香港浸會大學
HONG KONG BAPTIST UNIVERSITY

HKBU Scholars

DOCTORAL THESIS

Direct quantification of cancer biomarkers by fluorescence microscopy

Ho, See Lok Ashley

Date of Award:
2015

Link to publication

General rights
Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

Download date: 04 Dec, 2021

Direct Quantification of Cancer Biomarkers by Fluorescence Microscopy

HO See Lok Ashley

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Principal Supervisor: Dr. LI Hung Wing

Hong Kong Baptist University

February 2015

DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature: ___________________

Date: February 2015

ABSTRACT

As a high-resolution wide-field near-surface microscopy, total internal reflection fluorescence microscopy (TIRFM) has been widely applied for the study of biomolecules. Unlike those costly, sample consuming and time consuming traditional detection assays, the application of TIRFM enable the direct quantification of biomolecules in a sample pretreatment and enrichment free fashion. Taking advantages of the TIRFM imaging system, in this thesis we have applied the TIRFM imaging system to directly quantify the content of different cancer associated biomarkers. Four different detection approaches for direct cancer biomarkers quantification with the aid of TIRFM were herein presented respectively.

In Chapter 2, a direct quantification of nasopharyngeal carcinoma associated miRNAs was described. In the assay, five different miRNAs were chosen as the target analytes, which hybridized with the synthetic complementary LNA, probe in solution. The duplex was labeled with intercalating fluorescence dye YOYO-1 and the signal was then detected by the TIRFM-EMCCD imaging system. The LNA probe exhibited a high binding affinity towards the complementary target miRNAs and a limit of detection of 8 pM was achieved. Since the LOD is far below the reported concentration of miRNAs found in body fluids, this developed assay is of high potential to serve as a tool for non-invasive detection of miRNAs for early disease diagnosis.

In Chapter 3, an advanced single-molecule based assay for direct circulating miRNAs detection was developed. The assay was demonstrated to be capable of

differentiating the expression of a nasopharyngeal carcinoma (NPC) up-regulator hsa-mir-205 (mir-205) in serum collected from patients of different stages of NPC. To overcome the background matrix interference in serum, locked nucleic acid modified molecular beacon (LNA/MB) was applied as the detection probe to hybridize, capture and detect target mir-205 in serum matrix with enhanced sensitivity and specificity. A detection limit of 500 fM was achieved. The as-developed method was capable of differentiating NPC stages by the level of mir-205 quantified in serum with only 10 μL of serum and the whole assay can be completed in an hour. The experimental results agreed well with reported and while the quantity of mir-205 determined by our assay was found comparable to that of quantitative reverse transcription polymerase chain reaction (qRT-PCR), supporting that this assay can be served as a promising non-invasive detection tool for early NPC diagnosis, monitoring and staging.

In chapter 4, a self-assembled protein nanofibril based online pre-concentrating sensor was developed. This solution-based hybridization assay was applied to quantified the amount of target miRNAs, mir-196a. Biotinylated locked nucleic acid (LNA) of complimentary sequence was served as the probe to capture the target miRNA analyte. The target hybridization duplex was immobilized on the backbone of the nanofibril through the biotin-streptavidin interaction. The quantification was achieved by the fluorescence intensity measured with total internal reflection fluorescence microscopy. A detection limit of 1 pM was achieved with trace amount of sample consumption. This assay showed efficient single-base mismatch discrimination. The applicability of quantifying circulating

mir-196a in both normal and cancer patient's serums was also demonstrated.

In chapter 5, a magnetic nanoparticles based sandwich immunosensor with carbazole-based cyanine as the fluorescence labeling dye for the direct quantification of prostate cancer related antigen, PSA, was developed. Taking benefit of the magnetic property of the nanoparticles, the target sandwich immunocomposites can be easily online separated from the sample matrix. The as-developed assay can efficiently discriminate the target PSA from other disease related antigens and achieve a LOD of 400 fM (13 pg/mL) and a LOQ of 2 pM (0.66 ng/mL). As the whole detection assay can be completed in 1 h with only 10 μL of sample, this assay is fast and cost effective and of high potential for early disease and cancer diagnosis, staging and monitoring.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my profound gratitude to my supervisor, Dr. Hung-Wing Li, who has been very supportive since my first day working as a RA in her group and throughout my whole graduate study with her patient and knowledge whilst giving me freedom to work in my own way. I appreciate her vast knowledge and assistance in writing proposal, scholarship application, manuscript, and this thesis. I also appreciate a lot for her enthusiasm in teaching and research and her open-mindless in brainstorming research ideas. I would never be able to complete my graduate study without her help and guidance.

