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厦门大学

博　士　学　位　论　文

超高灵敏（光谱）流式检测装置的研制及其在纳米颗粒定量表征中的应用

Development of High-Sensitivity (Spectral) Flow Cytometer for Quantitative Characterization of Individual Nanoparticles

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Development of High-Sensitivity (Spectral) Flow Cytometer for Quantitative Characterization of Individual Nanoparticles

A Dissertation Submitted to the Graduate School of Xiamen University for the Degree of

Doctor of Science

By

Shuo Wang

This work was carried out under the supervision of

Prof. Xiao-Mei Yan

At

Department of Chemistry, Xiamen University

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摘 要

流式细胞术（Flow Cytometry, FCM）是一种对悬液中的细胞或细胞大小的颗粒进行高通量定量分析或分选的技术。FCM 通过对单个样品颗粒散射光及多色荧光信号的同时检测，实现细胞尺寸、内部颗粒度、蛋白含量、酶活性等物理、化学及生物性质的多参数同时测定，具有统计精确性高、数据可靠、实用性强等优点。传统流式分析仪的检测通量高达每秒数万个细胞，但难以对粒径小于 500 nm 或者荧光亮度小于 200 个荧光素分子的信号进行检测。生命科学研究的不断深入和纳米科技的飞速发展均对纳米颗粒的表征技术提出了更高的要求。基于纳米粒子固有的多分散性，需要在单颗粒水平对其多种物理和生化性状进行定量表征。然而，国际上没有一种技术能够对如此微小的纳米粒子在单颗粒水平进行多参数综合表征。如果能大幅度提升传统流式细胞仪的检测灵敏度，纳米颗粒的检测将享有流式细胞术快速、多参数、定量、天然状态悬液检测等优势。

结合瑞利散射和鞘流单分子荧光检测技术，本课题组研制成功超高灵敏流式检测装置（HSFCM），采用独特的流体动力学聚焦系统在低流速下实现稳定液流聚焦。通过减小探测区体积降低背景，延长样品颗粒穿越探测区时间、提升激发光功率密度、采用单光子探测器等措施增加信号，于 2014 年成功实现了粒径仅为 7 nm 的单个纳米金颗粒和粒径为 24 nm 的单个二氧化硅纳米颗粒散射信号以及单个藻红蛋白荧光信号的超高灵敏检测，检测灵敏度较传统 FCM 提升数万倍。HSFCM 的研制为人工合成纳米粒子及天然生物纳米粒子（细菌、病毒、线粒体等）的快速表征提供了强有力的分析手段，在疾病诊断、生物传感、环境分析等领域具有重要意义。

本研究的重点是进一步提升仪器检测灵敏度及开发高维度信号检测模式，以拓展超高灵敏流式检测技术的应用范围。基于实验室原有的 HSFCM，通过对液路系统、集光系统、信号采集及软件分析系统等的一系列改进，进一步提升仪器检测灵敏度。通过对单个量子点微弱荧光信号的定量检测，发展了量子点发光亮度均一性、量子点浓度、量子点团聚程度以及环境因素对其发光性能影响等快速表征手段。此外，为了获取更丰富的单个纳米颗粒的生化信息，我们在 HSFCM

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基础上搭建了超高灵敏光谱流式检测装置（S-HSFCM），实现了人工合成纳米颗粒及微生物的单颗粒荧光光谱检测。本论文的主要工作包含以下几个方面：

第一章为文献综述。本部分主要介绍流式细胞术的发展历程、最新研究进展及发展方向，并对本论文选题思路及研究内容进行论述。

第二章为 HSFCM 检测装置的系统优化。通过对仪器光路、液路系统的升级，进一步提高仪器的稳定性，仪器检测灵敏度较原有仪器提升 2.8 倍，检测信噪比（S/N）提高~1.7 倍。使用仪器对单个藻红蛋白（R-PE）分子进行表征，信噪比高达 34。仪器灵敏度的提升对于纳米颗粒的多参数定量检测具有重要意义。

