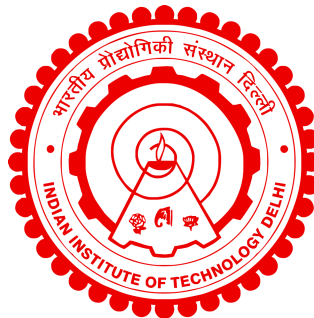


DEVELOPMENT OF QUANTITATIVE PHASE AND TIRF MICROSCOPE FOR BIO-PHOTONICS APPLICATIONS

ANUJ SAXENA



**CENTRE FOR SENSORS, INSTRUMENTATION AND CYBER
PHYSICAL SYSTEM ENGINEERING (SeNSE)
INDIAN INSTITUTE OF TECHNOLOGY DELHI
JUNE 2026**

© Indian Institute of Technology Delhi (IITD), New Delhi, 2026

DEVELOPMENT OF QUANTITATIVE PHASE AND TIRF MICROSCOPE FOR BIO-PHOTONICS APPLICATIONS

by

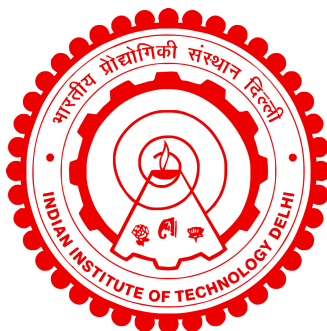
Anuj Saxena

Centre for Sensors, Instrumentation and Cyber Physical System
Engineering (SeNSE)

Submitted

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

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

June 2026

Dedicated to my family

Certificate

This is to certify that the thesis entitled '**DEVELOPMENT OF QUANTITATIVE PHASE AND TIRF MICROSCOPE FOR BIO-PHOTONICS APPLICATIONS**', submitted by **Anuj Saxena** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy** in Centre for Sensors, Instrumentation and Cyber Physical System Engineering (SeNSE), is a record of the original, bona fide research work redcarried out by him 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.

Professor Dalip Singh Mehta
Department of Physics,
Indian Institute of Technology Delhi.

Professor Satish Kumar Dubey
Centre for Sensors, Instrumentation and Cyber Physical System Engineering (SeNSE),
Indian Institute of Technology Delhi.

Professor Balpreet Singh Ahluwalia
Department of Physics and Technology,
UiT The Arctic University of Norway.

Date:

Acknowledgements

It is with immense gratitude and humility that I reflect upon the journey culminating in this thesis. Over these years, I have been guided, supported, and inspired by many individuals whose contributions have shaped both my academic and personal growth.

I would like to express my profound respect and sincere gratitude to *Prof Dalip Singh Mehta*, whose mentorship has been the cornerstone of my academic journey. His visionary guidance, deep scientific insight, and constant encouragement have been invaluable throughout the course of my research. From my initial exposure to his course PYL-760 (Biomedical Optics and Biophotonics) during my master's studies at IIT Delhi, his passion for optics and biophotonics has been a continuous source of inspiration. His persistent support, generosity with ideas, and unwavering belief in my abilities have profoundly shaped my scientific outlook and personal growth. I am deeply indebted to him for his trust, patience, and for setting a lasting example of intellectual rigor and humility.

I owe special thanks to *Prof. Balpreet Singh Ahluwalia* for his exceptional mentorship, scientific insight, and constant encouragement. His thoughtful guidance and constructive feedback have been instrumental in shaping the direction and depth of this research. The Nanoscopy Course under his supervision offered a new perspective on research and significantly influenced the development of my super-resolution imaging work. His valuable advice on experimental design, data interpretation, and scientific writing greatly enriched this thesis. His genuine kindness, clarity of thought, and enthusiasm for science continue to inspire me deeply.

I would also like to express my sincere thanks to *Prof. Satish Kumar Dubey* for his valuable guidance, constructive suggestions, and continued encouragement during the course of my research. His insightful inputs and supportive attitude have been greatly appreciated and have contributed meaningfully to the progress of this work.

