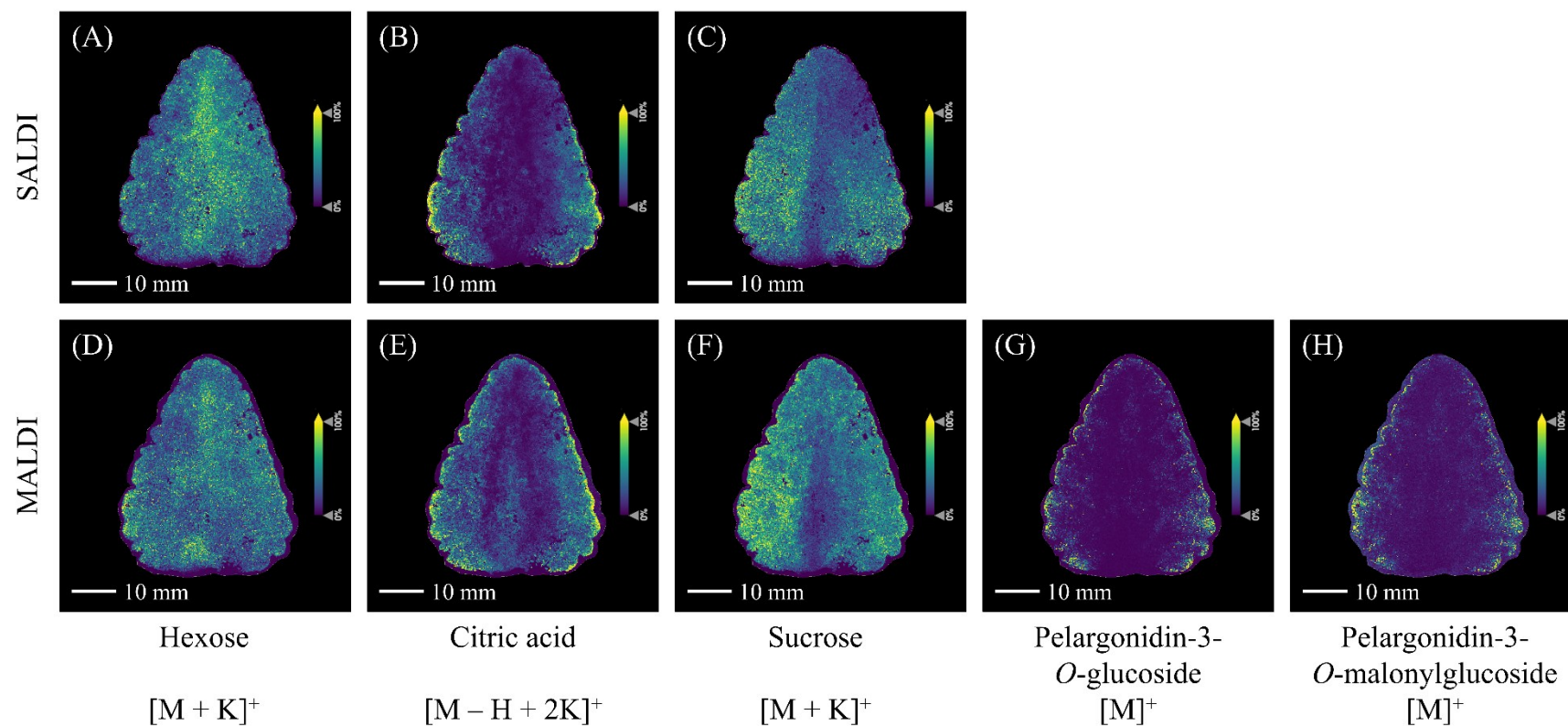


Figure 3.4. Ion images of the strawberry metabolites obtained via SALDI-MSI (A–C) and MALDI-MSI (D–H): (A, D) m/z 219.0 (hexose $[M + K]^+$), (B, E) m/z 268.9 (citric acid $[M - H + 2K]^+$), (C, F) m/z 381.1 (sucrose $[M + K]^+$), (G) m/z 433.1 (pelargonidin-3-*O*-glucoside $[M]^+$), and (H) m/z 519.1 (pelargonidin-3-*O*-malonylglucoside $[M]^+$). The color gradient corresponds to the relative intensity scaled by SCiLS.

The MALDI-MSI ion images of the three constituent ions are shown in Figure 3.4D–F. As described above, SALDI and subsequently MALDI were performed on the same transferred sample; therefore, spatial variability associated with the transfer process was eliminated. The distribution characteristics in Figure 3.4A–C and 4D–F showed qualitative agreement, indicating that the initial SALDI-MSI measurement had no substantial effect on the MALDI-MSI ion images, in accordance with the conclusions based on the S/N comparison. As shown in Figure 3.4G–H, the MALDI-MSI ion images of pelargonidin-3-*O*-glucoside and pelargonidin-3-*O*-malonylglucoside, exhibit their localization at the skin of the fruit. These results are consistent with the previous MALDI-MSI studies on strawberries^{2,3}. Therefore, the absence of these ion images in the prior SALDI-MSI measurement is attributed not to the transfer process of the strawberry cross-section, but to the difference in ionization efficiency between SALDI and MALDI. These findings validate that thin-section-free MALDI-MSI can be performed when a matrix is sprayed onto the sample after being transferred to the transfer plate.

3.3.3 Characteristics of thin-section-free MSI using a transfer plate

No significant delocalization of ion images was observed owing to manual matrix spraying for MSI measurements recorded with a step size of 200 μm . For this spatial resolution, the ion images obtained by SALDI and MALDI are considered qualitatively comparable, indicating that the migration of transferred constituents on the transfer plate is restricted to the step size used during matrix application. Figure 3.5 shows SEM images of the active surface before (3A) and after (3B–C) matrix spraying on the transfer plate. Figure 3.5B confirms that the matrix completely covers the active surface. Furthermore, as shown in the magnified image Figure 3.5C (Figure 3.5B magnified five times), more of the matrix was deposited at the neck between the glass beads than on the spherical surface of the glass bead. Thus, the neck could prevent horizontal matrix migration between the glass beads. The microstructure of the transfer plate, which increases solvent absorption, may enable the transfer of moist samples and contribute to the suppression of delocalization within the corresponding step size upon manual matrix spraying.

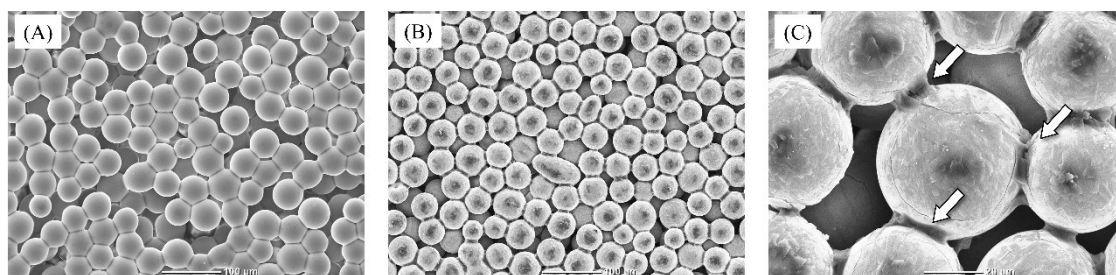


Figure 3.5. SEM images of the surface of a transfer plate (A) before and (B) after matrix-spraying. (C) is an SEM image of (B) magnified five times. The arrows shown in (C) indicate matrix deposition on the neck between glass beads.

MSI using a transfer plate is based on thin-section-free sampling and offers the flexibility to select and integrate other preparation methods and ionization techniques according to the specific analytical purpose. This study demonstrated the feasibility of performing both SALDI, which requires no additional preparation, and MALDI, which involves matrix spraying, on the same platform. SALDI-MSI using a transfer plate offers advantages such as simplified sample preparation (requiring only the transfer process) and delivers less-complex spectra that do not contain matrix-derived interfering peaks. In contrast, MALDI-MSI on a transfer plate employs matrix-assisted soft ionization, which may lead to different ionization outcomes compared to SALDI-MSI. Moreover, the MALDI-MSI experiments used to obtain the images shown in Figure 3.4D–H were conducted on the same transfer plate that had previously been used for SALDI-MSI. These results indicate that SALDI–MALDI multimodal MSI can also be achieved sequentially on a single transfer plate. This sequential approach applied to an identical transferred sample is considered a practical strategy for acquiring both SALDI and other modalities, particularly in cases where thin-sectioning is not feasible and sample duplication is not possible.

3.4 Conclusion

In this chapter, Thin-section-free MALDI-MSI measurements were performed on a strawberry cross-section using a transfer plate. Using strawberry cross-sections as a model sample, SALDI-MSI measurements were first performed on the same transferred sample, followed by matrix application and subsequent MALDI-MSI analysis. This experimental design enabled the direct comparison of SALDI-MSI and MALDI-MSI under conditions that eliminated the variability arising from the transfer process.

The ion images of hexose, citric acid, and sucrose obtained by SALDI-MSI using a transfer plate reproduced the results reported in Chapter 2. The signal-to-noise ratios of these ions in the averaged spectra were comparable for SALDI-MSI and MALDI-MSI, and the corresponding ion images showed qualitative agreement. These results indicate that the prior SALDI-MSI measurements did not exert a substantial influence on the MALDI-MSI results obtained in this study. In contrast, anthocyanins localized in the strawberry skin, which were not detected with sufficient sensitivity by SALDI-MSI, were successfully visualized by MALDI-MSI after matrix application. This finding demonstrates that, in thin-section-free MSI using a transfer plate, the combination of MALDI with SALDI-based measurements enables complementary visualization of molecular species that are difficult to detect using SALDI alone. Furthermore, delocalization caused by manual matrix spraying was not significant when the MSI step size was set to 200 μm because the microstructure between the glass beads in the transfer plate effectively suppressed matrix migration.

This chapter demonstrates the practical feasibility of thin-section-free MALDI-MSI using a transfer plate. The transfer plate not only enables straightforward thin-section-free sampling but also allows different ionization modalities, such as SALDI and MALDI, to be employed on a common experimental platform. These findings provide a technical foundation for the broader use of the transfer plate as a versatile MSI platform and support its further application and development in diverse MSI workflows.

References for Chapter 3.

1. Ikeda T, Kotani M. Thin-section- and matrix-free mass spectrometry imaging: Reproducible sample transfer using novel platinum-coated porous plate formed of glass beads. *Rapid Commun Mass Spectrom*. 2024;38(6):e9697. doi:10.1002/rcm.9697
2. Enomoto H, Sato K, Miyamoto K, Ohtsuka A, Yamane H. Distribution Analysis of Anthocyanins, Sugars, and Organic Acids in Strawberry Fruits Using Matrix-Assisted Laser Desorption/Ionization-Imaging Mass Spectrometry. *J Agric Food Chem*. 2018;66(19):4958-4965. doi:10.1021/acs.jafc.8b00853
3. Wang J, Yang E, Chaurand P, Raghavan V. Visualizing the distribution of strawberry plant metabolites at different maturity stages by MALDI-TOF imaging mass spectrometry. *Food Chem*. 2021;345:128838. doi:10.1016/j.foodchem.2020.128838
4. Enomoto H, Kotani M, Ohmura T. Novel Blotting Method for Mass Spectrometry Imaging of Metabolites in Strawberry Fruit by Desorption/Ionization Using Through Hole Alumina Membrane. *Foods*. 2020;9(4):408. doi:10.3390/foods9040408
5. Lee PY, Yeoh Y, Omar N, Pung YF, Lim LC, Low TY. Molecular tissue profiling by MALDI imaging: recent progress and applications in cancer research. *Crit Rev Clin Lab Sci*. 2021;58(7):513-529. doi:10.1080/10408363.2021.1942781
6. Unsihuay D, Mesa Sanchez D, Laskin J. Quantitative Mass Spectrometry Imaging of Biological Systems. *Annu Rev Phys Chem*. 2021;72(1):307-329. doi:10.1146/annurev-physchem-061020-053416
7. Qiao Z, Lissel F. MALDI Matrices for the Analysis of Low Molecular Weight Compounds: Rational Design, Challenges and Perspectives. *Chem. Asian J*. 2021;16(8):868-878. doi:10.1002/asia.202100044
8. Harkin C, Smith KW, Cruickshank FL, et al. On-tissue chemical derivatization in mass spectrometry imaging. *Mass Spectrom Rev*. 2022;41(5):662-694. doi:10.1002/mas.21680

9. Lim MJ, Yagnik G, Henkel C, Frost SF, Bien T, Rothschild KJ. MALDI HiPLEX-IHC: multiomic and multimodal imaging of targeted intact proteins in tissues. *Front Chem.* 2023;11:1182404. doi:10.3389/fchem.2023.1182404
10. Caprioli RM, Farmer TB, Gile J. Molecular Imaging of Biological Samples: Localization of Peptides and Proteins Using MALDI-TOF MS. *Anal Chem.* 1997;69(23):4751-4760. doi:10.1021/ac970888i
11. Bunch J, Clench MR, Richards DS. Determination of pharmaceutical compounds in skin by imaging matrix-assisted laser desorption/ionisation mass spectrometry. *Rapid Commun Mass Spectrom.* 2004;18(24):3051-3060. doi:10.1002/rcm.1725
12. Mullen AK, Clench MR, Crosland S, Sharples KR. Determination of agrochemical compounds in soya plants by imaging matrix-assisted laser desorption/ionisation mass spectrometry. *Rapid Commun Mass Spectrom.* 2005;19(18):2507-2516. doi:10.1002/rcm.2078
13. Goto-Inoue N, Hayasaka T, Taki T, Gonzalez TV, Setou M. A new lipidomics approach by thin-layer chromatography-blot-matrix-assisted laser desorption/ionization imaging mass spectrometry for analyzing detailed patterns of phospholipid molecular species. *J Chromatogr A.* 2009;1216(42):7096-7101. doi:10.1016/j.chroma.2009.08.056
14. Valdes-Gonzalez T, Goto-Inoue N, Hirano W, et al. New approach for glyco- and lipidomics - Molecular scanning of human brain gangliosides by TLC-Blot and MALDI-QIT-TOF MS: New approach for glyco- and lipidomics. *J Neurochem.* 2011;116(5):678-683. doi:10.1111/j.1471-4159.2010.07152.x
15. Zaima N, Goto-Inoue N, Adachi K, Setou M. Selective Analysis of Lipids by Thin-Layer Chromatography Blot Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry. *J Oleo Sci.* 2011;60(2):93-98. doi:10.5650/jos.60.93
16. Klein AT, Yagnik GB, Hohenstein JD, et al. Investigation of the Chemical Interface in the Soybean–Aphid and Rice–Bacteria Interactions Using MALDI-Mass Spectrometry Imaging. *Anal Chem.* 2015;87(10):5294-5301. doi:10.1021/acs.analchem.5b00459
17. Fournelle F, Yang E, Dufresne M, Chaurand P. Minimizing Visceral Fat Delocalization on Tissue Sections with Porous Aluminum Oxide Slides for

Imaging Mass Spectrometry. *Anal Chem.* 2020;92(7):5158-5167.
doi:10.1021/acs.analchem.9b05665

18. Hapuarachchige PNP, Tat VT, Lee YJ. Advanced Tissue Imprinting With Pneumatic Press for Mass Spectrometry Imaging of Plant Tissues. *J Mass Spectrom.* 2026;61(1):e70007. doi:10.1002/jms.70007

Chapter 4.

Multi-Perspective Internal Observation in Product Development Teams: Practical Knowledge and Motivation Dynamics.