第三章为 HSFCM 检测装置对量子点的表征。在量子点的生产合成及实际应用中，需要准确地了解量子点的浓度、发光性能及其均一性、生物试剂偶联后是否导致量子点聚集，以及量子点所处缓冲液氛围对其发光性能的影响等物理、化学性质。我们采用性能优化后 HSFCM 对量子点进行单颗粒荧光检测，建立了高效的单颗粒水平量子点表征方法，仪器对 Qdot655 量子点检测信噪比（SNR）高达 88，可轻松实现量子点发光强度、单分散性、团聚程度、颗粒浓度等的快速表征，该表征技术的建立对于指导量子点的合成、量子点质量控制和生化应用标准化流程的建立具有重要的意义。

第四章为 S-HSFCM 检测装置的研制。为满足生命科学前沿研究、生物医学和纳米科技对于发展高灵敏、高通量的多参数定量分析技术的迫切需求，我们利用 HSFCM 独特的低流速流体聚焦系统和高灵敏性，通过采用高效率集光系统、高性能的分光系统和光谱型 EMCCD，成功研制超高灵敏光谱流式检测装置（S-HSFCM），实现单个纳米颗粒和细菌的荧光光谱全光谱检测。仪器采用光栅分光，在 0.27 nm 的光谱分辨率下实现与传统流式细胞仪带通检测模式相当的检测灵敏。S-HSFCM 的研制将推动单细胞、单个纳米颗粒光谱检测技术的发展，为基础生命科学研究、生物医学和纳米科技的发展提供创新的表征分析技术。

第五章为聚球藻的 S-HSFCM 检测。聚球藻是海洋蓝细菌的重要组成部分，广泛分布于世界大洋、河流及湖泊，丰度高且多样性丰富，在全球初级生产力和碳循环中发挥着重要作用。聚球藻细胞内含有特征色素藻胆素，具有独特的荧光特性。我们采用 S-HSFCM 在单细胞水平对不同种类聚球藻进行高通量光谱流式

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检测，通过对其荧光色素组分的鉴定及含量的分析，建立聚球藻生理研究及生态多样性分析的快速表征方法。

第六章为总结与展望。

关键词：超高灵敏流式检测装置；流式细胞术；单颗粒光谱；量子点；聚球藻

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Abstract

Flow cytometry (FCM) is a well-established technique for the rapid, multiparameter, and high-throughput quantitative analysis or sorting of individual cell and other microscopic particle in aqueous suspension. Information regarding size, internal granularity and surface roughnessof particles can be gathered via light scatter measurements, and biochemical attributes such as the nucleic acid content, enzymatic activity, and antigenic determinants of biological cells can be characterized via fluorescent labeling. In traditional FCM analysis, tens of thousands of cells can be detected per second for rare cell analysis and high-speed cell sorting. Such highthroughput detection requires transit time of sample particles within microseconds, which greatly limits the detection sensitivity of the instrument. Great challenge exists for the conventional FCM, in attempt to detect NPs smaller than 500 nm in size or dim particles with less than several hundreds of fluorescent molecules. The rapid development of nano-technology and the research of life science have put forward higher demand for the characterization of nanoparticles. Based on the inherent multi-dispersibility of nanoparticles, a variety of physical and biochemical characters need to be quantitatively represented at the single particle level. However, there is no technique for the multi-parameter analysis of such nanoparticles at the single-particle level. If the detection sensitivity of FCM could be greatly enhanced, the detection of nanoparticles would have the advantages of rapid, multi-parameter, quantitative and natural state suspension detection.

