TOTAL INTERNAL REFLECTION FLUORESCENCE AND SCATTERING IMAGING FOR BIOLOGICAL APPLICATIONS

ARTI TYAGI

DEPARTMENT OF BIOCHEMICAL ENGINEERING
AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

FEBRUARY 2023

TOTAL INTERNAL REFLECTION FLUORESCENCE AND SCATTERING IMAGING FOR BIOLOGICAL APPLICATIONS

by

ARTI TYAGI

Department of Biochemical Engineering and Biotechnology

Submitted

in partial fulfillment of the requirements of the degree of Doctor of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

FEBRUARY 2023

Dedicated to Amma, Maa and Papa

Certificate

This is to certify that the thesis entitled **'Total internal reflection fluorescence and scattering imaging for biological applications'**, submitted by **Arti Tyagi** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy** in February 2023, is a record of the original, bonafide research work carried out by her under my supervision and guidance. The thesis has reached the standards fulfilling the requirements of the regulations related to the award of the degree.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma to the best of my knowledge.

Prof. Ravikrishnan Elangovan
Department of Biochemical Engineering and Biotechnology,
Indian Institute of Technology Delhi

Acknowledgements

PhD has been a very challenging phase of my life, academically, physically, mentally and emotionally. It would not have been possible to achieve this monumental feat without crucial support from a lot of people. The last five and a half years have taught me so many things and shaped me into someone with a never-ending curiosity and zeal to understand and learn.

The list of people I would like to thank could be very long. I will begin by expressing my deepest gratitude to my parents, Mr. Khushpal Singh and Mrs. Neelam Tyagi, for always standing by me in all my decisions, for having faith in me, and encouraging me to keep on doing better. Next, I would like to thank the one person who has been the biggest driving force behind this journey, my supervisor, Prof. Ravikrishnan Elangovan. I remember how naive and inexperienced I was when I entered his laboratory. He has been extremely patient and thoughtful with me. I will forever be thankful to him for introducing me to applied optics, a field I have come to love and enjoy. Other than research aptitude and acumen, I have learnt important skills of time management, delegation, planning, execution and documentation from him, which will help me wherever I go.

My SRC members, Prof. Kedar Khare, Prof. Ritu Kulshreshtha and Prof. Ashish Misra, have also been very forthcoming with constructive criticism as well as encouragement and appreciation. The discussions I have had with Prof. Khare have been extremely informative and helpful for my work. I was also involved in a collaborative project with AIIMS and through that I received critical support from mebers of the Oncology department at AIIMS, Dr. Sachin Singla, Dr. Prabhat Singh Mallik and Dr. Amit Dinda. Without their contributions, it would have been nearly impossible to carry out the SNC-TIRS work. Similarly, I received a great deal of help from an inter-disciplinary team of exceptional engineers from one of our lab projects. Mr. Pabhu Balsubramanian, Mr. Bramha Tripathi, and Mr. Tushar Jeet brought along their individual expertise and helped me develop the *i-scope* system.

Other than my professors and colleagues, several of my labmates and friends have been instrumental in completion of my thesis. During my initial years, I acquired a lot of hands-on-experience and instrument knowledge from my seniors, Dr. Pooja

Singh, Dr. Saumya Singh, Dr. Vidhu Soman, and Ms. Jyoti Sharma. These people helped me understand how to effectively function inside a laboratory atmosphere and inculcated a healthy working environment. I have received considerable support and help from the members of our neighbouring laboratory of Prof. Prashant Mishra. His students, Dr. Sanjay Singh and Dr. Senthil Murugan were always ready to share their knowledge as well as laboratory resources with us. I have also been fortunate enough to have amazing labmates and friends, Ms. Shefali Singh, Ms. Neha Khaware, Mr. Jagat Narayan, Ms. Sneha Kumari, Ms. Neelam Upadhyay, and Ms. Ada Zwetlana. They have all taught me so many new things in such varied disciplines, helped me carry out experiments, and always been there for me when I needed them for both work as well as fun!

Other than the academic influences and support, I have been the subject of tremendous moral support and aid from my brother, Mr. Dharmanshu Tyagi and long time friends, Ms. Shalini Goyal, Mr. Shubham Gupta, Ms. Vismaya Vinay, Mr. Himanshu Pandey, and Mr. Ashish Gopalakrishnan. It is very well known that a PhD can be an emotionally taxing time, and without these people it would not have been possible to go through all the ups and downs I have faced throughout this journey.