A portion of the content in this chapter has been submitted as a peer-reviewed article to *The Journal of Organization and Discourse*. Copyright for the unpublished manuscript belongs to *The Journal of Organization and Discourse*. As this chapter deals with social science topics, the citations and references use an APA-based style.

4.1 Introduction

Chapters 2 and 3 examined the technological principles of the transfer plate and its potential applications. However, product development as a business practice does not progress solely through resolving technical challenges. Instead, it advances through the accumulation of practical activities involving collaborative work among multiple members, decision-making, trial and error, and evolving motivational processes. Chapter 4 therefore focuses on the team practices embedded in the product development project and aims to clarify the methodological characteristics and forms of motivation inherent in these practices from a managerial perspective.

This section first reviews prior research in technology management and then outlines the theoretical frameworks of internal observation and the theory of *katari*. It subsequently introduces a new conceptual framework, the *multi-perspective internal observation*, which extends conventional internal observation by incorporating the perspectives of all team members. The background and rationale for applying this framework in the present study are also explained.

For companies whose core competence lies in technology, the continuous pursuit of new business opportunities and the development of technological innovations are essential corporate activities. In Japan, long described as a technology-oriented nation, many firms achieved postwar growth through manufacturing capabilities. However, the stagnation of Japanese companies from the collapse of the economic bubble to the present highlights the difficulty of sustaining innovation. In today's business environment, the technology management practices that once supported Japanese competitiveness have largely faded, and new business development and innovation rarely occur. Aside from a small number of highly capable firms, many workplaces lack inherited methodologies for practicing technology management at the operational level.

A substantial body of research has examined the technology management that supported the success of "Japan as number one." Clark and Fujimoto (2009), for example, conducted an empirical study of organizational capabilities and competitiveness in product development in the automotive industry. Numagami (1999) analyzed technological innovation in liquid crystal displays as a process of social consensus building among actors inside and outside the firm. Kawakami (2005) discussed the relationship between marketing and technology development, as well as its management, through case studies of the consumer electronics industry. Much of this prior research relied on publicly accessible data, newspaper and magazine articles, and interviews conducted with companies.

Beyond interview-based studies, some cases have been written by insiders who describe their companies' practices and experiences from a managerial standpoint. Azuma (1999), for example, drew on 40 years of knowledge at a chemical manufacturer to present a panoramic managerial account of business development and its management. Urakawa (2010) reflected on research and development at a rubber manufacturer and developed a practical management framework linking business strategy with technology strategy. Although their approaches differ, these studies share the aim of extracting managerial insights from the management viewpoint and contribute to the development of macro-level technology management theories.

Despite the many advantages of technology management, new business development and innovation are still large challenges for Japanese companies. Overcoming roadblocks like this requires practical methods that function effectively at the work site. Urakawa (2010) also observed that successful technology management practices depend on a *collective guidance system* grounded in free discussion among all involved parties, including supervisors and staff. He further emphasized that this system arises not from top-down instruction but from organizational culture. However, macro-level studies do not reveal the concrete content of such practical methodologies, including shared understandings and actual interactions. Approaching individual practices instead requires a micro-level perspective rooted in the realities of the workplace. From a research standpoint, investigating such practices is difficult. External researchers have limited access to day-to-day activities, and even internal researchers cannot obtain a truly micro-level perspective unless they themselves are frontline practitioners.

The author believes that many managerial insights are embedded in the practical activities occurring inside organizations. To uncover such insights, researchers must adopt an internally situated standpoint and be embedded within the practices themselves. For example, when in-house practitioners observe the practices in which they are involved, the distinction between researcher and practitioner dissolves. The *internal observation perspective* is therefore well suited to this purpose. Masuda (2007, 2013) formalized internal observation as a method in organizational research and showed that because an observer and observed are inseparable, the act of observing becomes a form of motivation management for the group being observed. Masuda (2013) further developed the theory of *katari* as a conceptual tool for applying internal observation to field research. Since then, internal observation and the theory of *katari* have successfully captured a wide range of internal corporate practices.

As one illustrative example, Fujiwara and Masuda (2015) examined corporate bootlegging (i.e., unofficial technical development) from an internal observation

perspective. They identified a process in which repeated attempts, including failures, accumulate and lead to further attempts. They also demonstrated a practical methodology showing how a sense of contributing to the organization reshapes explicit evaluations by others, whether approving or disapproving. Similarly, Kato and Masuda (2016), in their investigation of new product development processes, proposed the concept of *interfacilitation* as a form of mutual motivation derived from internal observation. They verified a case in which internal observation itself functioned as motivation management during project team formation.

Studies like these show that internal observation and the theory of *katari* can identify practical processes in organizations with precision, as well as effective management practices at the operational level. Apart from Masuda's own formalized studies, prior research on internal observation has typically involved corporate practitioners who maintained their original standpoint and conducted the investigation as researchers, even without specialized knowledge in management studies. To ensure research quality, these studies involved management scholars during data analysis and interpretation.

In Chapter 4, the author proposes a new approach called multi-perspective internal observation as a means of extending and complementing conventional internal observation. Traditional internal observation has involved observing and documenting practices from the viewpoint of a single individual who is both practitioner and researcher. That is, only those practical activities that the researcher could personally witness were recorded. However, practical activities within companies are typically performed by multiple people as a team, and such activities are inherently shaped by each member's internal background, or "another story," including motivations and experiences. From the standpoint of a single researcher, it is impossible to observe the "another story" held by other members. To understand these backgrounds comprehensively, conventional internal observation must be expanded.

Accordingly, this study broadens internal observation from one researcher to all team members and attempts to observe and analyze practical activities from multiple perspectives. In this approach, the practitioner team also becomes the research team, and members collectively observe and reflect on their own practices. The aim is to elicit, as fully as possible, each member's "another story." Moreover, as with conventional internal observation, multi-perspective internal observation functions not only as a method for investigating practical activities but also as a form of motivation management, since the act of observation itself stimulates members' engagement and willingness to act.

The motivation for this managerial study arose from the review of a product development project in which the author served as team leader. Although details will be described later, the project advanced from the initial concept proposal to final commercialization and market introduction. Naturally, project outcomes are influenced by a wide range of internal and external factors and cannot be reduced to simple explanations. Nevertheless, in this case the author sought a clear understanding of the team's condition as a foundation for future learning. As such, the author met a researcher external to the project team specializing in internal observation and initiated a collaborative study of the author's own team. However, the author began to question whether the team situation perceived through the author's viewpoint truly represented the actual team situation and thus recognized the limitations of relying solely on personal observation.

This recognition led the author to seek the cooperation of team members and to attempt multi-perspective internal observation aimed at eliciting their "another stories." It should be noted that although the researcher was the team leader, this role was not part of upper management or executive leadership; it was one of a midlevel employee working on the operational front line. Thus, the researcher was simultaneously a practitioner, and the study was conducted from the perspective of internal observation, examining practical activities occurring within the team.

The first purpose of this study is to identify and articulate practical methodologies effective in project environments through multi-perspective internal observation conducted by project members. The distinctive feature here is that project activities are examined from the multiple perspectives of those directly involved. This approach makes it possible to draw out each member's "another story" and to build shared interpretations through consensus. The practical knowledge that proved effective for the project team therefore emerges as a collective understanding. While it is unlikely that this study will generate entirely new managerial knowledge, clarifying which forms of practical knowledge were useful to the members and specific to this project offers a path for reconstructing on-site methodologies for technology management, many of which have largely disappeared. This type of knowledge differs from the managerial insights derived from the macroscopic perspective of executives.

The second purpose of this study is to demonstrate whether the multi-perspective internal observations reported here serve as motivators for transforming team members, including the author. Because the observations recorded in this study are made within a practitioner team, the insights are by definition "internal." If one member voices a new perspective, the others have the opportunity to gain a deeper understanding of one

another's motivations, leading to new team dynamics. These shifts, in turn, influence the member who originally spoke through others' reactions and responses. Such reciprocal interactions are inseparable from the act of observation in multi-perspective internal observation. If these processes help motivate team members, they constitute motivation management. In this sense, the theoretical stance of internal observation—that observing is itself motivation management—extends to multi-perspective internal observation. If this approach is effective as motivation management, it may be applicable to project activities in other companies as well.

In summary, the novelty and originality of this study lie in applying multi-perspective internal observation to a project team. This approach enables the articulation of the practical knowledge that proves effective in the project environment while simultaneously functioning as motivation management for team members.

The structure of this chapter is as follows. Section 4.2 introduces the theoretical framework for this study—internal observation, the theory of *katari*, related prior research, and theoretical perspectives on motivation. Section 4.3 outlines multi-perspective internal observation and the specific research methods employed. Section 4.4 presents the results of the multi-perspective internal observation, Section 4.5 discusses these findings in relation to the objectives of this study, and Section 4.6 concludes the chapter.

4.2 Theoretical Framework

4.2.1 Internal observation and the theory of *katari*

The conceptual foundation of this study lies in field research framed by the observation-oriented theory developed by Masuda (2007, 2013). Within this framework, Masuda introduced internal observation—originally articulated in the “theory of life” (Gunji 2006)—as a research perspective for one's own workplace. The premise suggests that the observer and the observed are not separate characters. However, the interactions and transactions between the roles alters the object of observation, which could either create bias or help the researcher overcome it. Hence, internal observation must clearly recognize that observations generate reciprocal transformations. In contrast, conventional participant observation assumes that the observer does not influence the research site.

Masuda (2013) also developed the theory of *katari* as a conceptual tool for applying the theory of life to field research. *Katari* does not refer to narrative as understood in social constructionism; rather, it aligns with Boje's (2001) idea of *antenarrative* that precedes a coherent story. According to Masuda (2007, 2013), the

linguistic act of *katari* creates a dynamic relational structure in which the *katarite* (teller), the *kikite* (listener), and the *mono* (that which is told) form a triadic relation of two elements mediated by a third. When the *mono* changes through *katari*, both *katarite* and *kikite* also change. This dynamic mirrors the continuous interplay between observation and the observed in internal observation, making *katari* an appropriate conceptual tool.

Masuda (2013) further extended motivation research by grounding it in the theory of life. Moving beyond mechanistic and organismic models, he proposed a life-theoretic approach based on an observation-oriented framework. Its defining claim is that motivation is not a preexisting internal resource but a relational emergence that arises in conjunction with events and actions. Motivation becomes most visible in nonroutine or unexpected situations, where it is discovered and constructed through action. This process of discovery = construction occurs through *katari*. Motivation is embedded in *katari* and made visible through it. Moreover, although field research seeks to clarify behavioral patterns and their origins in organizations, research conducted from an internal observation perspective inevitably influences those very patterns and origins. Thus, field research conducted through internal observation (and its tool, *katari*) is inherently an act that shapes the field—and this act is, in essence, motivation management.

In the theory of *katari*, linguistic acts are organized into multiple modes based on *temporal orientation* and *intentionality* (Masuda 2007, 2013; Morishita and Masuda 2018; Miyamoto and Masuda 2019). These modes provide an analytical lens for examining the quality of communication and the dynamics of interaction. In this study, applying these modes to the analysis allows a structural examination of how members' recognition and emotions shifted through group discussions and how future-oriented *katari* emerges. The author also clarifies how multi-perspective internal observation functions as motivation management by analyzing the sequential transitions among these modes of *katari*. Table 4.1 summarizes the modes of *katari* identified in prior research.

Table 4.1. Modes of *katari* identified in prior research.

Mode	Temporal Orientation	Intentionality
<i>Katari-kikase</i>	Past	A mode in which a scripted story is told. This mode does not appear in the present study.
<i>Katari-naoshi</i>	Past	A mode in which a recalled past is reinterpreted through present interactions.
<i>Katari-tsugi</i>	Past	A mode oriented from the past toward the future, guiding the next generation through emergent linguistic acts.
<i>Katari-tsutae</i>	Past	A mode in which past experiences are conveyed vividly, generating empathy in the listener.
	Present	A mode in which present situations are conveyed vividly, generating empathy in the listener.
<i>Katari-ai</i>	Present	A mode of <i>katari</i> in which mutual understanding is already shared.
<i>Katari-awase</i>	Present	A mode in which mutual understanding is not initially shared but a new <i>katari</i> emerges through the alignment of viewpoints.
<i>Katari-dashi</i>	Present	A mode that leads to new actions within the present context.
	Future	A mode that creates a new future-oriented context and connects to <i>katari-tsukuri</i> .
<i>Katari-tsukuri</i>	Future	A mode in which a future-oriented self is constructed and future actions are articulated.

Source: Adapted and partially revised from Miyamoto and Masuda (2019), Table 2.

Katari-kikase is a mode in which a narrative with a predetermined conclusion is delivered. It corresponds to authoritative discourse (Bakhtin 1975), where leaders or senior members convey their philosophies or hero narratives to junior members. In a multi-perspective internal observation conducted within a frontline team, as in this study, linguistic acts falling under *katari-kikase* do not appear.

Katari-naoshi is a mode in which the meaning of past events is reconstructed through the intersection of multiple interpretations. When the same event comes to be understood differently, a transformation of recognition occurs—a “rewriting of the past.”

This resembles reframing therapy in psychotherapy and provides new contextual grounding for present and future actions.

Katari-tsugi is a mode in which the past is carried forward as values and lessons that guide future decisions and actions rather than treated as mere recollection. Experiences become shared resources for the team and inform subsequent choices and behaviors.

Katari-tsutae is a mode in which past or present experiences are conveyed vividly so that the listener can feel and understand them. Rather than reporting facts, it expresses emotions and concrete details that directly shape the listener's understanding and feelings. Identified by Miyamoto and Masuda (2019), this mode plays an important role in building relationships within teams engaged in innovation.

Katari-ai is a mode in which tellers and listeners already share an understanding of processes and experiences. Here, a deepened mutual perspective supports their relational stability and psychological safety.

Katari-awase is a mode in which multiple tellers with different perceptions and interpretations can align their *katari* to build shared understanding. Unlike *katari-ai*, differences in understanding surface and are adjusted through dialogue. This process often generates updated meanings and new insights.

Katari-dashi, identified by Morishita and Masuda (2018), is a mode in which a person shows a clear intention to participate actively and a willingness to act. It tends to reflect personal motivation and intrinsic drive. Although they also suggested that future-oriented *katari-dashi* may lead to *katari-tsukuri*, concrete examples based on actual cases have not yet been reported.

Katari-tsukuri is a mode in which concrete future actions and future-oriented self-conceptions are constructed based on earlier *katari-awase* or *katari-dashi*. It verbalizes direction toward the future and produces “seeds of future practice.” This mode is closely tied to the emergence of intrinsic motivation within organizational practice and represents the future-oriented phase in the chain transformation of *katari*.

4.2.2 Previous studies using internal observation and the theory of *katari*

Internal observation and the theory of *katari*, formalized by Masuda, have since developed into a methodological foundation for qualitative, practice-embedded research. Specific research methods include *self-ethnography*¹ (Alvesson 2003), in which

¹ The more general method of autoethnography (Ellis & Bochner 2000) focuses primarily on reflecting on one's own experiences through autobiographical description. In contrast, self-ethnography (Alvesson 2003)

practitioners document team practices in real time, as well as interviews with project participants. The works introduced below—together with those cited in Section 4.1—illustrate how internal observation and the theory of *katari* have been applied in prior studies.

Masuda (2010) used the *katari* framework to analyze how polysilicato-iron developed within his company came to be accepted as a sustainability-oriented Reduce–Reuse–Recycle material in both the waterworks and agriculture industries, showing how its value shifted contextually within managerial practice. Masuda (2014) examined a small company that recovered rapidly after the Great East Japan Earthquake and demonstrated that routine *katari-tsugi* played a critical role in crisis decision making.