I would also like to thank my co-supervisor, Prof. Ricky Ngok-Shun Wong, for giving me the opportunity to start my graduate study and being very kind and supportive throughout all these years. I would not be able to finish most of the work without his help.

Many thanks to all the collaborators for all their supports and valuable discussions to my work presented in this thesis. Thanks Amber Wai-Yan Ha from Prof. Ricky N.S. Wong's group, the Department of Biology, HKBU, for providing the NPC patients' serum samples and performing the qRT-PCR assay; Prof. Ricky M.S. Wong, Dr. Wanggui Yang, Mr. Di Xu from the Department of Chemistry, HKBU, for synthesis of the carbazole-based cyanines and performing the ultra-violet measurements.

I must also express my appreciation to all the current and former members in Dr. Li's group, Yi Wong, Kin-Man Lo, Chung-Yan Poon, Dr. Zhongping Li, Jiali Zhang, Hei-Nga Chan and especially Dr. Ho-Man Chan for their precious

discussion and help on all the projects. I would also like to thank other lab-mates in OEW 1304, including Dr. Yeuki Tsoi, Amy Ho, Judy Lum from Dr. Kelvin Sze-Yin group and my group-mates for all the laughter, joyful moment and an excellent working environment during my time at HKBU.

I greatly appreciate all the generous technical support from the department and faculty, especially Mr. John S.L. Ng from the Department of Chemistry and Ms. Louise L.H. Ng from the Department of Biology for helping to order all the chemicals that I needed for my research, thank you for always responding my request so promptly. Thanks Mr. Benson S.C. Leung for helping us to perform the TEM imaging on the iron oxide nanoparticles.

The most importantly, I would like to devote my deepest appreciation to my parents for their support, scarify and unconditional love. Thank you for giving me everything and the opportunity to pursue my undergraduate studies in the U.S., it has been a very great experience for me and really broadened my horizon. I know it was uneasy and "expensive" to raise a kid like me, thank you very much!

The last but by no mean the least, many thanks to all the funding agents for the finical support throughout my whole PhD study. The work presented in this thesis were fully supported by Young Scientists Fund from the National Science Foundation of China (21205006), the University Grants Council of Hong Kong Special Administrative Region, China (GRF/HKBU201612, AoE/M06/08), Faculty Research Grant of Hong Kong Baptist University (FRG2/12-13/035, FRG2/11-12/126). The FEI-Technai G2 TEM used in this work is supported by the Center for Surface Analysis and Research (CSAR) of HKBU with funding

from the Special Equipment Grant from the University Grant Committee of the Hong Kong Special Administrative Region of China, (SEG_HKBU06).

TABLE OF CONTENTS

DECLARATION i

ABSTRACT ii

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS viii

LIST OF TABLES xi

LIST OF FIGURES xii

LIST OF SYMBOLS xviii

LIST OF ABBREVIATIONS xix

CHAPTER 1. INTRODUCTION 1

1.1 Cancer biomarkers 1

1.2 Total internal reflection fluorescence microscopy 4

1.3 References 10

CHAPTER 2. SINGLE MOLECULE DETECTION OF MICRORNA WITH LNA PROBE BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY 13

2.1 Introduction 13

2.2 Experimental 17

2.3 Results and discussion 23

2.4 Conclusions 33

2.5 References 34

CHAPTER 3. DIRECT QUANTIFICATION OF CIRCULATING MIRNAS IN DIFFERENT STAGES OF NASOPHARYNGEAL CANCEROUS SERUM SAMPLES IN SINGLE MOLECULE LEVEL WITH TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY 37

3.1 Introduction 37

3.2 Experimental 42

3.3 Results and discussion...48

3.4 Conclusions 64

3.5 References 65

CHAPTER 4. SELF-ASSEMBLING PROTEIN PLATFORM FOR DIRECT QUANTIFICATION OF CIRCULATING MICRORNAS AT FEMTOMOLAR LEVEL IN SERUM WITH TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY 68

4.1 Introduction 68

4.2 Experimental 73

4.3 Results and discussion...79

4.4 Conclusions 95

4.5 References 96

CHAPTER 5. MAGNETIC NANOPARTICLES BASED IMMUNOPLATFORM FOR DIRECT QUANTIFICATION OF PROSTATE CANCER ANTIGEN (PSA) WITH CARBAZOLE-BASED CYANINE FLUOROPHORE 99

5.1 Introduction 99

5.2 Experimental **103**

5.3 Results and discussion **108**

5.4 Conclusions **127**

5.5 References **128**

GENERAL CONCLUSIONS **131**

LIST OF PUBLICATIONS **133**

ACADAMIC ACHIEVEMENTS **137**

CURRICULUM VITAE **138**