学　位　論　文　の　要　旨

エクソソーム高精度計数技術と同時多重計測応用に関する研究

（High-precision counting technology for exosomes and its application of simultaneous multiplex measurement）

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本論文は、エクソソームを単一粒子レベルで計数可能な高精度計測技術、ならびにエクソソームの多様な性質を同時に解析可能な多重計測技術応用について述べたものである。

近年、エクソソームと呼ばれる細胞が分泌する微粒子が注目され、多くの研究が行われている。エクソソームは分泌過程で細胞由来の脂質、タンパク質、核酸等を取込み、細胞間の情報伝達に寄与している。しかし、ヒトの体液中のエクソソームは極めて微小で低濃度、かつ多様な性質を持つため、既存技術では単一粒子レベルでの高精度な解析が困難である。本研究では、これらの課題を解決するため、光ディスク技術とナノ粒子標識によるサンドイッチ型免疫アッセイを組み合わせた新規のエクソソーム高精度計数技術を提案し、光学シミュレーションに基づいたナノ粒子検出信号の理論的解析とエクソソーム検出技術の確立と評価を行った。

第一章では、本論文の緒言として、エクソソームの性質について概説した。その後、エクソソーム研究の重要性に焦点を当て、先行研究を踏まえて現状のエクソソーム計測における難しさと課題を示した。これらの課題を解決する新規エクソソーム計数技術と、その

応用技術の提案が本研究の目的であることを述べた。

第二章では、提案したエクソソーム高精度計数技術について詳細に述べた。検出に用いるレーザー光波長以下の微小な領域で生じる電磁場の厳密な計算が可能な有限差分時間領域法（FDTD method: Finite-difference time-domain method）と、伝搬光の光応答を高速計算可能な高速フーリエ変換（FFT: Fast Fourier Transform）を組み合わせた光学シミュレーションを確立し、エクソソーム標識用磁性ナノ粒子（FG ビーズ）由来の光パルス応答を解析した。その中で、近接する FG ビーズで生じる近接場の光現象を見出し、その特徴的な光パルス応答に基づいた高精度エクソソーム計数システムを構築した。さらに、表面抗原（タンパク質）の特異的な捕捉、標識によるエクソソームの高精度計測を実証し、疾患と関連するエクソソーム表面抗原の特異的な計測による疾患診断への応用可能性についても示した。

第三章では、エクソソーム同時多重計測を実現するため、FFT-FDTD シミュレーションを用いて、FG ビーズと同径で異なる複素屈折率の金（Au）および銀（Ag）のナノビーズが多重検出に適した素材となることを見出し、計算結果と実験結果が一致することを示した。そして、金属ナノビーズ表面へのタンパク質吸着メカニズムに基づいて、FG ビーズとは異なる抗体固定方法を確立し、排他的な表面抗原を持つ 2 種類の細胞株由来精製エクソソームを用いて、３種類のナノビーズを用いたエクソソーム同時多重計測性能を示した。また、臨床応用への可能性を示すため、エクソソーム以外の複雑なタンパク質が多量に含まれるヒト血液試料に精製エクソソームを添加して実用的な定量性が得られる事も実証した。本研究で構築したシステムの検出限界濃度（LOD）は 210 exosomes/μL であり、他方式の同時多重計測技術と比較して、最も優れた性能が得られた。

第四章では、本論文の総括として、エクソソーム高精度計数技術と同時多重計測応用に関する研究の成果をまとめ、今後の展望についても言及した。

以上の研究結果より、理論的計算に基づいた測定アルゴリズムとプラットフォームの構築、細胞由来エクソソームを用いたシステムの基本特性の検証を通じて、提案した技術の有用性を実証した。本研究で確立した技術は、従来のエクソソーム計測における「精製に伴うサンプル前処理」、「特異的かつ高精度なハイスループット計測」、「同時多重計測」という課題を解決する画期的なエクソソーム計数技術である。この技術は、エクソソーム計測による疾患診断への応用可能性、複雑な性質を持つエクソソームの多様性に関する新たな洞察など、エクソソーム研究の進展に貢献できるものである。

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In this research, the author proposes a novel high-precision counting technology for exosomes at the individual particle level, which can be further applied to enable simultaneous and multiplex analysis of their heterogeneous properties.