Kato and Masuda (2016), as noted earlier, conceptualized interfacilitation as a process in which members autonomously motivate one another without relying on a facilitator and showed that it functions as motivation management. Morishita and Masuda (2018) extended the theory by introducing *katari-dashi* and analyzed how practitioners entered a community of practice during early market research for new business development. Miyamoto and Masuda (2019) applied the theory of *katari* to a successful interdisciplinary technological development project and identified features of dialogic communication involved in innovation creation.

Efforts have also been made to integrate internal observations with other field research approaches to broaden its theoretical scope. Fujiwara and Masuda (2015) proposed emergent business field research, which incorporates internal observation into developmental work research (Engeström 1991), and used it to examine the practical processes of corporate bootlegging. Kato and Masuda (2017) applied internal observation to new product concept development and, drawing on expansive learning theory (Engeström 1987), developed the Abstract–Action–Communication–Dialogue–Practice–Community–Decision–Plan–Concept–Development–Product (ACDP) cycle as a conceptual model suited to real development environments. Goto and Masuda (2021) applied the *emergent business field research* method to a 27-year product development history in a research institute and identified previously unarticulated tacit knowledge, while also clarifying qualitative differences between practical knowledge that had been successfully inherited and that which had not.

emphasizes not only the researcher’s experiences but also the organizational, social, and cultural contexts that shape them, and the two methods are therefore distinguished methodologically. In internal observation, because practitioners serve as researchers observing their own practice, they often adopt a self-ethnographic stance in which descriptions begin from the self while incorporating the surrounding situations and interactions. The descriptive framework used in this study follows this self-ethnographic approach.

4.2.3 Theoretical framework of motivation

This study seeks to describe the process of problem solving in workplace settings through multi-perspective internal observation and to theorize the interactions and motivational phenomena that emerged among the members. Internal observation and the theory of *katari* (Masuda 2013), the core analytical frameworks of this study, already provide effective perspectives for examining how interactions arise and transform in practice. However, the motivational phenomena identified here involve multilayered psychological and social dynamics that cannot be fully explained by *katari* itself.

This section expands the theoretical framework by introducing several related motivation theories, such as Deci's *theory of intrinsic motivation*, Csikszentmihalyi's *flow theory*, and Bakhtin's *dialogic* and *carnival theories*. These theoretical perspectives help build the foundation for novel and useful understanding of motivational mechanisms in workspaces.

4.2.3.1 Deci's theory of intrinsic motivation and Csikszentmihalyi's flow theory

Intrinsic motivation refers to a psychological state in which people engage in activities for their own sake, rather than obtaining rewards or new obligations. Deci (1975) suggested these activities for which no obvious reward exists beyond the activity itself, explaining that people are driven by the desire to feel *competent* and *self-determined* through the activity. In this sense, intrinsic motivation is strongest when individuals value the activity itself and when the situation affords autonomy and a sense of effectiveness.

Csikszentmihalyi's (1990) flow theory describes a state of deep immersion in which individuals continue acting in a self-purposive manner. Flow emerges when challenges and skills are balanced and the activity provides clear feedback. In this state, concentration intensifies, peripheral concerns recede, and the experience becomes inherently rewarding. Recent organizational research (Kato and Masuda 2016) has viewed this status as a prototypical form of intrinsic motivation, indicating that it enhances creativity and problem-solving.

4.2.3.2 Bakhtin's dialogic theory and carnival theory

The Russian philosopher Mikhail Bakhtin viewed language not as a fixed system with predetermined meanings but as one that dynamically manifests through the activity of communicating. According to Bakhtin (1975), words arise as organic responses from dialogue, manifesting from the interaction of multiple human words. Even when propositions may be deductively identical, meanings shift depending on the social and

cultural context. In meetings, which have a formal context, statements made by superiors are typically received as *authoritative discourse*. In informal contexts, the same content is more likely to be seen as *internally persuasive discourse*, which can result in a totally different understanding. This phenomenon is considered *polyphonic*, and its perspective of dialogue is essential for understanding how group communication affects individuals and organizations.

Bakhtin (1965) extended this perspective into *carnival theory*, which examines the transformative power of extraordinary and unusual workspaces. Drawing on medieval European carnivals, he considered situations where everyday hierarchies and authority structures are temporarily suspended as capable of offering people a sense of freedom and equality: a *carnival sense of the world*. This worldview relativizes established truths and authority, resists *fixity* (a condition in which meanings, roles, norms, and authority structures become rigid, stable, and taken for granted), and opens a space in which creative thought emerges once constraint is lifted. Two elements are central to this worldview: *the public square*, where social ranks and roles are momentarily dissolved, enabling open and equal interaction; and *the jester*, who disrupts entrenched values through humor and paradox, offering alternative perspectives.

Bakhtin's dialogic and carnival theories together show how the settings in which language is produced shape its meaning and how the relativization of authority creates conditions for free and generative interaction. These perspectives demonstrate that communication within groups or organizations can be understood only by attending not just to what people say but also to the situations and relationships in which their utterances occur.

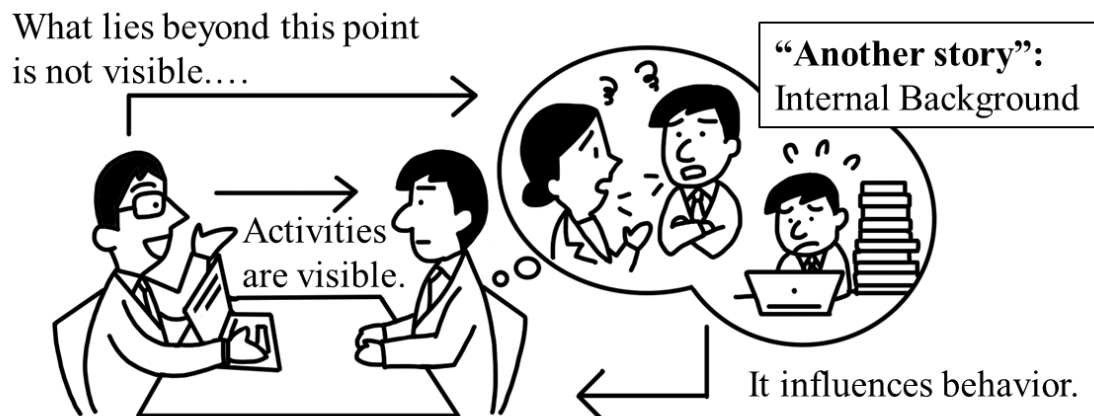
4.3 Multi-Perspective Internal Observation and Specific Research Methods

4.3.1 Multi-perspective internal observation

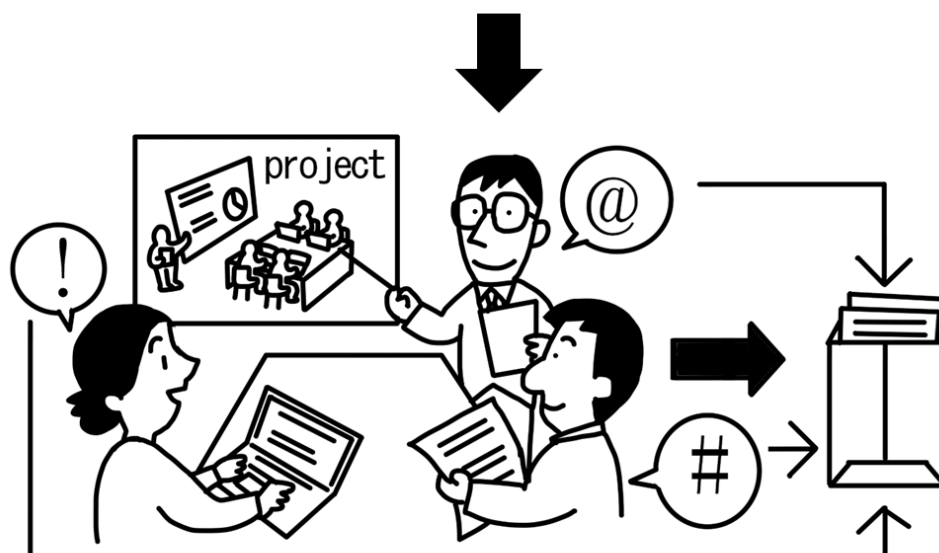
In this study, the author proposes the multi-perspective internal observation concept, which extends internal observation to all team members. Conventional internal observation follows the practitioner = researcher framework, in which a single practitioner observes the organization and examines how the act of observing influences both the organization and the observer. According to internal observation theory, organizational members other than the researcher are also affected by being observed. These transformations arise from the interaction between the researcher's observational acts and each member's internal background—personal history, personality, and the path

that led them to the project. These internal backgrounds shift through day-to-day activities, much of which is not visible to the researcher. In this study, such unseen backgrounds and activities are referred to as each member's "another story," and they exert significant influence on team practice.

Under traditional internal observation, however, a single researcher can perceive only the outcomes of interaction and cannot directly observe these "another stories." If internal observation could be expanded to include these stories as objects of observation, team practice could be understood far more comprehensively. In conventional internal observation, the practitioner serves as the researcher; when internal observation is used to capture "another stories," this one-to-one relationship expands into a view in which the practitioner team becomes the research team. In this expanded framework, team practice is observed from multiple perspectives by all members, and the effects of observation reflect back onto the team in multiple forms. The author therefore defines multi-perspective internal observation (Figure 4.1) as internal observation extended to the whole team and implements it as a new form of organizational observation capable of capturing "another stories."



Internal observation conducted by a single researcher may fail to capture “another stories.” However, these “another stories” can still influence members’ behavior.



Multi-perspective internal observation.

By integrating the “another stories” held by different members, the team can understand its practices from a collective perspective.

Figure 4.1. Overview of multi-perspective internal observation. “Another stories” that cannot be observed from the viewpoint of a single researcher are captured through multi-perspective internal observation, conducted from the team’s viewpoint.

The basic requirements of multi-perspective internal observation are:

- Eliciting each member's "another story" as fully as possible from their own perspective; and
- Integrating these stories and building shared agreement among members.

The primary significance of this approach is that it enables a broad understanding of practice grounded in "another stories," making their effective elicitation essential. Equally important is the process of integrating these stories, as consensus building deepens the team's understanding of its own practice. To meet the intent of multi-perspective internal observation, the research method must therefore satisfy both conditions.

As in conventional internal observation, "another stories" are elicited through *katari* and dynamically interact with team members. Consequently, field research conducted through multi-perspective internal observation functions as an act that generates change within the team and operates as motivation management.

4.3.2 Specific research methods

The project examined in this study had already been completed when this thesis was written; hence, no real-time records of practice (e.g., self-ethnography) were available. Consequently, there were no existing discourses on practical activity that could serve as research material. As such, the author conducted a postproject group discussion where members jointly recounted past events.

Using this method, interactions naturally emerge among the group members, allowing recounted perceptions to be documented, allowing the team leader and others to perceive past events they had not witnessed first-hand. This differs from documented first-hand testimony as each member acts as an internal observer, recounting their activities and sharing perspectives, including their "another stories." This approach mitigates bias that arises when internal observation is limited to one researcher's viewpoint. Kato and Masuda (2016) referred to the motivational phenomenon generated through *katari-awase* as interfacilitation, and this concept is applied here to the group discussion used to review project practice.

To satisfy the two conditions outlined in Section 4.3.1, the team conducted two group discussions designed as follows. The first discussion (D1) was held without the team leader and included only the other members. The team leader communicated the topics to be addressed beforehand but did not participate. In the absence of the leader, who normally serves as facilitator, the discussion proceeded through interfacilitation

among the members. This irregular setting, combined with interfacilitation, was expected to elicit “another stories” effectively and satisfy Condition 1.

The second discussion (D2) included the team leader and was conducted based on the content of D1. While reflecting on D1, D2 aimed to satisfy Condition 2—integrating “another stories” and building shared agreement within the team. After both D1 and D2, the team leader and the researcher external to the project team reviewed the discussion content and added managerial perspectives. In D2, the views of the researcher external to the project team on the content of D1 were also shared with the team.

Through these two rounds of discussion and subsequent reflection, the author sought to emulate effective multi-perspective internal observation. An overview of the process used in this study is shown in Figure 4.2.

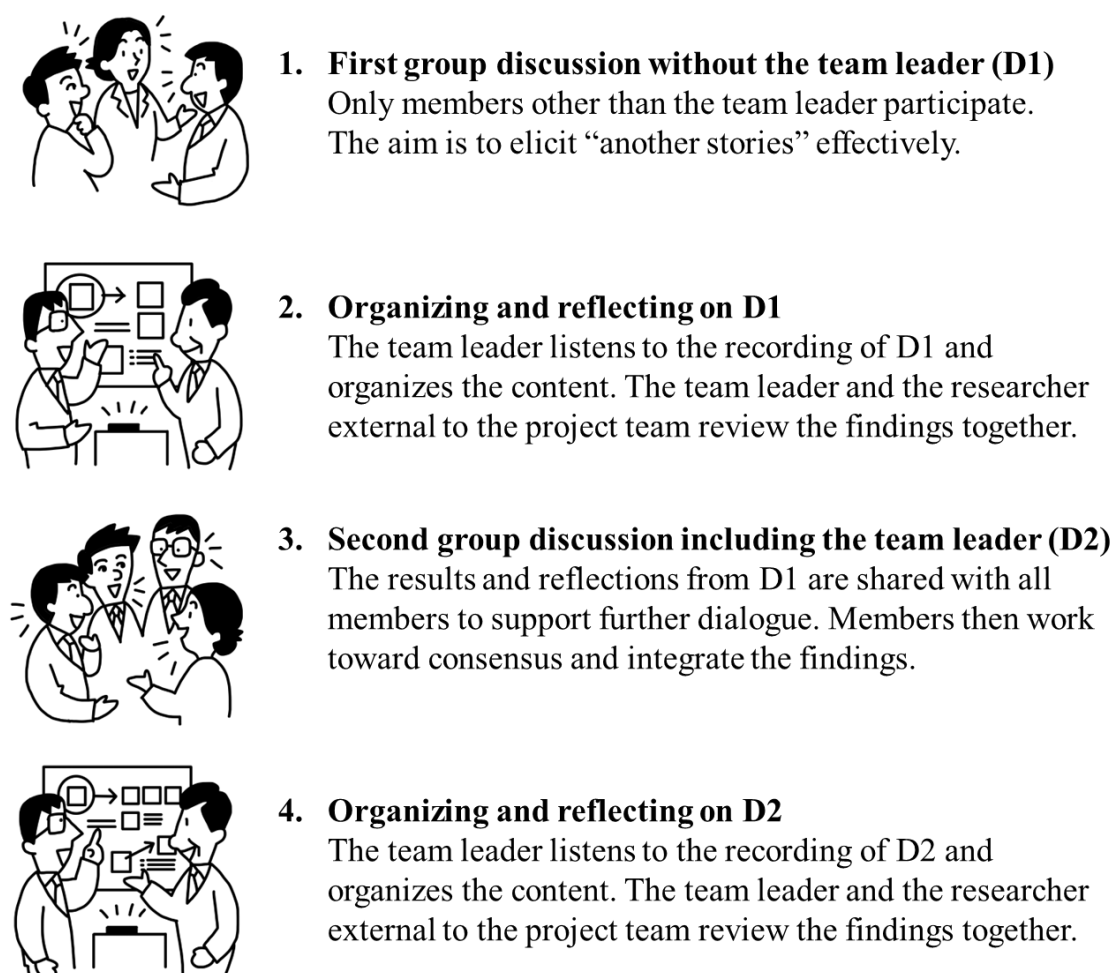


Figure 4.2. Specific research methods for the multi-perspective internal observation conducted in this study.