At last, I would express my sincere thanks to the Indian Institute of Technology Delhi and especially to the Department of Biochemical Engineering and Biotechnology for choosing me to be part of the IITD family and providing me with the means and opportunities to carry out this work. I would also thank the Ministry of Human Resource and Development, Government of India for providing me with financial support through the five years of my PhD.

सारांश

जैविक इमेजिंग एक अत्यंत उपयोगी और लोकप्रिय विश्लेषणात्मक उपकरण है। इसने सूक्ष्मदर्शी तकनीकों में होती प्रगति से महत्वपूर्ण लाभ उठाया है और गुणात्मक के साथ-साथ मात्रात्मक जानकारी प्राप्त करने के एक विश्वसनीय स्त्रोत के रूप में उभरा है। जैविक इमेजिंग में कंट्रास्ट उत्पन्न करने के दो व्यापक साधन प्रतिदीप्ति (फलूरेसेंसे) और प्रकाश प्रकीर्णन (स्कैटरिंग) हैं। ये अलग-अलग तरीके हैं जिनसे प्रकाश, एक विद्युत चुम्बकीय विकिरण, पदार्थ के साथ संपर्क करता है। प्रतिदीप्ति में इलेक्ट्रॉनिक राज्य संक्रमणों के परिणामस्वरूप चयनात्मक तरंगदैर्ध्य का अवशोषण और उत्सर्जन शामिल है। दूसरी ओर, प्रकीर्णन ज़्यादातर कंपन संक्रमणों के परिणामस्वरूप होता है, जिसमें एक नमूने पर पड़ने वाली प्रकाश किरणे अलग-अलग दिशाओं में विक्षेपित होती है। इमेजिंग विधियों और अनुप्रयोगों के संबंध में दोनों तकनीकें अद्वितीय लाभ और हानि की पेशकश करती हैं। अनुसंधान और वाणिज्यिक उद्देश्यों के लिए उपलब्ध मौजूदा इमेजिंग सिस्टम द्वारा प्रदान किए जाने वाले विभिन्न लाभों के बावजूद, इनमें से अधिकांश मामलों में एक सामान्य सीमित कारक है उपकरण और रखरखाव की उच्च लागत। ये प्रणालियां अक्सर भारी उपकरणों पर आधारित होती हैं और प्रशिक्षित ऑपरेटरों की आवश्यकता रखती हैं| इसलिए, आज हमें कॉम्पैक्ट, लागत-प्रभावी, तेज़ और उपयोग में आसान इमेजिंग आधारित प्लेटफॉर्म की आवश्यकता है।

इस काम में मैंने कुल आंतरिक प्रतिबिंब-आधारित ऑप्टिकल मॉड्यूल को नियोजित करके व्यापक संभावित जैविक अनुप्रयोगों के साथ दो इमेजिंग सिस्टम विकसित किए हैं। पहले मामले में, एक पूरी तरह से स्वचालित और लघु सूक्ष्मदर्शी प्रणाली, जिसे आइ-स्कोप कहा जाता है, विकसित किया गया है| यह उच्च आवर्धन एवं संकल्प, बड़े क्षेत्र के दृश्य और बड़े क्षेत्र नमूना स्कैनिंग जैसी सुविधाएं प्रदान करता है। सिस्टम में अधिकांश उद्दत या स्वविक्सित थ्रीडी- प्रिंटेड घटकों के साथ एक सरलीकृत डिज़ाइन शामिल है। यह एक ब्राइटफ़ील्ड और (कुल आंतरिक प्रतिबिंब) प्रतिदीप्ति मोड में नमूना इमेजिंग की सुविधा देता है। आइ-स्कोप का उपयोग दूध दैहिक कोशिकाओं और जीवाणुओं की कोशिका गणना के लिए किया गया है। यह पारंपरिक प्रतिदीप्ति सूक्ष्मदर्शी (फ्लोरोसेंस माइक्रोस्कोप) के स्थान पर एक लागत-प्रभावी, पोर्टेबल, स्वचालित और सुविधाजनक विकल्प प्रदान करता है।

