RGC Ref.: N_HKUST631/11 NSFC Ref. : 21161160557
(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Photochemical properties of endogenous biological molecules: fundamental and application (内源生物分子光化学特性的基础和应用研究)

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Jianan Qu	Prof. Luo Yi
Post	Full Professor	Full Professor
Unit / Department / Institution	Biomedical Engineering Program/Electronic and Computer Engineering/ HKUST	Bio-X Division/HeFei National Laboratory for Physical Sciences at the Microscale/USTC
Contact Information	eequ@ust.hk	yiluo@ustc.edu.cn
Co-investigator(s) *(with title and institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2012		
Project Completion date	31/12/2014		
Duration *(in month)*	36		
Deadline for Submission of Completion Report	15/12/2015		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. *To systematically study the photochemical properties of multiphoton excited hemoglobin fluorescence and understand the excited state dynamics of hemoglobin in different biological environment.*
2. *To optimize the multiphoton imaging technique based on hemoglobin fluorescence and to explore its possible applications in life science research and medical diagnosis.*
3. *To synthesize new near-IR non-toxic fluorescence probes based on the understanding of hemoglobin fluorescence and to develop the multiphoton*

microscope system with advanced photonic technique for in vivo deep tissue imaging.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

In this research project, we conducted systematic investigation of endogenous fluorescence from the blood. We demonstrated a label-free *in vivo* flow cytometry in zebrafish blood vessels based on two-photon excited autofluorescence imaging. The major discovery in this work was the strong autofluorescence emission from the plasma in zebrafish blood. The plasma

autofluorescence provided excellent contrast for visualizing blood vessels and counting blood cells. In addition, the cellular nicotinamide adenine dinucleotide autofluorescence enables in vivo imaging and counting of white blood cells (neutrophils). We demonstrated that two-photon excited endogenous fluorescence enabled label-free morphological and functional imaging of various human blood cells. Specifically, we achieved distinctive morphological contrast to visualize morphology of important leukocytes, such as polymorphonuclear structure of granulocyte and mononuclear feature of agranulocyte, through the employment of the reduced nicotinamide adenine dinucleotide (NADH) fluorescence signals. In addition, NADH fluorescence images clearly revealed the morphological transformation process of neutrophils during disease-causing bacterial infection. Our findings also showed that time-resolved NADH fluorescence can be potentially used for functional imaging of the phagocytosis of pathogens by leukocytes (neutrophils) in vivo. In particular, we found that free-to-bound NADH ratios measured in infected neutrophils increased significantly, which is consistent with a previous study that the energy consumed in the phagocytosis of neutrophils is mainly generated through the glycolysis pathway that leads to the accumulation of free NADH. Future work will focus on further developing and applying label-free imaging technology to investigate leukocyte-related diseases and disorders. Zebrafish has rapidly evolved as a powerful vertebrate model organism for studying human diseases. We first demonstrated a new label-free approach for in vivo imaging of microvasculature, based on the recent discovery and detailed characterization of the two-photon excited endogenous fluorescence in the blood plasma of zebrafish. In particular, three-dimensional reconstruction of the microvascular networks was achieved with the depth-resolved two-photon excitation fluorescence (TPEF) imaging. Secondly, the blood flow images, obtained by perpendicularly scanning the focal point across the blood vessel, provided accurate information for characterizing the hemodynamics of the circulatory system. The endogenous fluorescent signals of reduced nicotinamide adenine dinucleotide (NADH) enabled visualization of the circulating granulocytes (neutrophils) in the blood vessel. The development of acute sterile inflammation could be detected by the quantitative counting of circulating neutrophils. Finally, we found that by utilizing a short wavelength excitation at 650 nm, the commonly used fluorescent proteins, such as GFP and DsRed, could be efficiently excited together with the endogenous fluorophores to achieve four-color TPEF imaging of the vascular structures and blood cells. The results demonstrated that the multicolor imaging could potentially yield multiple view angles of important processes in living biological systems. Hemoglobin, one of the most important proteins in the human body, is composed of "heme" groups (iron-containing rings) and "globins" (proteins). We investigated the two-photon excited fluorescence of hemoglobin and its subunit components (heme and globin). We measured the hemoglobin fluorescence lifetime by using a streak camera of ps resolution and confirmed that its lifetime is in femtosecond scale. In the study of the fluorescence properties of heme and globin, the experimental results reveaedl that heme is the sole fluorophore of hemoglobin. Hemoglobin fluorescence can be effectively excited only via two-photon process, because heme has a centrosymmetric molecular structure and two-photon allowed transition is forbidden for single-photon process and vice versa due to the Laporte parity selection rule.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