4.4 Observation Results in Multi-Perspective Internal Observation

4.4.1 Research participants

The participants in this study comprised a four-member product development team, including the team leader. The scope included members from the initial concept proposal through to the formal launch of the project. Details of each member are presented in Table 4.2, and in the following sections each member is identified by the letters **X**, **A**, **B**, or **C**. The researcher external to the project team is referred to as **Y**. Members **A**, **B**, and **C** concurrently held other responsibilities outside the project and determined their own level of contribution at their discretion.

Table 4.2. Details of project members. Age and company tenure are fixed based on the date of the group discussion.

Member	Age	Company Tenure	Notes
Author X	Late thirties	9	Team leader of the product development project
Technical Staff A	Early thirties	12	Responsible for the production process
Sales Staff B	Early thirties	9	Sales contact including predecessor products and market research; joined the company in the same year as X
Technical Staff C	Early twenties	4	Responsible for manufacturing predecessor products and managing processing operations

This section describes the project background under examination. The purpose of the project was to develop a new product that compensated for weaknesses in the predecessor product. The informal team that preceded the formal project began in 2020 with two members, including the author. The trigger was **X**'s creation of the technical concept and completion of the proof of principle, after which **X** and technical staff member **A** initiated preliminary studies to determine how the concept could be developed into a product. Shortly thereafter, **B**, the sales representative for the predecessor product, joined the effort. Based on the outcomes of these preliminary studies, the product development project was formally launched in 2021.

One year after the official start, however, the predecessor product was discontinued. As a result, **C**, the technical staff member previously responsible for that product, joined the project. The discontinuation created uncertainty about whether the development effort could continue, but the team persisted, managing internal coordination while resolving the challenges of product development. Two and a half years after the project's launch, the new product was completed and released in 2024. Although the formal project ended at that point, the same members continued working as a product team, engaging in product improvement, manufacturing process development, and business activities. At the time of the investigation, the team had already achieved commercialization and was working toward further improvement and sales expansion. This project thus represents a practical setting of technical product development.

The four members listed in Table 4.2 served as the core practitioners who played essential roles throughout the project. Although many stakeholders contributed, these four individuals were selected for multi-perspective internal observation because they directly engaged in field-level work and continually devised and adjusted methods on site.

Each member had expertise in their assigned role, but none, including the team leader, had formal management training. Although **X** had experience in technical development, this was his first time leading an internal project team. Likewise, neither **A**, **B**, nor **C** regarded themselves as having a wealth of practical project management knowledge and experience. Therefore, the project's success can be attributed to the steady accumulation of members' interaction around a shared goal.

As noted, the team conducted no formal reflection during the project period, and no discourse documenting their practical activities had been preserved. In this study, however, the author seeks to reconstruct those practices through multi-perspective internal observation conducted by the practitioners themselves. Given these characteristics, the project and its team members provide the author with a very suitable model for understanding the efficacy of multi-perspective internal observations.

4.4.2 First group discussion (D1)

4.4.2.1 Overview of the D1 investigation

D1 was conducted by members **A**, **B**, and **C** without **X**. The session was recorded, and all participants were informed that the content would later be reviewed with **Y** and used solely for this study. All members consented. The author presented three themes and coordinated the session so that it functioned as a semistructured group discussion.

† Reflect on the circumstances under which you joined the team and recall that period.

† Reconstruct the product development story from your own memory.

† Describe how you now view and feel about the completed product development.

Participants were also given the following requests:

‡ Speak based only on memory (do not consult records).

‡ Include the emotions you felt at the time.

‡ Actively discuss matters the leader would not know, such as behind-the-scenes episodes.

These semistructured guidelines were built to elicit people’s “another stories” and to promote further interaction. Indeed, this is central to multi-perspective internal observation, where, when practical topics arise, each member speaks from their own “another story” perspective (including memory, emotion, and behind-the-scenes experience). This tends to prompt similar levels of insight from others. These exchanges allow their “another stories” to interact dynamically.

No time limit was set, but the audio recording indicates that the discussion lasted 90 min. After transcription using generative language model (ChatGPT), the author reviewed the transcript and proceeded with analysis. Speaking ratios were **A**: 25%, **B**: 45%, and **C**: 30%, indicating no significant imbalance. **B**’s slightly higher proportion likely reflects the numerical difference between technical staff (**A** and **C**) and the single sales staff member (**B**).

The research results that follow comprise an overview of the discussion, individual member statements, and supplemental information relevant to those statements. During editing, care was taken to preserve the intent of each utterance. For incomplete expressions or self-corrections, wording was refined and sentences were reorganized within the same topical unit to improve readability, without altering their intended meaning.

Although the discussion began with the three initial themes, it naturally broadened. When a member introduced a memorable incident, it became a new topic that prompted others to recall related practices and circumstances.

To prepare the data for coding, each topic in D1 was assigned a label in the format, D1-x, where x denotes the topic number. These topics are summarized in Table 4.3. Each individual utterance within a labeled topic was assigned a sequential identifier using the D1-x-y format, where y reflects the order of the utterance. The same

approach was used in D2, with topics labeled D2-x and utterances labeled D2-x-y. This system ensured consistent referencing, traceability, and clarity in comparing narratives.

Table 4.3. Topics discussed in the first group discussion.

Numbering	Topic
D1-1	Project participation history
D1-2	Development Challenge i
D1-3	Development Challenge ii
D1-4	Development Challenge iii
D1-5	Sales Challenge
D1-6	Motivation
D1-7	Communication
D1-8	Future prospects

The topics identified in D1 are discussed next. Topic D1-1 proceeded as planned, with members commenting on how they joined the team. In reconstructing the product development story, three Development Challenges (i–iii) emerged spontaneously, although the author had recalled four. The remaining challenge was later discussed in D2-1. Notably, the reflections captured in D1-2 through D1-5 closely matched X’s understanding, indicating remarkably high reproducibility.

Topics D1-6, D1-7, and D1-8 related to the theme of “how members now see and feel about the completed development,” but their scope expanded to include analysis of team dynamics and prospects for future projects. These topics were therefore classified separately. Their emergence was entirely member driven, arising naturally through interaction during the discussion.

4.4.2.2 [D1-1] Project participation history

A had already been extremely busy, responsible for a wide range of tasks. He was single-handedly operating one manufacturing process for another product and had no available time. Under these circumstances, his direct supervisor in the manufacturing division,

together with the section managers of both the manufacturing and development divisions, instructed him to take on this project as well. He recalled the situation as follows:

A: Honestly, I had absolutely no time. I was told this would not be that hard, but from experience I knew things never go that smoothly, so I thought, well, I guess I have to brace myself for this. And to be honest, I even wondered, is something like this really going to sell? (D-1-1)

With no real ability to decline, **A** joined the effort and began informal technical verification with **X**. He did not comment on the timing of the transition from the informal team to the formal product development project.

B, meanwhile, was mainly responsible for sales in another product category but also handled part of the sales work for the predecessor product. At the time, sales for the predecessor product were managed by two people, including **B**. Although assigned to the current project, he initially regarded it as merely an extension of the predecessor product and took it only half seriously when it became a formal development project. Later, the other salesperson left, and without realizing it **B** had become the sole dedicated salesperson for both the predecessor product and the new project. He described the situation as follows:

B: Since discussions about ending the predecessor product started around the same time as this project, I remember thinking, how long is this even going to last? (D-1-2)

C had been responsible for manufacturing the predecessor product and was engaged in routine, repetitive tasks. When sales of the predecessor product ended, he suddenly had far more time available, and it was at that point that he was asked to join the project. Because he had found the manufacturing work for the predecessor product quite burdensome, he carried similar concerns into this new assignment.

C: I was pretty tense about it. I kept wondering how much work it would be, and whether it would turn into something I'd have to be constantly tied to. (D-1-3)

As these accounts show, **A**, **B**, and **C** joined the project under what Bakhtin (1975) would call authoritative motivation. In this discussion topic, because **C** joined the project later than the others, he asked **A** and **B** about their situations out of genuine interest. Their responses prompted both **A** and **B** to recount their own experiences from

that time. It also appeared that **A** and **B** were hearing each other's joining "another stories" for the first time, and they spoke while actively recalling the circumstances of that period.

4.4.2.3 [D1-2] Development challenge i

Development Challenge i was the first issue addressed by **X** and **A** in the informal team. At the time, **X** was producing samples using the initial method he had devised, but defects occurred frequently, making it impossible to transition the method directly into a manufacturing process. **X** and **A** therefore worked closely to explore alternative approaches and eventually overcame this challenge. **A** and **B** recalled the situation as follows:

***A:** I tried out an approach I had thought through on my own, and it worked surprisingly well. (...) Nobody in my division had any know-how, so it was tough to move forward alone. I reported my results to **X**, and he would give me feedback and advice, and we went back and forth like that. If I had been alone, I probably would have gotten stuck wondering what to do next. (D1-2-1)*

***B:** I think we were able to work really closely as a team, but once I stepped away from the team and went back to my own department, there was no one I could talk to about the development product. I just had to handle everything myself, and that was honestly pretty tough. (D1-2-2)*

The technology used in this development product was entirely different from that of the predecessor product. As a result, no one in **A**'s division had sufficient knowledge of it. For **A**, too, the technology was new, and he described overcoming Challenge i by internalizing the technology through close collaboration with **X**. **B** highlighted the psychological strain of having few colleagues to consult because the product under development was unfamiliar. Because the events **A** described occurred before **B** and **C** joined the project, both were hearing this information for the first time.

Challenge i did not disappear entirely, and further improvement was achieved after **C** joined the project. Until then, **A** had been solely responsible for all manufacturing process development for the new product, but **C**'s participation allowed the two to divide tasks based on their respective areas of expertise. By examining one of the processes in depth, **C** was able to fully resolve Challenge i. **A** and **C** described the process as follows:

A: *At the beginning, doing everything on my own was just too much. I said I wanted to reduce that burden, and C joined in, which made a huge difference. (D1-2-3)*

C: *When I joined, they were talking about wanting to improve Challenge i. I was told something like, “You can just help out a bit,” but I thought there was no way it would be fixed like that, so I worked on improving it. The current process is based on listening to the ideas from X and A. (D1-2-4)*

Before C joined, A alone handled all development and execution of the manufacturing process. Because C specialized in a part of the process related to Challenge i, he was able to resolve the issue completely by improving the process that A had not been able to refine thoroughly on his own.

4.4.2.4 [D1-3] Development challenge ii

The discussion then turned to Development Challenge ii. This defect had been present since the early stages, and even after C joined the project, no clear path to a solution had been identified. To achieve a fundamental improvement, X, A, and C examined the phenomenon together and tested various countermeasures. Although new issues emerged temporarily during this process, all problems were ultimately resolved. The discussion of Challenge ii unfolded as follows.

A: *When I tried to figure out how to eliminate Challenge ii, I noticed that the shape originating from the old process seemed to be the cause. (...) I saw that another product was finished very cleanly, so I thought, why not just imitate that process? And when we tried it, it worked surprisingly well. (D1-3-1)*

C: *The three of us were really excited working on it. We tried all kinds of things. There were other methods that looked like they might also solve Challenge ii, but in the end we settled on the one that was most efficient. (D1-3-2)*

B: *This is the first time I’m hearing any of this. It sounds like you were having a lot of fun. I honestly think its better when we can work with that kind of freedom. (D1-3-3)*

C: *There was that time when strange noise appeared, right? X told me it might be caused by residue from the tool we were using. So I went and improved all of*

the tools, and that's what we use now. And when you change one thing, something else pops up somewhere. (D1-3-4)

The process by which **X**, **A**, and **C** worked through the challenge was vividly reconstructed through the exchange between **A** and **C**. Although **B** was hearing this information for the first time, he actively joined the conversation, prompting further elaboration from the others.

4.4.2.5 [D1-4] Development challenge iii

The discussion then moved to Development Challenge iii. This issue emerged in the final stage of product commercialization and was critical because it had the potential to affect the final product price. As a result, addressing it became an all-out effort that involved parties outside the company. Because **A** and **C** had limited experience coordinating with external organizations, **X** assumed the lead throughout the process. The members recalled the situation as follows.

B: *Wasn't Challenge iii the toughest one? You were looking for all kinds of vendors, right? (D1-4-1)*

A: *At first, I looked for options myself, involving people from other departments to see whether we could find something suitable, but because of the product specifications, there really weren't many options that met the requirements. (D1-4-2)*

C: *We had meetings with the vendors that were listed for us to consider, and I was allowed to sit in on those meetings as well. (D1-4-3)*

A: *What **X** was really particular about was the impact on the final product price. Honestly, I thought it would be fine if we just used something off the shelf that the vendor proposed. Even when I talked to people around me, they said things like, "That's not something our department should be doing." (...) But I came to realize that **X**'s insistence on that point was important. I learned that we really do have to consider those detailed cost implications. (D1-4-4)*

C: *I learned a lot from that too. Like how you put out feelers to multiple places first, keep a backup plan running at the same time, and only make the final decision at the very end. Before hearing this, I thought there would be one option*

decided from the start and that we should just focus on that one. I had never been involved in anything like that before. (D1-4-5)

As **A** noted, there was originally a division responsible for external coordination, and company culture suggested that such matters should normally be handled by that group. However, because this was a new product with unique requirements, the existing division could not fully address the issue. The project team therefore assumed direct responsibility for negotiations. In this context, **A** and **C** reflected on how **X** worked intensively to minimize the impact on the final product price. Both stated that they learned from **X**'s approach and way of thinking toward Challenge iii.

4.4.2.6 [D1-5] Sales challenge

Alongside technical challenges, sales-related issues emerged as recurring topics. Because the new product differed significantly from the company's existing array, it required a new business strategy. However, existing sales practices were deeply rooted, and not all newly proposed strategies could be implemented. For **B**, this meant bold actions were difficult to pursue.

For example, most existing products followed a business-to-business model, with large transaction values that allowed for significant upfront investment before returns were realized. In contrast, the new product had a business-to-customer character, where the end user was the direct customer. As a result, opinions between this project and other departments did not always align regarding business strategy, and spending on sales activities was closely scrutinized.

Compounding this, the termination of the predecessor product created an even stronger internal headwind. Despite these pressures, **B** and **X** worked closely together, taking every feasible step to advance the business strategy and ultimately bring the product to market. Many of the specific sales efforts—such as trade show exhibitions and internal and external negotiations—were unfamiliar with **A** and **C** and are recounted here in detail.

A: *X actually went out and met customers directly to gather feedback, right?*
(D1-5-1)

B: *Even though I am in sales, it is not easy for me to go all the way to the end users, so it was hard to grasp the real market situation. But X would give me technical insights from the user side, so I could build a solid understanding. That*

really helped me explain things to people in my department in my own words. (D1-5-2)

C: *How is it actually going?* (D1-5-3)

B: *The fact that we have a partner helping us develop the market already puts us in a good place. Right now, it is not about increasing sales. The priority is building the environment where sales can grow. We are steadily accumulating progress in the market development phase, so it is not something that can be evaluated by sales numbers alone. As for how the overall market will react, we need a bit more time before we know.* (D1-5-4)

A: *Hearing that we are in that kind of phase helps me understand that what we are doing on our side also has meaning.* (D1-5-5)

C: *After commercialization, since it is not selling in large numbers yet, without hearing this kind of explanation I honestly do not know what to think.* (D1-5-6)

B: *We have been able to build a partnership structure that we could not achieve with the predecessor product. That alone is a major accomplishment.* (D1-5-7)

C: *That is a big step forward.* (D1-5-8)

Because **A** and **C** did not have direct contact with customers, this portion of the discussion became a rare opportunity for them to understand the business conditions surrounding the product. Given that both the product and its business strategy differed fundamentally from anything the company had previously handled, **B** explained that sales results would not appear immediately, though steady groundwork was beginning to generate progress beneath the surface. Although **A** and **C** had felt uneasy about the postlaunch situation, they expressed understanding once they learned the project was still in a phase where visible sales outcomes could not yet be expected.