Adopting strategies for single-molecule fluorescence detection in a sheathed flow, we recently developed a high-sensitivity flow cytometry (HSFCM) method. The method uses a unique sheath flow system in which the sample fluid is hydrodynamically focused to a very narrow stream at low flow rate, so that the transit time of analyte particles can be as long as milliseconds. Cooperating with other sensitivity improvement strategies, such as reducing the size of the detection region

and using single photon detection module, the detection limit of HSFCM is much lower than that of the traditional FCM. Real-time light-scattering detection of single 7-nm gold nanoparticles, 24 nm SiO_2 nanoparticles, and fluorescence detection of single R-PE molecule have been achieved. Clearly, the development of advanced flow cytometry enabling rapid and multiparameter characterization of physical and chemical properties of individual nanoparticles is of great importance to nano-biotechnology and bioscience studies. Present study focuses on the further improvement of HSFCM sensitivity and the upgrade of its detection mode for the purpose of expanding its applications.

We optimized the optical and fluidics systems of the laboratory-built HSFCM instrument, so the detection sensitivity is further improved. By employing the improved HSFCM, we have developed a rapid and highly sensitive method for the precise quantification of the fluorescence intensity of single quantum dots. In addition, we added a spectral detection module to the HSFCM for the development of a high sensitivity spectral flow cytometer (S-HSFCM). The S-HSFCM inherits the high sensitivity advantage of HSFCM, achieving high S/N ratio detection for single particle fluorescence spectra of nanomaterials and microorganisms. The thesis consists of the following parts:

In chapter one, the development history of FCM was briefly introduced. Then, recent advances and development trends on FCM were reviewed. The motivation and the research contents were also included in this part.

The second chapter describes the system optimization of HSFCM. The sensitivity and stability of instrument are greatly improved by the upgrading of the optical and fluidics systems. The detected signal strength of the instrument was 2.8 times higher than that of our original setup, and the SNR ratio was ~1.7 times higher. The sensitivity enhancement of HSFCM will be of great significance to the quantitative characterization of nanoparticles.

The third chapter describes the rapid and quantitative measurement of single

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quantum dots via HSFCM. Semiconducting quantum dots (QDs) are used in a wide range of biomedical applications due to their intense fluorescence brightness and long-term photostability. Here, we report precise quantification of the fluorescence intensity of single QDs on a laboratory-built high-sensitivity flow cytometer (HSFCM). By analyzing thousands of QDs individually in 1 min, intrinsic polydispersity was quickly revealed in a statistically robust manner. Applications of this technique in QD quality assessment, study of metal ion influence, and evaluation of aggregation upon biomolecule coupling are presented. Moreover, an accurate measurement of the QD particle concentration was achieved via single-particle enumeration. HSFCM is believed to provide a powerful characterization tool for QD synthesis and application development.

The fourth chapter describes the development of S-HSFCM. Taking advantage of the unprecedented sensitivity of HSFCM, we developed the S-HSFCM by adding a spectral detection module on HSFCM. Multi-PMT detection module and spectral detection module are placed on both sides of the flow channel perpendicular to the laser incident beam and to the flow of the sample. Single nanoparticle spectrum is acquired by an EMCCD attached to the imaging spectrograph through the trigger generated by the PMT signal of particles' side scatter or fluorescence. The sensitivity of the system was evaluated by analyzing fluorescent SiO_2 nanoparticles with intensities quantified in the unit of molecules of equivalent soluble fluorochrome (MESF) per nanoparticle. Auto fluorescence of single bacteria was also examined. S-HSFCM could become a powerful tool for life science and nanobiotechnology studies.

The fifth chapter describes the spectra analysis of *Synechococcus* autofluorescence at the single-cell level by S-HSFCM. *Synechococcus* are the important and major group of cyanobacteria. They are widely distributed in various aquatic environment including ocean, river and lakes. *Synechococcu*s are abundant in the ocean with high biodiversity, contributing highly to marine primary production and global carbon cycling. The phycobilin pigments in *Synechococcus* cells result in

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the unique *in vivo* fluorescent properties of them. We established a method for high throughput spectra detection of the autofluorescence of single *Synechococcus* cells via using S-HSFCM. By detecting the autofluorescence spectra of single cell *Synechococcus*, we identified the pigment components and contents of different *Synechococcus* species. Given the advantages of high-throughput and high resolution analyses, there would be wide applications of S-HSFCM in the study of *Synechoccoccus* physiology and ecology.