In recent years, a growing interest in exosomes, extracellular vesicles secreted by cells, has driven extensive research. Exosomes play a vital role in intercellular communication, as they incorporate various cell-derived components such as lipids, proteins, nucleic acids, and others during the secretion process. However, employing conventional measurement methods for measuring exosomes at the single-particle level with high precision is difficult due to their small size, low concentration, and heterogeneity in human body fluids. Therefore, in response to solving the problems, the study proposes a novel high-precision exosome counting technology by combining optical disc technology with a sandwich immunoassay using nanobeads labeling. The research encompasses theoretical analysis of nanobeads detection signals through optical simulation, as well as the establishment and evaluation of the exosome detection technology.

In Chapter 1, serving as an introduction to this research, an overview of the properties of exosomes was presented. Subsequently, the focus shifted to the importance of exosome research and highlighted the difficulties and issues inherent in the current state of exosome measurement, building upon previous research. The purpose of this study is to propose a novel exosome counting technology and its application technology to address and overcome these challenges.

In Chapter 2, a detailed explanation was introduced regarding the proposed high-precision exosome counting technology. Initially, the optical simulation was established by combining the Finite-Difference Time-Domain (FDTD) method, which facilitates precise calculations of the electromagnetic field in tiny regions below the wavelength of the laser used for detection, with Fast Fourier Transform (FFT), enabling fast computation of the optical response in the far-field. This simulation aimed to analyze the optical pulse response derived from magnetic nanobeads (FG beads) applied for exosome labeling. Subsequently, optical phenomena in the near-field generated by two consecutive FG beads were identified. Utilizing the distinctive optical pulse response of these

phenomena, a high-precision exosome counting system was developed. Furthermore, the system demonstrated specific capture and labeling of exosomes through surface antigen (protein) recognition, thereby validating the accuracy of exosome measurements. Moreover, the potential application of this technology in disease diagnosis, particularly in measuring disease-associated exosome surface antigens, was both discussed and demonstrated.

In Chapter 3, for achieving simultaneous multiplex measurement of exosomes, the author employed FFT-FDTD simulations to demonstrate that gold (Au) and silver (Ag) nanobeads, possessing the same diameter as FG beads but different complex refractive indexes, are suitable materials for multiplex detection. The computational results were demonstrated to be consistent with experimental results. Based on the protein adsorption mechanism onto the surface of metal nanobeads, a different antibody fixation method from FG beads was established. Using exosomes purified from two cell lines with exclusive surface antigens, and utilizing three types of nanobeads, the performance of simultaneous multiplex measurement of exosomes was demonstrated. To demonstrate its potential for clinical applications, practical quantifiability was demonstrated by adding purified exosomes to human serum samples containing abundant complex proteins other than exosomes. The developed system achieved a limit of detection of 210 exosomes/μL in this research, representing the highest performance compared to other methods of simultaneous multiplex measurement.

In Chapter 4, serving as a conclusion of this research, the results of the high-precision exosome counting technique and its application for simultaneous multiplex measurements were summarized. Additionally, further research in this field was discussed.

Building upon the aforementioned research results, the efficacy of the proposed technology was demonstrated through the development of measurement algorithms and platforms based on theoretical calculations, and the validation of the fundamental characteristics of the system using cell-derived exosomes. This study demonstrates the potential of the exosome counting technology to provide a groundbreaking solution to challenges in conventional exosome measurements, including "sample pre-processing associated with purification," "specific and high-precision high-throughput measurements," and "simultaneous multiplex measurements." This technology contributes to the advancement of exosome research by offering possibilities for disease diagnosis applications through exosome measurements, providing practical insights into the heterogeneity of exosomes with complex properties.