4.4.2.7 [D1-6] Motivation

Although the discussion topic was framed as “how members now see and feel about the project after its completion,” the conversation expanded to include spontaneous reflections on the characteristics and nature of the project and the team itself. Such comments arose throughout the session, and related statements are summarized here. Because they did not follow a systematic sequence, they are presented below in two

categories: motivation and team communication. The author begins with statements related to motivation.

B: *How do I feel about being in this team now... I'm actually having a pretty good time.* (D1-6-1)

A: *For me, I'm genuinely glad I took on this project. I really leveled up. Nobody had done this before, but there was no real reason not to try. And because the product was relatively simple, the technical challenges were things I could handle myself and make work. In a way, the difficulty level was a really good match for me.* (D1-6-2)

B: *It made me feel like, oh, we're actually allowed to try things like this.* (D1-6-3)

C: *Being able to have that kind of mindset was huge.* (D1-6-4)

(...)

B: *Now that the product is out, do you personally feel like things are finished?* (D1-6-5)

C: *No, honestly I don't feel done at all. There are still way too many things left unresolved.* (D1-6-6)

A: *If anything, it feels like things are just getting started. There's still a ton left to do.* (D1-6-7)

B: *Commercialization is not the goal. The real question is what we do to make it actually sell. We also need to continue improving it. And the fact that the technical members feel the same way is incredibly encouraging from a sales perspective. Commercialization is just one step. I feel like from the beginning it was never treated as the final goal, and that was really good.* (D1-6-8)

C: *Back in the day, I used to think once the documentation was registered, the job was basically done.* (D1-6-9)

Because the project had ended, each member looked back positively on having participated. They noted that they learned to adopt a constructive, challenge-oriented mindset, which contributed to their own growth. Even at this stage, none of them felt the

project was *finished*, and all expressed a strong willingness to continue. However, as described, their initial motivation was not particularly high. These comments indicate that members' motivation changed substantially over the course of the project.

4.4.2.8 [D1-7] Communication

This section presents statements related to communication. Here, the core topic is *progress management*. Throughout the project—and still today—the members meet roughly once a week to share updates and ongoing tasks. Because the team is small and each member inevitably works independently, these meetings serve as an open communication venue where members freely share feedback for collective review.

Early in the project, their interactions occurred one-on-one between **X** and each member. Over time, **A**, **C**, and **X** began meeting together, and eventually **B** joined. This led to the establishment of a regular progress meeting that became the project's central gathering. Although the technical and sales issues were generally shared, time constraints limited discussion of the concrete actions underlying each topic. **A** and **C** focused on technical work, while **B** handled sales, and although **X** communicated closely with both groups, many operational details remained unknown between the technical side (**A**, **C**, **X**) and the sales side (**B**, **X**).

Members expressed the following thoughts about the progress meetings:

B: *I actually think the way we do progress management works really well. (D1-7-1)*

A: *Meeting once a week, keeping things simple... the visualization of tasks is great. The "To Do list" helps a lot. (D1-7-2)*

C: *Breaking things down toward the larger goal makes everything easier to understand. (D1-7-3)*

A: *What I really think **X** does well is balancing things. Like showing that we've made progress, but also pointing out where we still need to dig deeper. (D1-7-4)*

B: *And proper feedback always comes back. (D1-7-5)*

C: *It becomes so clear what needs to be improved. Once you know what to fix, all you have to do is push toward it. Anytime we did something, **X** responded instantly with an evaluation. (D1-7-6)*

A: *Because X devoted himself to the development product and gave us quick feedback, I think that was a major factor in getting from development to commercialization so quickly. (D1-7-7)*

(...)

C: *Even a small amount of shared knowledge across everyone really makes things run smoother. Maybe it is only possible because we are such a small team. (D1-7-8)*

B: *I feel like we really understand each other, and someone is always keeping an eye on the whole picture. Meeting regularly and sharing not only updates but the atmosphere and context of the moment is really valuable. (D1-7-9)*

A: *Exactly. It genuinely feels like a team. But if we tried to use this same style for another project, I do not think it would work the same way. (D1-7-10)*

(...)

A: *X sends samples to all sorts of places and always shares the reactions with us. Thanks to that, I gradually started to feel that we were making something good. Without that feedback, I probably would have stayed doubtful the whole time. (D1-7-11)*

B: *Getting so many kinds of feedback made me feel that what we are doing really has meaning. (D1-7-12)*

A: *It made me feel that the time I was putting in actually mattered. Not knowing what your work means is the toughest thing. (D1-7-13)*

B: *At first I was pretty skeptical, but hearing those stories helped me empathize and really commit. That seems important for the team to function. And interestingly, everyone has naturally fallen into that mindset. (D1-7-14)*

Through these exchanges, members reflected on how the project was managed and identified several success factors, including task segmentation, clear goal setting, and rapid feedback. Their statements consistently showed trust in X's management approach during the weekly meetings. Members shared not only status updates but also the atmosphere surrounding their work, and they perceived themselves as a tightly connected

team with strong mutual understanding. They also noted that X's steady feedback on external reactions and experiment results played a major role in sustaining their motivation.

In addition to progress meetings, members discussed broader topics such as how the project concept was formed and how the project should be run:

C: *When X first explained the development product to me, he talked about the entire future outlook. The commercialization flow, the customer base, the business development. I remember thinking, he has thought this through so thoroughly. (D1-7-15)*

B: *Because we made use of a lot of information from the predecessor product, X had already structured a solid business strategy and concept. I think we all partly resonated with it, like, yes, this makes sense. On the sales side, we supplemented key points and helped with ideation. We had a concept for the predecessor too, but the team back then struggled to steer things properly. (D1-7-16)*

A: *Having the predecessor as a reference was probably a big factor. (D1-7-17)*

B: *Exactly. We are making use of the feedback from that time. We are not doing anything wasteful. It feels like everything is aligned toward moving in the right direction. (D1-7-18)*

(...)

A: *There are cases where development finishes and then someone says, okay, now hand it over, but we cannot mass produce it. Just because something worked once does not mean it is good enough. (D1-7-19)*

B: *In that sense, X really understands that. If we cannot actually manufacture the product properly, it means nothing. And it is not like we are saying everything should be completed before handing it off. We just need an overlap between development and production readiness. (D1-7-20)*

A: *That is why I really appreciated that X oversaw everything consistently from start to finish. (D1-7-21)*

C: *Even after commercialization, when we are mainly focused on production, he still listens to our opinions. (D1-7-22)*

Members seemed to appreciate how **X** incorporated lessons from failed product into the new concept. He also did well in securing consensus among stakeholders and took responsibility for the product after commercialization. They recognized that the combination of these favorable elements helped sustain motivation and foster healthy communication within the team. Each member independently reflected that such conditions were essential to the project's successful completion.

4.4.2.9 [D1-8] Future prospects

Finally, a topic not originally included in the agenda emerged during the discussion. Now that the initial product has been commercialized, a derivative product is being prototyped within the team, led primarily by **C**. Members voluntarily shared their thoughts on future prospects and expressed their impressions as follows.

C: *So what about the new product? I mean, is it supposed to be positioned as a kind of high-performance version? (D1-8-1)*

B: *The new product will benefit from the foundation we have already built with the current one. (D1-8-2)*

A: *If we keep moving at this pace, I think it will succeed. (D1-8-3)*

C: *Anyway, I'll just try making it first. From there, I'll keep pushing it forward bit by bit. The technology behind this product seems like it could be used in lots of places. (D1-8-4)*

B: *Yeah, I think it can be used for other applications too. (D1-8-5)*

A: *Honestly, I also feel like it could work in various ways. Since we've developed this technology, it would be great if we could apply it to something else. (D1-8-6)*

B: *For now, we should focus on the current product, but I really hope we can take on those new directions in the future. (D1-8-7)*

In this exchange, members reaffirmed their intended development goals and expressed positive perspectives on achieving them. They also expressed the desire to leverage extant technological capabilities and extend them to other applications.

4.4.3 Second group discussion (D2)

4.4.3.1 Overview of the D2 investigation

D2 was conducted with all members present, including **X**. As in D1, the discussion was recorded, and all members consented to its use solely for this study, with the understanding that the recording would later be reviewed with **Y**. In D2, several topics were raised based on the findings from D1, and members were encouraged to speak freely about each. Because **X** had already discussed the D1 results with **Y**, D2 also behaved as a venue for reporting the discussions. The D2 topics are summarized in Table 4.4.

Table 4.4. Topics discussed in the second group discussion.

Numbering	Topic
D2-1	Review of D1 Development Challenge iv
D2-2	Team cohesion
D2-3	Changes in motivation

According to the audio recording, the session lasted 115 min, with **B** departing at around the 105-min mark. As in D1, members' statements were transcribed and used for analysis. Notably, the share of statements was **X**: 57%, **A**: 13%, **B**: 12%, and **C**: 18%, indicating that **X** accounted for more than half of the narrative. This distribution reflects **X**'s role as project leader and discussion facilitator, as well as the fact that approximately 40% of **X**'s statements consisted of reporting on D1. A small portion of the transcript (about 5%) consisted of digressions and anecdotal remarks, which were included in the word count. In contrast, the proportions of statements among the nonleader members were roughly comparable.

4.4.3.2 [D2-1] Reviews of D1 and development challenge iv

D2 began with a review of D1. **X** first highlighted the high reproducibility of the reconstructed development story and expressed surprise that members had reflected on the team itself, going beyond the originally planned topics. **X** then thanked the members

for their cooperation in D1 and for the motivation they expressed toward the ongoing project. The “another stories” extracted from D1 (Section 4.5.1.1) and the discussion between **X** and **Y** regarding D1 (corresponding to Sections 4.5.2 and 4.5.3) were also shared. Through this review, members confirmed that there were no discrepancies in their understanding of the D1 results.

When **X** asked about Development Challenge iv, which had not emerged in D1, the members explained that they had simply forgotten it. The group then collectively revisited that challenge.

X: *I thought the development story we reconstructed was pretty comprehensive, but did we forget to talk about Development Challenge iv?* (D2-1-1)

A and C: *Yeah, we thought the same thing.* (D2-1-2)

X: *Is there anything you remember about Development Challenge iv?* (D2-1-3)

A: *At first we used the equipment that was readily available, but defects kept showing up. So we went on business trips to check out different options, hoping we could find some way to fix it.* (D2-1-4)

B: *Going on business trips as a customer is actually pretty fun. Sales trips have a completely different kind of enjoyment.* (D2-1-5)

(...)

X: *Unfortunately, everything we tested on that trip failed. But I remembered that, years ago, we tried a completely different machine and it had seemed promising. When we tried that one again, the defects disappeared.* (D2-1-6)

C: *That was lucky. If we hadn't had that option, I think we would've given up.* (D2-1-7)

In addressing Development Challenge iv, **X**, **A**, and **C** took several business trips to evaluate new equipment, and episodes from those experiences were shared in the discussion. Because **A** and **C** rarely traveled outside the company due to the nature of their technical roles, these trips provided valuable opportunities to gather external information and observe equipment demonstrations. In contrast, **B** frequently traveled with **X** for external coordination. The discussion therefore broadened into a wider reflection on external interactions. Ultimately, it became clear that Challenge iv was

resolved because **X** recalled past experimental experience that fortuitously led to a viable solution.

4.4.3.3 [D2-2] Team cohesion

In D1-7, **A** had noted that the team felt a strong sense of unity. To explore how that unity developed, members were asked to reflect again on the process.

X: *I'd like to know what led you to feel that a sense of unity had emerged. For example, was it the way we communicated? If anything comes to mind, let me know. (D2-2-1)*

C: *I think it comes down to having a shared sense of purpose. You feel the goal, you move toward it, and the results come back to you. It builds up over time. (D2-2-2)*

X: *I see. But goal setting itself is something most teams probably do. (D2-2-3)*

B: *Maybe so, but even if a goal is set, whether people actually empathize with it is... I think that sense of ownership probably comes from empathy or something similar. (D2-2-4)*

A: *I feel like the number of people might matter. In my department, nine of us meet weekly for process discussions, and I really feel a sense of unity there. (D2-2-5)*

C: *But that is because that group gets along well. Even with a small group, you don't always have that sense of unity. (D2-2-6)*

X: *So it's not about team size? (D2-2-7)*

A: *Maybe it is the so-called "small talk"? Everyone says what they want, then once that's done, we share updates and give each other advice. (D2-2-8)*

B: *Yeah, I used to have a different project where we had a 30-minute check-in every morning, and there was a lot of casual talk. It felt very cohesive. That environment is similar to this one. (D2-2-9)*

(...)

A: *If X were not someone who talks a lot, we probably wouldn't even have these meetings in the first place. (D2-2-10)*

X: *Communicating with emotion matters. It can be about private things or about work. (D2-2-11)*

A: *Whether we can joke around makes a difference. (D2-2-12)*

C: *In the end it all comes back to psychological comfort. More people mean more eyes on you, which can make it harder to speak freely. (D2-2-13)*

B: *Having people, you feel safe talking to really matters. (D2-2-14)*

Each member spontaneously shared personal views on what creates a sense of team unity. Ideas included a shared purpose, empathy, team size, and everyday casual conversation. As the discussion progressed, the group converged on the view that a condition resembling *psychological safety* was essential. Because members felt safe speaking openly, a sense of unity developed naturally.

This led to comments about members' first impressions of **X**.

A: *At first, I thought X wasn't interested in anything outside of work. I felt like saying the wrong thing might be risky, so in the beginning, I reported things very plainly. (D2-2-15)*

C: *My first interaction with X was in this room. I remember X speaking passionately, but even so, I still had this preconception that I shouldn't say anything careless. (D2-2-16)*

X: *Really? You felt that way? (D2-2-17)*

C: *Yeah. I didn't know how what I said would be taken. (D2-2-18)*

X: *B never felt that way. (D2-2-19)*

B: *Well, we joined the company the same year. (D2-2-20)*

Both **A** and **C** initially felt they should not speak carelessly to **X**. Although **X** had been mindful of his interactions from the beginning, psychological safety was not present at the outset. **B**, being **X**'s cohort, did not experience this distance. It therefore

appears that a meaningful amount of time was required before the team environment evolved into one where everyone felt comfortable speaking openly.

4.4.3.4 [D2-3] Changes in motivation

Although the members now view the team positively (D1-6), their motivation at the time they joined was not high (D1-1). To explore how their motivation changed, members reflected on contributing factors and key turning points.