We plan to conduct the similar study in different class of animal models, such as transgenic mice. This will allow us to test the biocompatibility of new fluorescence probes in the animal model closer to human, and to take the advantage of transgenic technology to label key biological structures for the study of interaction of new probes with them.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Hemoglobin is an essential protein found in red blood cells that is responsible for binding oxygen in the lungs and delivering it to all tissues and organs in the body. Our recent discovery revealed that under short wavelength two-photon excitation, hemoglobin emits strong fluorescence with an extremely short lifetime. In this multidisciplinary research, we have aimed to develop a significant understanding of the photochemical properties of hemoglobin, to explore the applications of hemoglobin fluorescence for life science research and to design and synthesize a new class of fluorescence probe for multiphoton fluorescence imaging. The knowledge acquired from the systematic study of hemoglobin can help us to design fluorescence probes with high excitation efficiency and clear differentiation from the autofluorescence of biological samples. *In vivo* visualization of living biological systems can provide the information on how cells develop, function, communicate, and even die. These are crucial information for life science and the development of medical technology. The success of this study highlights the importance of the basic knowledge about endogenous biological molecules on the development of new fluorescence probes. The new class of probes may advance the multiphoton fluorescence imaging in the applications of life science research and medical diagnosis.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) *(**bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2015				**Qiqi Sun, Wei Zheng, Jiannong Wang, Yi Luo and Jianan Y. Qu***	"Mechanism of two-photon excited hemoglobin fluorescence emission" /Journal of Biomedical Optics (in press)	31-Dec.-2014	Yes.	Yes.	Yes.

2014				**Yan Zeng, Bo Yan, Qiqi Sun, Sicong He, Jun Jiang, Zilong Wen* and Jianan Y. Qu***	"In vivo micro-vascular imaging and flow cytometry in zebrafish using two-photon excited endogenous fluorescence" Biomedical Optics Express, V.5, 653-663 (2014)	31-Dec.-2014	Yes.	Yes.	Yes.
2013				**Yan Zeng,** Bo Yan, **Qiqi Sun, Seng Khoon Teh, Wei Zhang,** Zilong Wen, and **Jianan Y. Qu***	Label-free in vivo imaging of human leukocytes using two-photon excited endogenous fluorescence/Journal of Biomedical Optics, V. 18, 040103 (2013)	30-Jun.-2013	Yes.	Yes.	Yes.
2012				**Yan Zeng,** Jin Xu, **Dong Li,** Li Li, Zilong Wen, **and Jianan Y. Qu***	Label-free *in vivo* flow cytometry in zebrafish using two-photon autofluorescence imaging/ Optics Letters, V.37, 2490-2492 (2012)	30-Jun.-2013	Yes.	Yes.	Yes.

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
San Francisco, USA, Feb. 2-7, 2014	Mechanism of two-photon excited hemoglobin fluorescence emission	Multiphoton Microscopy in the Biomedical Sciences, BiOS 2014, SPIE	31-Dec.-2014	Yes	Yes	Yes
San Francisco, USA, Feb. 2-7, 2013	Two-photon excited endogenous fluorescence for label-free in vivo imaging ingestion of disease-causing bacteria by human leukocytes	Multiphoton Microscopy in the Biomedical Sciences, BiOS 2013, SPIE	30-Jun.-2013	Yes	Yes	Yes
Miami, USA, April 29- May 2, 2012	Invited Talk: Two-photon Excited Blood Autofluorescence for In Vivo Imaging and Flow Cytometry	Meeting on Biomedical Optics, Optical Society of America	30-Jun.-2013	Yes	Yes	Yes
Miami, USA, April 29- May 2, 2012	In vivo cytometry using two-photon autofluorescence microscopy	Meeting on Biomedical Optics, Optical Society of America	30-Jun.-2013	Yes	Yes	Yes
June 2-5, 2013 Lake Tahoe, California, USA	Label-free in vivo imaging of leukocytes and their interaction with bacteria	Advances in Optics for Biotechnology, Medicine and Surgery XIII	30-Jun.-2013	Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Yan, Zeng	Ph.D.	2009	2014

Sun, Qiqi	Ph.D.	2011	2016
Zhang, Wei	M.Phil.	2010	2012

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

N/A