In chapter six, the research progresses were summarized and the future prospects of HSFCM and S-HSFCM were discussed.

Keywords: High sensitivity flow cytometer; flow cytometry; single particle spectral analysis; quantum dot; *Synechococcus*

厦门大学博硕士论文摘要库

目录

摘要......i

Abstract......v

第一章 绪论......1

1.1 引言......1

1.2 流式细胞术及发展史......1

1.2.1 流式细胞术......1

1.2.2 流式细胞术发展史......2

1.3 流式细胞仪的构造......4

1.3.1 流动室：流体动力学聚焦的实现......5

1.3.2 液流驱动：鞘液系统与进样系统......7

1.3.3 光源：激光、光束整形以及多激光激发......9

1.3.4 分光系统：透射与反射......12

1.3.5 细胞分选系统......14

1.3.6 检测器：量子效率与增益......15

1.4 流式细胞仪的发展方向......17

1.4.1 成像型流式细胞仪......17

1.4.2 流式细胞术的多参数检测......22

1.5 本论文的选题思路及研究内容......31

参考文献......34

第二章 超高灵敏流式检测装置的系统优化......45

2.1 引言......46

2.2 实验部分......46

2.2.1 仪器主要零部件......46

2.2.2 零部件设计及仪器三维建模......46

2.2.3 试剂和药品......46

2.3 超高灵敏流式检测装置的性能优化......46

厦门大学博硕士论文摘要库

2.3.1 光学系统 47
2.3.2 液流驱动系统 51
2.3.3 信号采集与数据处理系统 54
2.3.4 HSFCM 的性能提升 62
2.4 本章小结 67
参考文献 67
第三章 超高灵敏流式检测装置对量子点的表征 71
3.1 引言 71
3.2 实验部分 72
3.2.1 仪器与设备 72
3.2.2 试剂与药品 74
3.2.3 样品制备 74
3.3 实验结果与讨论 76
3.3.1 HSFCM 对量子点的超高灵敏检测 76
3.3.2 激光功率对量子点检测的影响 79
3.3.3 缓冲液酸碱性对量子点检测的影响 80
3.3.4 金属离子对量子点检测的影响 81
3.3.5 HSFCM 对不同公司生产量子点的检测 82
3.3.6 量子点团聚表征 83
3.3.7 使用 HSFCM 对量子点的浓度测定 85
3.3.8 HSFCM 对有机相量子点的检测 86
3.4 本章小结 87
参考文献 88
第四章 超高灵敏光谱流式检测装置的研制 93
4.1 引言 93
4.2 实验部分 94
4.2.1 仪器主要零部件 94
4.2.2 零部件设计及仪器三维建模 94
4.2.3 试剂和药品 94

厦门大学博硕士论文摘要库

4.3 超高灵敏光谱流式检测装置的研制及性能优化 95
4.3.1 多通道检测模块 96
4.3.2 光谱检测模块 96
4.3.3 数据处理与谱图分析 101
4.3.4 S-HSFCM 的采样条件优化 105
4.4 本章小结 110
参考文献 110
第五章 聚球藻单细胞荧光光谱的超高灵敏流式检测 113
5.1 引言 113
5.2 实验部分 114
5.2.1 仪器与设备 114
5.2.2 实验材料及试剂 115
5.2.3 样品制备 117
5.3 实验结果与讨论 118
5.3.1 聚球藻菌种选择与常规表征 118
5.3.2 聚球藻光谱流式检测中激发波长的选择 121
5.3.3 S-HSFCM 对聚球藻单细胞荧光光谱的检测 122
5.3.4 S-HSFCM 对聚球藻混合样品的表征 124
5.3.5 S-HSFCM 对聚球藻生长状态的评估 125
5.4 本章小结 126
参考文献 127
第六章 总结与展望 129
6.1 总结 129
6.2 展望 130
作者攻读博士期间发表的论文和获得的奖项 131
致谢 133

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