X: *None of you were very positive about joining the project at first. Was there a turning point? Or was it more like you suddenly realized your mindset had changed?* (D2-3-1)

A: *At the beginning, I had no starting point, and everything felt like a hassle. But this project had a lot of freedom. I could try anything, starting with something simple. As I made things and went through trial and error, the product gradually got better. What I made kept getting refined. That was really fun.* (D2-3-2)

C: *That is the essence of making things.* (D2-3-3)

X: *So as you made progress, it started to feel fun? And through that work, you shifted from “being made to do it” to “doing it yourself”?* (D2-3-4)

A: *Exactly. Seeing results improve was really rewarding. Until now, most of my work involved following instructions. I hardly ever reflected my own ideas in anything.* (D2-3-5)

C: *I think I am similar to A. It is not that there was a specific turning point. It was through the work itself, seeing things gradually improve, that my mindset changed.* (D2-3-6)

X: *You also mentioned that feedback was quick. You could immediately see the results, so you could run many short trial-and-error cycles. That kept motivation moving.* (D2-3-7)

A: *Other product improvements can take months. Here, the speed was really helpful.* (D2-3-8)

C: *Whenever I do something, I want to know the results right away. Even if it is bad, I am satisfied because then I know I just need to revise it. How about you, B?* (D2-3-9)

B: *I think I am close to A. When I had parts I could think through myself, it really got my adrenaline going. Even small things, like designing a flyer, thinking through it myself and talking with the team about it. That was great. I am not fully confident the company recognizes those efforts as results, but now that we finally have an environment where the product can sell, and I can have intense conversations with customers, my motivation has gone up.* (D2-3-10)

(...)

X: *At the beginning, everyone was pretty skeptical. In D1-8, you said the new product would likely succeed. Hearing you say that now makes me really glad.* (D2-3-11)

B: *What I remember most is realizing that “it is okay to try things.” That really broke a lot of fixed assumptions for me.* (D2-3-12)

C: *I felt that it was okay to do what I wanted to do.* (D2-3-13)

X: *The fact that you shifted to thinking that way—and are now openly saying so—is a big change, even though I had sensed it already.* (D2-3-14)

The members reflected on what enabled their shift from having little motivation to feeling motivated. Their views were largely consistent: because the project allowed a high degree of freedom and encouraged trial and error based on their own ideas, they gradually came to enjoy the work. Their willingness to express strong motivation toward the new product in D1-8 stemmed from their recognition that this was a team in which they could experiment and progress autonomously.

4.5 Discussion

4.5.1 Extraction and integration of “another stories”

4.5.1.1 Extraction of “another stories”

From the perspective of extracting “another stories,” this section revisits D1. Although the initial aim was to uncover “another stories” unknown to leader X, the discussion

revealed that **A**, **B**, and **C** also held many “another stories” unknown to one another. By sharing and aligning these individual “another stories,” members gained a deeper understanding of each other and an opportunity to rediscover the meaning and significance of their own roles. The extraction of “another stories” is reviewed for each topic below.

In D1-1, each member described the circumstances that led them to join the project and their initially low motivation. While **X** had sensed this lack of motivation, **X** did not know the detailed backgrounds. As seen in D1-1-1 through D1-1-3, **A**, **B**, and **C** expressed skepticism about participating in the project. The circumstances of **A** and **B** at the time of joining were unknowable to **C**, the last to join, unless **C** asked directly—and indeed **C** elicited these narratives. Likewise, **A** and **B** did not know each other’s joining stories and heard them for the first time in D1. Thus, these accounts functioned as “another stories” not only for **X** but for all members.

D1-2, which concerns Development Challenge i, had been handled jointly by **A** and **X**, making the content completely new to **B** and **C**. Moreover, **X** had not fully understood how **A** felt during the period when **A** worked alone on the technical task, as expressed in D1-2-3. Early in the project, **A** and **X** worked only at an experimental level, without strict process management. As the project progressed, however, each process became more specialized, and **A**’s workload increased. **X**’s perception remained anchored in the early stage, preventing a full appreciation of **A**’s burden. This constituted, quite literally, *unseen labor*.

In D1-3 (Development Challenge ii), **X**, **A**, and **C** experimented together, and **X** was familiar with nearly all aspects of the process. For **B**, however, the content was entirely new. **A** and **C** recreated the atmosphere of that period through their exchanges, conveying their experiences with a strong sense of presence. **B** joined in, prompting further storytelling from **A** and **C**.

In D1-4 (Development Challenge iii), although **X** had taken the lead, the reflections revealed how **A** and **C** had felt at the time. As stated in D1-4-4, **A** had experienced a sense of resignation, but seeing **X** persist without compromise taught **A** the importance of cost. **C** likewise noted in D1-4-5 that **C** learned the value of running multiple plans in parallel to pursue the best outcome. **X** had worried that such insistence might have frustrated others and had not imagined that **A** and **C** viewed these efforts positively.

D1-5 (Sales Challenge) was led by **B** and **X**, making its details normally inaccessible to **A** and **C**. **B** explained how he worked within existing business constraints while steadily building sales activities. Although **X** already understood this through daily

communication with **B**, comments such as D1-5-5 and D1-5-6 showed that the discussion helped reduce **A** and **C**'s anxieties. It also became clear that the product-development narratives reconstructed in D1-2 through D1-5 were highly reproducible and nearly identical to **X**'s recollection. **X** had not expected the members' understanding to be so high and learned this only through D1. Because the members shared this depth of understanding, their narratives carried weight even for those who did not know the details, strengthening mutual interaction among "another stories."

D1-6 and D1-7 contained members' spontaneous reflections on the project and team—content **X** had not anticipated. Their observations on team structure and management were points **X** rarely heard. The comments were largely positive, including satisfaction with having participated in the project (D1-6-1 through D1-6-4) and appreciation for the progress meetings (D1-7-1 through D1-7-14). While some differences toward **X** cannot be ruled out, hearing concrete views about aspects **X** had been unsure of was valuable.

D1-8 also emerged spontaneously and consisted of future-oriented talk about the newly developing product. Members expressed positive impressions and showed eagerness to pursue further technical advancement. **X** had been concerned that members might have lost motivation once the main project concluded, particularly because new developments demanded additional effort. Seeing their enthusiasm renewed **X**'s motivation. In short, D1 contained "another stories" not only for **X** but also for **A**, **B**, and **C**.

For the purpose of extracting "another stories," D1 was conducted without **X**. Members served as their own facilitators, and their speaking time was nearly evenly distributed. This contrasted with D2, where **X** participated. Removing **X** made it easier for members to discuss topics that **X** knew but that they had never shared with each other. Thus, the "leader absent" condition effectively supported the extraction of "another stories." The semistructured format provided thematic guidance while allowing discussion to expand beyond preset topics. The group discussion enabled real-time interaction, alignment of understanding, and the emergence of new "another stories" through mutual influence. A written questionnaire would not have enabled such interaction. Therefore, the semistructured, leader-absent group discussion implemented in D1 can be considered an effective method for extracting "another stories" from team members.

4.5.1.2 Integration of “another stories”

D2 functioned as the process through which the “another stories” extracted in D1 were integrated by all members. In D2, the leader **X** also participated, reflecting on the D1 results alongside **X**’s own “another stories,” while the team collectively discussed newly emerging questions and issues.

The session began with an overview of the “another stories” extracted in D1, including **X**’s interpretation and the discussion between **X** and **Y**. This step integrated those “another stories” with **X**’s own, creating a shared understanding of how members’ perspectives had changed since their initial involvement and of the overall development trajectory. Missing elements, such as those addressed in D2-1, were supplemented through collective reflection. For **X**, **A**, and **C**, this meant revisiting content they already knew, whereas for **B** it constituted an “another story.” At the same time, **B**’s remarks (e.g., D2-1-5) introduced **B**’s perspective into the topic, generating reciprocal interaction among “another stories” similar to that observed in D1.

As seen in D2-2 and D2-3, members then engaged in deeper discussions on topics of particular significance, strengthening their understanding of the team. In D2-2, they examined the origins of their sense of unity. Statements such as D2-2-1 through D2-2-9 show how members exchanged views and responded actively to one another, ultimately arriving at a collectively satisfying conclusion, as reflected in D2-2-10 through D2-2-14. In D2-3, they revisited how their motivation had changed over time. Although each member described their motivation as having risen gradually (D2-3-2, D2-3-6, D2-3-10), articulating these experiences in their own words deepened individual understanding and established shared recognition of the process across the team.

In sum, D2, conducted with all members present, enabled the integration of the “another stories” extracted in D1 and supported consensus formation regarding the team’s practical activities. The two group discussions demonstrated the two essential components of multi-perspective internal observation. D1 showed that team members observe practical activity from their own perspectives and that each member’s “another stories” can be fully elicited. Together, D1 and D2 achieved the integration of these stories through dialogic alignment and the establishment of shared conclusions about practical activity as research findings. Through this study, the concrete procedures used in D1 and D2 were shown to function as an effective approach for realizing multi-perspective internal observation.

4.5.2 Practical knowledge identified from the product development project

4.5.2.1 Characteristics of the project and analytical perspectives

Throughout the progression of this project, from initiation to completion, members' conditions, interpersonal relationships, and the project structure changed markedly.² Early communication consisted mainly of one-to-one exchanges (D1-2-1, D1-2-2), and no framework existed for viewing the project collectively. As the project advanced, however, weekly progress meetings were introduced (D1-7), evolving into a structure through which information, issues, and emotions were shared across the entire team.

A comparable transformation occurred in members' motivational states. In the early phase (D1-1-1 through D1-1-3), none of the members expressed strong enthusiasm; skepticism and uncertainty were common. By contrast, at the time of project completion, members described themselves as working proactively with high motivation (D1-6-1 and D1-6-2). This reflects a substantial shift in motivational states over the course of the project.

As described in Section 4.1, although each member entered the project with a different technical background, the team did not share specialized expertise in project management or in methods related to internal observation or practice-based research. The project therefore began in a “non-ideal state.” Despite the absence of explicit theories or models, members achieved the positive transformations noted above through locally emergent processes grounded in day-to-day practice. This is a defining characteristic of the project.

This section examines how such state changes occurred through specific practical processes, addressing the central analytic question of this study. In doing so, it articulates the practical knowledge that proved effective in the actual context of the project, thereby fulfilling the study's first objective.

4.5.2.2 From individual exchanges to a multilayered team communication system

In the early stages of the project, communication among members took the form of one-to-one exchanges. When only **X** and **A** were working on the task, they “proceeded by communicating closely” (D1-2-1), and “the only person **A** could consult with was **X**”

² The concept also changed from its initial stage. Although **X** constructed the initial business strategy using information from the predecessor product (D1-1-1), the members did not fully agree with its direction at first (D1-1-2). However, roughly one year after the project began—when **C** joined—it was received as a concept that “made sense” (D1-7-15). This indicates that the concept evolved during the project into one that could be shared and accepted by all involved.

(D1-2-2). The community for technical discussion was therefore quite limited. This began to change when **C** joined. As seen in D1-2-4, D1-3-2, D1-3-4, and D2-1-4, collaborative technical work among **A**, **C**, and **X** emerged naturally, forming a localized community of practice. On the sales side, **B** and **X** jointly considered concrete measures (e.g., D1-5-2), establishing a separate communication channel for sales practice. Together, these episodes show that “highly immediate one-to-one communication,” enabling consultation and confirmation as needed, arose organically early in the project. Because these were informal field-level exchanges, they provided fertile ground for what Bakhtin (1975) calls “internally persuasive discourse,” which mediates mutual understanding.

As these individual exchanges accumulated, members’ practices became increasingly concrete and complex, creating a need for a shared, bird’s-eye view of “who is working on what, and where each person is struggling.” In response, regular progress meetings emerged spontaneously. As described in D1-7-8 and D1-7-9, these meetings worked effectively for cross-member information sharing and gaining an overview of the whole project. In addition, D1-7-11 through D1-7-14 show how the results of technical trials and external reactions were shared, enabling members to situate their own practices within a broader context. Moreover, as suggested by D1-7-10, the meetings also functioned as a psychologically safe space that fostered a “sense of team” and allowed members to share “how they felt at the time” (D2-2-4, D2-2-13, D2-2-14).

The nature of these meetings differed markedly from each member’s everyday practice, including interactions with external stakeholders. They correspond to the “extraordinary space” described by Bakhtin (1965), where members could be exposed to topics outside their own jurisdictions and influenced by others’ perspectives. For example, **A**’s technical practice normally lay outside **B**’s sales-related concerns. Yet when **A** shared his practice, **B** could respond from a different angle—as a kind of jester—and initiate new forms of interaction. The progress meetings thus created a space where members could step away from daily constraints, interact on an equal footing, and experience a carnivalesque sense of the project world.

A key factor enabling the continuity of these meetings was **X**’s consistent stance of actively engaging in conversation, as indicated in D2-2-10. Although **A** and **C** initially felt hesitant to speak freely with **X** (D2-2-15, D2-2-16), their impressions changed through daily one-to-one exchanges, and they came to view **X** as “someone they could consult.” This supports the observation that the progress meetings did not begin as a formal mechanism but emerged gradually as individual communication matured. The same pattern applied to the other members. As each became more willing to accept

interactions from new angles—and experienced that these interactions helped them overcome practical impasses—the meetings took shape as a team-level forum.

In summary, the project developed a multilayered communication structure consisting of immediate, flexible one-to-one exchanges at the micro-level and progress meetings that provided a macro-level overview. Such a structure is particularly important in exploratory product-development projects. It enabled the team to continue generating rapid trials while maintaining team-level coherence.

4.5.2.3 Acceleration of trial cycles through task decomposition and rapid feedback

A notable feature of practice in this project is that multiple members described feeling able to “proceed at their own discretion” (for example, D1-2-1, D1-2-4, D1-3-1, D1-4-2, D1-5-2). In D2 as well, this discretionary quality was explicitly identified as a major source of motivation (D2-3). However, such discretion did not arise because **X** simply adopted a hands-off approach. The group discussion data contain several indications of how discretion gradually became established.

For instance, **A**’s descriptions of work being “within my own capability” and of difficulty levels that “matched my level” (D1-6-2), together with comments that tasks had been “broken down” (D1-7-3) and set at a balanced level (D1-7-4), suggest that task decomposition and fine-grained segmentation were progressing in the field through daily one-to-one exchanges (as discussed in 5.2.2). In the early phase, members were unsure “where to start” (D2-3-11, D2-3-12), and **X** therefore needed to indicate “what to do next.” Yet these indications were not unilateral assignments. Rather, through ongoing conversations of the type “this part is difficult” and “then let us first try only this portion,” tasks were naturally decomposed through responsive dialogue. In this way, each task was reshaped into a “hurdle that could be cleared,” enabling members to set appropriate tasks for themselves and to feel “competent” as they overcame them. This aligns with Deci’s (1975) conceptualization of competence and self-determination.

By the middle stage of the project, as seen in D2-3-5, members themselves had begun proposing ideas, with **X** responding to their initiatives. Task decomposition and discretion were thus not predesigned elements of a managerial scheme. They emerged as the natural outcome of **X**’s responses to members’ consultations and the evolving division of roles within the team. This emergent quality was also reflected in the progress meetings, where the team collectively shared, decomposed, and reframed tasks, and considered next actions from multiple perspectives.

Another factor accelerating task decomposition was that **X** was responsible for evaluating the performance of the new product. Because **X** was assigned exclusively to this project, it became possible to “return evaluation results as quickly as possible” for each member’s trials. **A**, **B**, and **C** described the feedback as arriving “almost instantly” (D1-7-6, D1-7-7). Such rapid feedback promoted successive trials and the resetting of tasks, thereby increasing the pace of the trial cycle. Quick feedback not only raised the number of trials but also enhanced motivation. Several comments (D2-3-2, D2-3-6) indicate that the accelerating accumulation of trials became a source of stimulation, deepening members’ immersion in their practice. This directly corresponds to the emergence of a flow state (Csikszentmihalyi, 1990) and served as a core source of intrinsic motivation.

