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授与した学位	博　士
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学位論文の題目	Multimodal analysis of the *Caenorhabditis elegans* lipid molecular profile （*Caenorhabditis elegans* における脂質分子構成のマルチモード解析）
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学位論文内容の要旨

Chapter 1 introduces the thesis and research that was performed in this project as well as its main goals.

Chapter 2 explains the background of this research, advances in the relevant fields until now, and any present gaps that require further research. The background is separated into the biological explanations of *C. elegans* research and its potential and uses as a model organism. The second part expands on the technical aspects of this research, more specifically introducing nanodiamonds and their use as quantum thermometers in biological systems, and mass spectrometry imaging and its use in the visualization of lipid distributions in biological samples.

Chapter 3 explains the methodology used in this project. Some of the methods were performed based on previous publications with modifications specific to *C. elegans* analysis, while others were fully developed within the scope of this thesis. To visualize the distribution of lipids in the nematodes using MSI, we developed a sectioning method that allowed the preservation of internal structures of the nematodes which were later used for correlation in multi-modal analysis and 3D reconstruction. Protocols were developed for the local heating of nematodes, followed by their retrieval and staining to demonstrate lipid changes. The precision of the heating was additionally controlled and calibrated using nanodiamond-fed nematodes and an area heating system developed by the research group.

Chapter 4 presents the first major part of the results. The multi-modal analysis approach is explained, as well as how it was achieved. This part of the research also serves as the foundation for later experiments. The developed method was first tested for reproducibility to determine its suitability for further studies using larger amounts of samples. To show the usability of the preparation method for different analysis methods, the samples were analyzed using MSI, ORO and H&E staining, and further verified by LC-MS analysis. The results show the capability of multi-modal image analysis correlating internal nematode structures with a variety of lipids, as well as the subsequent 3D reconstruction of the nematode, allowing for more in-depth visualization of lipid distributions through the nematode body.

Chapter 5 dives into the temperature modulation of *C. elegans*. It demonstrates the effect of global temperature manipulation on the lipid profile of the nematodes, and current developments in the local temperature manipulation. To establish the applicability of the previously described MSI and ORO stain methods for detecting differences in lipid amounts, global temperature manipulation was performed using three different culturing temperatures and two strains of *C. elegans*. Here, it could be seen that the intensity of lipid signals can indicate the changes in lipid amounts in the nematodes. Once this was established, further work could be performed using local temperature manipulation that allowed for the heating of specific parts of the nematodes. Following the heating, the *C. elegans* were retrieved and stained using the ORO method to first show overall changes in the lipid levels.

Lastly, Chapter 6 will summarize the research and discuss both the current limitations and future prospects based on this work.

論文審査結果の要旨

本論文は、線虫 *Caenorhabditis elegans* (*C. elegans*) を用い、質量分析イメージング（MSI）と光学イメージングの多重モード計測によって脂質分子構成の体内分布、および脂質代謝の分子的理解を得ることを目的としている。特に、線虫が培養温度を記憶し、その温度に向かって移動する温度走性と、脂質代謝の温度依存性との関連に着目し、高解像度MSI法の構築と、線虫全体および局所温度変化に伴う脂質代謝変化の解析を行っている。本論文では、以下の二点において、生体分子メージング研究におけるMSI技術の有効性と新たな応用可能性を示す重要な成果を挙げている。

（１） 内部構造を保持した線虫MSI法の開発

従来、線虫の臓器構造を保持した状態でのMALDI (Matrix-Assisted Laser Desorption/Ionization) -MSI解析は困難であった。本研究では、マイクロ流体チップを用いた切片作製技術と、3次元断層像再構築技術を組み合わせることで、凍結による構造損傷を可能な限り回避しつつ内部構造を可視化可能なサンプル調製法を開発した。これにより、体長1 mm未満の線虫における脂質分子分布の高精度解析が可能となり、生体試料の分子解析技術を大きく前進させた。

（２） 培養温度依存的な脂質分布変化の解明

開発した手法を用い、野生株および脂質代謝経路に温度応答性変異を持つ*daf-2*変異株を対象に、培養温度（15, 20, 25 ℃）の違いによる脂質量および構成分子成分の変化を可視化した。従来、LC (Liquid Chromatography) -MSやOil Red O染色により総脂質量や全身での脂質構成温度依存性は知られていたが、本研究では脂質の種類と局在変化を線虫体内で直接捉えた点が新規である。変異株においても予想通りの変化が観測され、本手法の信頼性が示された。

以上の成果は、生体質量分析イメージングにおいて重要な技術的進展をもたらすと共に、線虫脂質代謝の分子機構解明および温度応答性代謝研究に新たな解析手法をもたらすものであり、当該分野の進展に大きく寄与する。また、論文発表会においても学位論文内容を明確に説明し、質疑応答にも適切に対応していた。本審査委員会は、本論文の審査および最終試験の結果に基づき、本論文著者に博士（学術）の学位を授与するにふさわしいと認めた。