दूसरे मामले में, एसएनसी-टीआईआरएस नामक एकल कण ट्रैकिंग पर आधारित एक लेबल-मुक्त नैनोपार्टिकल लक्षण वर्णन प्रणाली विकसित की गई है। सिस्टम एक इवनेसेंट फ़ील्ड (लुप्त होने वाली) के साथ नमूना कणों को रोशन करता है और कण प्रक्षेपवक्र को मैप करने के लिए बिखरे हुए प्रकाश को इकट्ठा करता है। कणों के हाइड्रोडायनामिक आकार का अनुमान लगाने के लिए एकल कण प्रक्षेपवक्र का उपयोग किया जाता है। इसके अलावा, नमूने में कणों की कुल मात्रा का अनुमान लगाने के लिए अवलोकन मात्रा निर्धारित की जाती है। एसएनसी-टीआईआरएस का मूल्यांकन अकार्बनिक नैनोकणों के साथ-साथ एक्सोसोम नामक जैविक नैनो-पुटिकाओं का उपयोग करके किया गया है।

इस थीसिस कार्य की एक एकीकृत विशेषता उन विधियों और प्रणालियों को विकसित करना है जो, विशेष रूप से सीमित-संसाधन क्षेत्रों में, अधिक सुलभ हैं| आइ-स्कोप और एसएनसी-टीआईआरएस दोनों की अवधारणा और विकास प्रक्रियाएं इस दृष्टिकोण से प्रेरित थीं। दोनों प्रणालियां कोई विस्तृत ढांचागत या नमूना पूर्व-उपचार आवश्यकताओं से बाध्य नहीं हैं| इसके अलावा, वे उपयोगी नैदानिक उपकरण के रूप में काम करने की क्षमता प्रदर्शित करते हैं।

Abstract

Biological imaging is an immensely useful and popular analytical tool. It has gained significantly with advances in microscopic techniques and has emerged as a reliable source of qualitative as well as quantitative information. Two of the most widely used means of contrast generation for biological imaging are fluorescence and light scattering. These are different ways in which light, an electromagnetic radiation, interacts with matter. Fluorescence involves absorption and emission of selective wavelengths as a result of electronic state transitions. Scattering, on the other hand, is more of a result of vibrational transitions wherein light incident on a sample is deflected in different directions. Both techniques offer unique pros and cons with regards to imaging methods and applications. Notwithstanding the various advantages offered by the existing imaging systems available for research and commercial purposes, a common limiting factor in most of these cases is the high cost of instrumentation and maintenance. These systems are also often bulky and require trained operators, and hence, there is a need for compact, cost-effective, rapid and easy-to-use imaging based platforms.

In this work I have developed two imaging systems with wide potential biological applications by employing a total internal reflection-based optical module. In the first case, a fully automated and miniaturized microscopy system, called the *i-scope*, has been developed which offers features such as a high magnification, resolution, larger field-of-view and a large-area sample scanning. The system comprises of a simplistic design with off-the-shelf or in-house 3D printed components. It allows sample imaging in a brightfield and (total internal reflection) fluorescence modes. The *i-scope* has been used for cell enumeration of milk somatic cells and bacteria. It offers a cost-effective, portable, automated and convenient alternative to conventional fluorescence microscopes.

In the second case, a label-free nanoparticle characterisation system based on single particle tracking, called SNC-TIRS, is developed. The system illuminates sample particles with a evanescent field and collects the scattered light to map particle trajectories as they diffuse through the observation volume. The single particle trajectories are used to estimate hydrodynamic sizes of the particles. Moreover, the observation volume is quantified to estimate sample concentration. SNC-TIRS

has been evaluated using inorganic nanoparticles as well as biological nano-vesicles called exosomes.

A unifying feature of this thesis work has been to develop methods and systems which are more accessible, especially in low-resource field settings. The conceptualisation and development processes of both *i-scope* and SNC-TIRS were driven by this approach. Both systems do not impose any elaborate infrastructural or sample pre-treatment requirements. Furthermore, they display potential to serve as useful diagnostic tools.

Contents

List of Figures

List of Tables

App Application
3D Three Dimensional
TIRF Total Internal Reflection Fluorescence
TIRFM Total Internal Reflection Fluorescence Microscopy
cTIRF Compact Total Internal Reflection Fluorescence
TIRS Total Internal Reflection Scattering
GUI Graphical User Interface
LED Light Emitting Diode
MJP MultiJet Printer
NA Numerical Aperture
SNR Signal-to-noise Ratio
Webcam Web camera
f/# F-number
v/v Volume-by-volume ratio
DMSO Dimethyl Sulfoxide
TB Tuberculosis
AFB Acid Fast Bacillus
NSCLC Non-Small Cell Lung Cancer
PCB Printed Circuit Board
TEM Transmission Electron Microscopy
HR-TEM High Resolution-Transmission Electron Microscopy
R.I. Refractive Index
RBC Red Blood Cell
WBC White Blood Cell
FOV Field-of-view