Through these mutually reinforcing processes of task decomposition and rapid feedback, members could maintain a continuous sense that “the next step is visible.” This structure did not result from a preplanned management intervention but emerged from practice. Over time, this accumulation elicited members’ autonomy and generated a dynamic of rising motivation. Similarly, the process functioned effectively only because it rested on the multilayered communication foundation described above. The coexistence of immediate, flexible one-to-one consultation (micro-level dialogue) and progress meetings that provided an overall project view (macro-level dialogue) allowed task decomposition and feedback to circulate smoothly and enabled members to adjust their actions to situational demands.

4.5.2.4 Managerial significance of the practical knowledge identified through multi-perspective internal observation

As described, this study extracted multiple forms of practical knowledge through multi-perspective internal observation. Each instance of practical knowledge was formed in response to specific events and interactions that occurred during the project and was continually adjusted to fit the situation. Thus, these findings are not merely a list of effective behaviors. They are context-sensitive forms of knowing that emerged through trial and error in practice.

It is important to note that these forms of practical knowledge arose and functioned only because certain team conditions were in place. Trust among members, mutual responsiveness, role awareness, and shared purpose had reached a sufficient level, and under these conditions the practical knowledge identified here could exert its intended effects and support the advancement of development activities.

Conversely, simply applying this practical knowledge in other settings where such conditions have not yet been established does not guarantee similar results. For example, the regular progress meetings that proved effective in this study might have generated burden or resistance had they been introduced at the very beginning of the project. In such a case, they may not have led to cooperative action based on members' understanding and agreement. Applying practical knowledge without regard to its generative conditions can even be counterproductive.

From this perspective, when attempting to apply practical knowledge to other projects or organizations, it is essential to examine background factors such as the maturity of the team, the quality of interpersonal relationships, and the nature of the tasks. Practical knowledge is inherently tied to the context in which it is generated. In this sense, it should not be viewed as something that can simply be *transplanted* but rather as something that must be *regenerated* through interaction with a specific local setting.

This study serves as a case that unpacks the processes through which practical knowledge functions and clarifies the origins of that knowledge. Much of the practical knowledge identified here aligns, to varying degrees, with existing frameworks and concepts discussed in prior research. However, the value of this study does not lie in whether the content of the practical knowledge itself is novel. Rather, it lies in having illuminated how that knowledge actually arose in a real-world context and under what conditions it operated. In this sense, detailed observation and description through multi-perspective internal observation help make visible the dynamics of practical knowledge generation in the field.

4.5.3 Motivational management effects of multi-perspective internal observation

4.5.3.1 Motivation moving toward a future orientation in D1

The method of multi-perspective internal observation in this study extends the internal observation formulated by Masuda (2007, 2013) and is characterized by the fact that multiple members mutually become observers. Prior research has already demonstrated that internal observation possesses the feature that *katari* itself functions as motivational management. In this study, however, because a reciprocal setting was created through group discussion, motivational acts mediated by *katari* occurred in a chain-like manner. Below, the members' statements in D1 are organized according to *katari* modes, and their motivational functions are examined.

In the participation histories and development story (D1-1–D1-5), each member presents their own “another story,” corresponding to a typical past-oriented *katari-tsutae*

mode. Miyamoto and Masuda (2019) argue that *katari-tsutae* generates empathy and forms a relational foundation. In this group discussion, as members shared these experiences with one another for the first time, mutual understanding and empathy visibly deepened.

The review of experiences that had already been shared between **A** and **C** (D1-2–D1-4) unfolded in the *katari-ai* mode, but this same *katari-ai* simultaneously functioned as *katari-tsutae* for **B**. In other words, the same act of *katari* operated as different modes depending on the listener's position and practical experience. While this multilayered functioning of *katari* is theoretically understood to occur universally within teams, prior research using the theory of *katari* has not sufficiently documented it. In this study, by incorporating each member's perspective as an object of observation through multi-perspective internal observation, it became possible to identify this layered structure of *katari* modes in detail.

In Development Challenge iii (D1-4), **A** and **C** described what they had learned from **X**'s stance toward the issue (D1-4-4, D1-4-5). This was not merely recollection, but a context in which past experience was connected to the present self and oriented toward future use, corresponding to *katari-tsugi*. **X**'s actions were reinterpreted by **A** and **C** and became part of the team's learning process.

In the Sales Challenge (D1-5), **A** and **C** initially interpreted the absence of sales negatively. However, through **B**'s explanations (D1-5-4, 5-6), this recognition shifted toward an understanding that “progress is occurring in the sales phase.” This process can be regarded as a “transformation of meaning” arising from *katari-awase*. Importantly, this shift generated a positive feeling—“we are doing something meaningful” (D1-5-5)—and thus functioned directly as motivation.

In the evaluation of team characteristics (D1-6–D1-7), members spontaneously articulated positive aspects of the team. This is a typical expression of the *katari-dashi* mode. Morishita and Masuda (2018) noted that *katari-dashi* may create new, future-oriented contexts through new actions, but earlier studies have not clearly demonstrated this phenomenon. In this study, the shared recognition that “the project is still ongoing” prompted explicit statements such as “there is still a lot left to do” (D1-6-5 to 9), revealing a distinctly future-oriented *katari-dashi*. This represents the first documented case in which a theoretically assumed future-oriented *katari-dashi* has been empirically observed.

In addition, statements such as “we felt that **X** continued to stay close to the members” (D1-7-19 to 22) generated empathy toward **X**'s stance in the current situation and therefore constitute present-oriented *katari-tsutae* rather than past-oriented. One

background factor underlying the *katari-dashi* discussed above may be that members genuinely felt this content of *katari-tsutae* in the present.

The subsequent Future Prospects (D1-8) explicitly concerns future action and shifts to the *katari-tsukuri* mode. The sequential development of *katari-dashi* → *katari-tsukuri* represents a process in which members, already in a motivated state, construct themselves toward the future. This sequence offers an empirical example of the mechanism proposed by Morishita and Masuda (2018).

To summarize the *katari* modes in D1, the discussion exhibited moment-to-moment shifts beginning with past-oriented *katari-tsutae*, moving to present-oriented *katari-ai* and *katari-awase*, and then to future-oriented *katari-dashi*. It further progressed to *katari-tsukuri*, in which members verbalized concrete future actions. This chain of modes emerged through changes in both *katari* content and the roles of “tellers” and “listeners” within internal observation. Importantly, the transformations that initiated this chain were triggered by “another stories” embedded in the *katari*. The *katari* of “another stories”—content that each member heard for the first time—constituted non-everyday and unexpected situations. In the theory of *katari*, such situations serve as cues for questioning motivation. The interaction of *katari*, in which “another stories” elicit further “another stories,” induced a chain-like development of *katari* modes, and within this process each member’s motivation became manifest.

In other words, D1, which intentionally sought to elicit “another stories,” functioned as motivational management for the team and simultaneously served as a catalyst that enabled motivation to unfold smoothly. Furthermore, the setting of D1 created an extraordinary situation due to the absence of the leader, producing for each member a carnivalesque sense of the world (Bakhtin, 1965) in which they were temporarily freed from everyday interactions with the leader.

In this way, the conditions of D1 and the “another stories” expressed there provided many nonroutine experiences for the members, inducing interfacilitation and mutual motivation. Miyamoto and Masuda (2019) also identified similar operative communication in another innovation-creation project, although in their case the research design contrasted with this study using interview surveys and the presence of an external facilitator.

Moreover, the future-oriented transformations in *katari* modes observed in D1 produced a meaningful shift even for X, who was not physically present in the *katari* setting. X had no direct access to how each member felt. Learning that each member had been motivated by the project led X to feel “motivated in turn to keep working hard.” Although this is not an immediate effect, it can be interpreted as a present-oriented *katari*-

tsutae through which **X**'s recognition changed based on *katari* concerning the team's current state.

4.5.3.2 Motivation through active recognition change in D2

D2 unfolded as a discussion that built on the shared understanding and relationships formed in D1. It involved reconfirming the content of D1, reconstructing the previously omitted Development Challenge iv, exploring the sources of team cohesion, and clarifying changes in motivation. As in D1, the statements in D2 are organized using *katari* modes to illuminate how they functioned motivationally.

At the beginning of D2-1, the content of D1 was reviewed. This process involved members mutually confirming that no discrepancies existed in their interpretations of the prior discussion. Because this review was grounded in shared understanding, it corresponds to *katari-ai*; however, its distinguishing feature is that members engaged in interactive confirmation of interpretive alignment rather than merely exchanging information.

Next, **X** presented supplementary insights based on discussions between **X** and **Y**. This constitutes past-oriented *katari-tsutae*, in which **X** conveys prior knowledge to evoke empathy. Similarly, because **X** introduced new perspectives on D1 that members had not previously considered, these statements also functioned as *katari-naoshi*, altering members' existing interpretations. The *forgotten* Development Challenge iv surfaced in this flow of discussion. Triggered by **X**'s remarks (D2-1-1), **A** and **C** recalled the issue (D2-1-2), and **A**, **C**, and **X** jointly reconstructed their earlier experiences—an instance of *katari-ai*. For **B**, who had not participated in Challenge iv, this same *katari-ai* operated as *katari-tsutae*, and **B** received it with empathy (D2-1-5). As in D1, a single *katari* simultaneously manifested in multiple modes depending on the listener's position.

In the discussion of team cohesion (D2-2), *katari* statements by **A**, **B**, and **C** stimulated one another. Members proposed factors such as shared purpose, empathy, group size, and informal conversation. Despite some differences in viewpoint, the discussion progressed and ultimately converged on psychological safety. This is a typical *katari-awase* mode. Through this *katari-awase*, members arrived at a shared understanding of the basis of team cohesion, further deepening their insight into team dynamics. Related to this, when members spoke about **X**'s characteristics (D2-2-15 to 20), **A** and **C** revealed their initial impressions of **X**, and **X** received these disclosures with surprise. Because psychological safety had not existed at the project's outset, **X** had consciously exercised care in communication, but this caution inadvertently created an

atmosphere in which A and C felt they “should not say the wrong thing.” These statements thus functioned as *katari-naoshi*, correcting X’s prior assumptions.

In the *katari* concerning motivational change (D2-3), members articulated internal histories that X had not recognized, and the discussion traced detailed fluctuations and transformations in their motivation. This process involved reconstructing “another stories” unknown to X and, because many aspects had not been mutually shared among the members, it operated as *katari-awase*. Through this *katari-awase*, elements previously identified in D1-6 and D1-7 were brought together, deepening the team’s collective understanding of motivational transitions and making visible the accelerated trial cycle and motivational growth described in 4.5.2.3. Moreover, X’s commentary on D1-8 (D2-3-11) served as *katari-naoshi*, reactivating the future-oriented *katari-tsukuri* from D1 and renewing its meaning in the present context. X’s evaluation that members came to feel “we are allowed to try things” (D2-3-14) reframed their recognition and granted it new significance.

Unlike D1, D2 featured frequent occurrences of *katari-naoshi* and *katari-awase*. This is natural given that D2 aimed to integrate “another stories,” making retrospective *katari-naoshi* and supplemental *katari-awase* central processes. Both modes transform the listener’s existing *katari*—past or present—and in D2 all members updated their “another stories” and their interpretations of the project. Through these exchanges, members renewed their understanding of both the project and one another, producing expressions of confidence such as “I can try anything” (D2-3-2) and “I am allowed to do it” (D2-3-12). Thus, D2 provided motivational effects through *katari* modes that actively transformed recognition, functioning in a manner distinct from the mode transitions that generated motivation in D1.

In summary, this study demonstrates that in both the extraction of “another stories” (D1) and their integration (D2), different *katari* modes interacted in complex ways to mutually motivate team members. This shows that multi-perspective internal observation—an extension of internal observation—operates as motivation management for team members, thereby fulfilling the second purpose of this study.

4.6 Conclusion

In this chapter, a managerial analysis of team practices in a product development project was conducted using multi-perspective internal observation. The distinctive feature of this study lies in extending conventional internal observation by expanding the role of observer to the entire team, thereby enabling the elicitation and integration of each

member's "another story" and allowing team practices to be examined from multiple perspectives.

The analysis drew on two group discussions: D1, a leader-absent session designed to elicit "another stories," and D2, a full-member discussion aimed at integrating them. Through these discussions, several forms of practical knowledge surfaced from concrete events and interactions during the project, including the formation of a multilayered communication system and the acceleration of trial cycles through task decomposition and rapid feedback. These forms of practical knowledge were not predetermined but were dynamically adjusted as the project evolved, and their generative processes were clarified through the analysis.

Furthermore, examining D1 and D2 through the theoretical framework of *katari* revealed characteristic *katari* modes. "Another stories" triggered chains of *katari* that linked past, present, and future, while integrative discussions actively transformed members' interpretations and understandings. This demonstrates that multi-perspective internal observation functioned not only as a research method for identifying practical knowledge but also as a form of motivation management that fostered mutual understanding and engagement among team members.

In summary, this chapter elucidated the practical knowledge, its emergence process, and the motivational dynamics embedded in a product development project through multi-perspective internal observation. These findings offer valuable insights not only for product development efforts but also for broader team-based activities and on-site management in organizational settings.

References for Chapter 4.

- Alvesson, M. (2003). "Methodology for close-up studies—Struggling with closeness and closure," *Higher Education*, 46(2), 167–193.
- Azuma, S. (1999). *Shin-kagaku-sangyo-sousei-no-management-Kenkyu-kaihatsu jigyoka-no-ron to jissai (Management for Creating a New Chemical Industry: Theory and Practice of R&D and Commercialization)*. Kagaku Kogyo Nipposha. [In Japanese]
- Bakhtin, M. M. (1965). *Творчество Франсуа Рабле и народная культура средневековья и ренессанса. Художественная Литература*, [In Russian]
- (Kawabata, K. (1995). *François Rabelais and the people's culture of the Middle Ages and Renaissance*. Serika Shobo.) [In Japanese]
- Bakhtin, M. M. (1975). *Вопросы литературы и эстетики. Художественная Литература*, [In Russian]
- (Ito, I. (1996). *Shosetsu no kotoba (The Language of the Novel)*. Heibonsha.) (In Japanese)
- Boje, D. M. (2001). *Narrative methods for organization & communication research*. SAGE Publications.
- Csikszentmihalyi, M. (1990). *Flow: The psychology of optimal experience*. Harper & Row.
- Deci, E. L. (1975). *Intrinsic motivation*. Plenum Press.
- Ellis, C., & Bochner, A. P. (2000). "Autoethnography, personal narrative, reflexivity: Researcher as subject." In N. K. Denzin & Y. S. Lincoln (Eds.), *Handbook of qualitative research 2nd ed.*, pp. 733–768. Sage Publications.
- Engeström, Y. (1991). "Developmental work research: A paradigm in practice." *The Quarterly Newsletter of the Laboratory of Comparative Human Cognition*, 13(4), 79–80.
- Engeström, Y. (1987). *Learning by expanding: An activity-theoretical approach to developmental research*. Orienta-Konsultit.

- Fujimoto, T., & Clark, K. B. (2009). *Zoho-ban seihin kaihatsuryoku: Jidosha sangyo no "soshiki noryoku" to "kyosoryoku" no kenkyu (Product Development Performance: Strategy, Organization, and Management in the World Auto Industry)*. (A. Tamura, Trans.). Diamond. [In Japanese]
- Fujiwara, H., & Masuda, Y. (2015). "The Methodology of Technology Development Practice without Eliminating the Chances of Innovation: A Case Study by Emergent Business Field Research," *Journal of Japan Society for Management Information*, 24(3), 169–195. [In Japanese]
- Goto, K., & Masuda, Y. (2021). "An Analysis on the Product Development Process of an In-House Research and Development Center Using Product Genealogy Based on an Emergent Business Filed Research: A Case Study on the Generation, the Transformation and the Succession of Knowledge in Practice," *Journal of Japan Society for Management Information*, 30(3), 151–166, [In Japanese]
- Gunji, P.-Y. (2006). *Semei-riron (The theory of life)*. Tetsugaku Shobo. [In Japanese]
- Kato, N., & Masuda, Y. (2016). "Formation of a community of practice through interfacilitation: Case study on new product development," *Japanese Journal of Communication Studies*, 44(2), 181–204. [In Japanese]
- Kato, N., & Masuda, Y. (2017). "Development of a new process model for creating a new product concept: A case study from a researcher–practitioner perspective," *Journal of Japan Association for Information Management*, 37(1), 63–75. [In Japanese]
- Kawakami, T. (2005). *Kokuyaku shikō no shinseihin kaihatsu: Māketingu to gijutsu no intafeisu (Customer Orientation for New Product Development)*, Yuhikaku. [In Japanese]
- Masuda, Y. (2007). "Dokizuke management ni okeru 'katari' no yukosei" (The Effectiveness of "Katari" in Motivation Management). *Keizai Kagaku Ronkyu*, 4, 39–51. [In Japanese]
- Masuda, Y. (2010). "A Study of 3R-Material 'Polisilicato-Iron' which Enables Sustainable 'Water Services and Agriculture' From a Viewpoint of

- ‘Katari=Antenarrative’ as Transforming Management Information,” *Journal of Japan Society for Management Information*, 19(3), 203–219. [In Japanese]
- Masuda, Y. (2013). *Sei no genba no “katari” to doki no shigaku: Kansoku sikougata riron ni teiisita genba kenkyuu=doukiduke manejimento (“katari” in the life-field of and poetics of motives: Field research based on measurement-oriented theory = methodology for motivation management)*, Hitsuji shobo. [In Japanese]
- Masuda, Y. (2014). “Strategic Speech Act ‘Katari=Antenarrative’ in the Management of Survival from Disaster: A Case Study in Field Research Approached by Life Theory,” *Journal of Japan Society for Management Information*, 23(3), 193–215. [In Japanese]
- Miyamoto, J., & Masuda, Y. (2019). “Characteristics of ‘Interactive’ Communication to Promote Innovation: A Case Study on ‘Katari=Antenarrative’ in a New Technology Development,” *Japanese Journal of Communication Studies*, 48(1), 5–27. [In Japanese]
- Morishita, K., & Masuda, Y. (2018). “Jissen ni sonzai suru monogatari to senryaku ga souhatsu suru ‘katari’: Shin-jigyo-kaihatsu ni okeru shijo-chousa no genba jirei (Narratives and Strategies Emerging in Practice: A Field Case Study of Market Research in New Business Development),” *Journal of Strategic Management Studies*, 18, 3–29. [In Japanese]
- Numagami, M. (1999). *Ekishō disupurei no gijutsu kakushinshi: Kōi rensa shisutemu toshite no gijutsu (History of Liquid Crystal Display Technology)*, Hakuto-Shobo. [In Japanese]
- Urakawa, T. (2010). *Innovation wo mezasu “jissen” kenkyu-kaihatsu management (Practice-Based R&D Management Aiming at Innovation)*. Hakuto-Shobo. [In Japanese]

Chapter 5.

Concluding Remarks

5.1. General Conclusion

5.1.1. Technological contribution

The technical contribution of this study lies in systematically demonstrating the technological foundation of thin-section- and matrix-free MSI, as well as the extensibility to thin-section-free MALDI-MSI, using a transfer plate composed of a platinum-coated porous body, with the aim of reducing the burden of sample preparation in MSI.

Chapter 2 demonstrates that thin-section- and matrix-free SALDI-MSI can be realized using a transfer plate consisting of a porous plate formed of glass beads and a platinum layer deposited on one side. The transfer plate combines sufficient mechanical strength to withstand the transfer pressure with a porous structure capable of absorbing sample-derived solvents, thereby enabling stable transfer regardless of the wetness of the sample. In addition, the platinum layer provides both ionization functionality based on SALDI and electrical conductivity, allowing the transfer plate to be used directly with commercially available MALDI-TOFMS. Consequently, a simplified MSI workflow without matrix application was achieved, with the advantage of avoiding matrix-derived background peaks in the low-mass region. Nevertheless, the results presented in Chapter 2 indicate that SALDI-MSI has limited sensitivity to certain classes of molecules, including anthocyanins. Accordingly, Chapter 3 investigates the feasibility of combining MALDI with thin-section-free MSI using a transfer plate.

In Chapter 3, strawberry cross sections were employed as a model sample, and an experimental design was adopted in which SALDI-MSI measurements were first performed on the same transferred sample, followed by matrix application and subsequent MALDI-MSI analysis. This approach enabled a direct comparison between SALDI-MSI and MALDI-MSI under conditions that eliminated variability arising from the transfer process. The ion images of metabolites such as hexose, citric acid, and sucrose obtained by both techniques showed qualitative agreement, and subsequent matrix spraying did not cause any substantial delocalization in the MALDI-MSI results. Furthermore, anthocyanins, which are difficult to detect by SALDI-MSI, were successfully visualized by MALDI-MSI on a transfer plate, demonstrating that the molecular coverage can be effectively expanded within a thin-section-free MSI framework based on the transfer plate.

When consolidated, the results of Chapters 2 and 3 demonstrate that the transfer plate not only enables straightforward thin-section-free sampling but also serves as a versatile MSI platform on which different ionization modalities, such as SALDI and

MALDI, can be employed in combination. The technical insights obtained in this study provide a foundation for expanding the applicability of MSI and lowering the barrier associated with sample preparation, thereby offering an important basis for the future development of diverse MSI applications.

5.1.2. Managerial contribution

The managerial contribution of this study lies in demonstrating that the introduction of multi-perspective internal observation enables a deeper understanding of team practices and motivational dynamics in product development research.

In Chapter 4, multi-perspective internal observation is implemented through the analysis of two sessions of group discussion conducted by the same team under different conditions: D1, conducted without the team leader; and D2, conducted with the team leader present. The analysis revealed the process by which the team members' previously undocumented "another stories" were integrated through dialogue and ultimately converged into a common understanding. Furthermore, the results show that the team acquired two forms of practical knowledge through multi-perspective internal observation. The first was a multilayered team communication system and the second was the acceleration of trial cycles achieved through task decomposition and rapid feedback. Multi-perspective internal observation also made it possible to visualize how these forms of practical knowledge were acquired by the team from the perspective of integrating "another stories". Understanding the background and processes through which practical knowledge emerges constitutes important insight into introducing and utilizing practical knowledge in project management at the operational level. In addition, multi-perspective internal observation promoted the reorganization of meanings that members had attributed to past events (*katari-naoshi*) and generated future-oriented *katari* (*katari-tsukuri*). The act of engaging in multi-perspective internal observation itself functioned as a source of motivation for the team, serving to corroborate the theoretical stance on internal observation which stipulates that observation equals motivation.

The multi-perspective internal observation approach adopted in this study was based primarily on two sessions of group discussions conducted by the same team and their subsequent analysis, without requiring specialized expertise or advanced skills. In this respect, the proposed approach is not limited to the present case but can be positioned as a methodology applicable to the analysis of a wide range of team activities and project contexts.

5.1.3. Business practices

The transfer plate served as the central theme of the new business practice, through which the author executed a series of phases, including idea generation, solution verification, business plan formulation, and business launch. As described in Section 1.4, the transfer plate was identified as a technological solution originating from a clear motivation to address customer requirements that had been expressed for DIUTHAME. Subsequently, the transfer plate was positioned as a product development project. Following the author's enrollment in GPI in 2022, the project advanced through the resolution of multiple technical challenges and the certification processes required for commercialization, leading to its launch in the Japanese market in January 2024 under the product name **Poropare™** (Figure 5.1). Furthermore, as of the doctoral thesis examination in February 2026, work on the transfer plate continues as an ongoing business within the author's affiliated company.

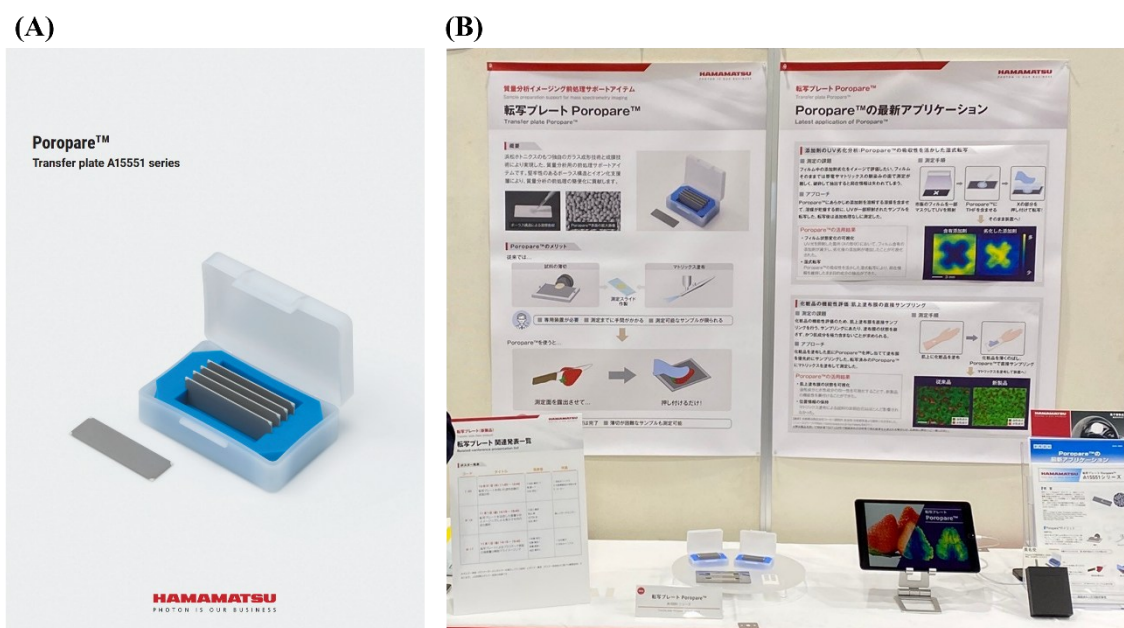


Figure 5.1. (A) Product brochure for the transfer plate. (B) Conference exhibition of the transfer plate.

As also noted in Section 1.5, MSI is an analytical technique that fundamentally applies photonic technologies at the core of its desorption and ionization processes and is therefore closely related to the development of the photonics industry. From this

perspective, the transfer plate can be positioned as a technology that promotes the further social implementation of MSI and as a business practice that contributes to *the creation of new photonics industries*.

The technical and managerial research presented in this study was conducted entirely as part of the business practice activities related to transfer plates. Technical investigations constitute concrete and case-specific technological studies within MSI; however, they are also positioned as practical processes for idea generation and solution verification embedded in new business practices. In parallel, this study advances the managerial perspective by demonstrating the effectiveness of a methodological approach that extracts practical knowledge from actual product development settings and applies multi-perspective internal observation as a framework for motivational management. This effectiveness was verified through its application in the product development phase analysis. The analytical concepts employed in this study are not dependent on a single case, but are generally applicable to a wide range of new business creation contexts. Accordingly, Chapters 2 and 3 focus on technical investigations corresponding to the solution verification phase, whereas Chapter 4 analyzes the practices of the product development project team during the business launch phase.

This doctoral thesis does not comprehensively describe all phases of the new business practice. Prior to establishing the platinum-coated porous body formed of glass beads as a transfer plate, multiple stages of idea generation and verification were undertaken. However, the details of these activities are confidential and are therefore not included in this thesis. In addition, the business scale and specific business plans related to the transfer plate are based on customer data owned by the author's affiliated company and thus cannot be disclosed in a publicly available doctoral dissertation. Under these constraints, this thesis focuses on the core technical verification and managerial analysis of new business practices within a scope that can be made public.

5.1.4. Overall conclusion

The major objective of this study was to acquire practical academic knowledge in both technology and management through the practical product development process of transfer plates, thereby contributing to *the creation of new photonics industries*. From a technical perspective, the study fulfilled its technical research objective by demonstrating the technological principles of the transfer plate for MSI and verified its applicability to thin-section-free MSI, including its extensibility to MALDI-MSI. These results establish a concrete foundation for reducing the burden of MSI sample preparation and for

expanding MSI applications. From a managerial standpoint, this study achieved its managerial research objective by analyzing the management of the product development team through multi-perspective internal observation and elucidating the practical methodologies and motivational processes involved. This analysis clarified how practical knowledge emerges within a team and how observation functions as motivation management at the operational level. These technological and managerial achievements were obtained in the context of a new business development activity that progressed from problem identification to product launch and continued operations within a technology-driven company. By integrating technological achievements, managerial insight, and business practice within a single research framework, this doctoral study presents a concrete model of photonics-driven industrial creation grounded in practical product development.

5.2. Future Perspectives

Building on the technological and managerial progress achieved in this doctoral study, several issues have emerged that warrant further investigation.

From a technological perspective, these issues include the reproducibility of the transfer operation, adaptation to higher spatial resolution, depth of analyte information obtained through the transfer process, and further improvements in sensitivity. In this study, spatial resolution was defined based on the diameter of the glass beads constituting the transfer plate (approximately 50 μm), and MSI measurements at spatial scales larger than this value were discussed. In practical measurements, however, it is necessary to consider not only the limitations imposed by the structure of the transfer medium itself but also the fluctuations inherent to the transfer operation. Further examination is required to determine the extent to which the molecular information from different depths of the sample surface is reflected in the transferred analytes. These issues are closely related to the reproducibility of the transfer process, and should be addressed in conjunction with the establishment of reliable transfer protocols. When aiming for a higher spatial resolution, improvements in both the transfer operation and the intrinsic spatial resolution limit of the transfer medium are required. With respect to sensitivity, although the feasibility of combining the transfer plate with MALDI was demonstrated in this study, it remains desirable to further enhancement of the intrinsic sensitivity of the transfer plate itself is required to preserve the original advantages of thin-section- and matrix-free MSI. Continued efforts to address these technological issues are essential for meeting the emerging user needs for transfer plates, and related investigations and developments are currently ongoing.

From a managerial perspective, several issues must be examined through continuous application and verification. First, a comparative analysis of the method employed in this study—two sessions of group discussion to elicit “another stories”—against alternative approaches is required. For example, a comparison with methods based on written questionnaires to collect “another stories” would provide valuable methodological insights. In addition, because multi-perspective internal observation is designed as a team-wide reflective practice, its applicability to different team sizes warrants further consideration. While the present study involved a team of four members, it remains unclear whether the same approach would be effective for larger teams, such as those consisting of ten members, or whether subdividing teams into smaller groups would be more appropriate. Furthermore, the effectiveness of multi-perspective internal observation is likely to depend on team characteristics such as the degree of familiarity or distance among members. Accumulating and examining application cases across diverse teams is necessary to clarify how such attributes influence the outcomes of multi-perspective internal observation. These managerial issues will be continuously examined through the author’s future participation in and observation of various project teams.

The author’s affiliated company has traditionally focused on developing and selling photodetectors and light sources as its primary business. In this context, the transfer plate represents a new business that differs substantially from the company’s existing operations in terms of technology, business models, and target customer base. In today’s rapidly changing business environment, product diversification and diversification of business models are of critical importance for corporate survival. New business practices such as transfer plates constitute an important challenge for technology-driven companies, and continued engagement in such practices is required. Finally, the author hope that this doctoral thesis, even in a modest way, will deepen the readers’ understanding and help cultivate a greater number of individuals who will take on the challenge of creating new industries, including those in photonics.