I owe my heartfelt gratitude to my parents, *Mr. Kuldeep Saxena and Mrs. Brij Lata Saxena*, for their unconditional love, sacrifices, and enduring faith in my pursuits. Their unwavering support and values have been my guiding strength throughout this journey. I am equally grateful to my siblings, *Mrs. Neha Saxena and Mr. Atul Saxena*, for their constant encouragement, affection, and belief in me. I acknowledge my nephew *Kiyansh* and my niece *Advika* for their joyful presence and positive energy throughout this journey.

My sincere appreciation goes to *Dr. Azeem Ahmad* for his consistent support, scientific inputs, and valuable advice during the early stages of my research. His patience, technical expertise, and constructive discussions played a significant role in the development of this thesis.

I also gratefully acknowledge the academic and infrastructural support provided by IIT Delhi and UiT Tromsø, which facilitated the successful execution of this research. I am thankful to *Dr. Vishesh Dubey and Dr. Shivani Bhardwaj* for their insightful suggestions, discussions, and constant motivation. I extend my appreciation to *Dr. Sathi Das, Mr. Kian Sartipzadeh, Mr. Keyvan Sartipzadeh, Ms. Jasmin, Mr. Abhishek Ranjan, and Dr. Komal Aggarwal* for their kind assistance and companionship, which made my stay in Norway both productive and memorable. I also wish to thank *Dr. Luis E. Villegas-Hernández, and Dr. Deanna Wolfson*, for their assistance with laboratory protocols, as well as *Dr. Anowarul Habib* for providing me with the opportunity to work in his lab. Special thanks are also due to *Dr. Jean-Claude Tinguely and Dr. Hong Mao* for providing biological samples and sharing their expertise, which greatly contributed to the completion of this work.

I would like to express my appreciation to my labmates and colleagues —*Anjika, Anand Kumar, Sachin Dhawan, Sunil Bhatt, Pramila Thapa, and Meenakshi*— for their cooperation, stimulating discussions, and shared commitment to research. I am also grateful to my friends —*Rishi Raj, Sanchayan Pal, Vyom Sharma, Sandeep Kumar, Vikas Kumar, Mohit Tomar, Prabhav Joshi, Arpit Gupta, Sheetal Devinder, Ashwani Sharma, Yatharth Tyagi, Rajneesh Kashyap and Nayeem* for their friendship, encouragement, and the many moments of laughter and motivation that lightened the challenges of this journey. I sincerely acknowledge my gym trainer and dear friend, *Mr. Deepak Bhatt*, for instilling discipline and motivation that strengthened my health, and for his constant support and joyful companionship.

Finally, I extend my heartfelt thanks to all those whose names may not have been mentioned here but who have, in various ways, contributed to this work. Each of you has played an important role in making this journey meaningful and fulfilling.

To all my mentors, colleagues, friends, and family, I express my sincere gratitude for your invaluable contributions and unwavering support throughout this doctoral endeavor.

Anuj Saxena

Abstract

This thesis presents the development and application of advanced label-free and fluorescence-based optical imaging techniques for high-contrast, quantitative, and super-resolved visualization of biological and industrial samples. The work focuses on the design, implementation, and validation of novel microscopy systems that address key challenges in optical imaging, including low contrast, temporal instability for dynamic events, and diffraction-limited resolution.

Firstly, a calcite-based common-path quantitative phase microscopy system for accurate phase measurements and high-contrast imaging of semi-transparent biological samples has been presented. The system demonstrated high temporal stability and robust performance in monitoring dynamic cellular events, such as red blood cell membrane fluctuations and mouse embryonic fibroblast cell dynamics, with ~ 20 mrad phase stability under ambient conditions. Building on this, a partially spatially coherent light source-based single-shot quantitative differential phase contrast microscopy system was developed to overcome the limitations of conventional differential phase contrast and differential interference contrast phase-shifting approaches. The use of a partially coherent source significantly reduced laser coherence noise and speckle artifacts, improving image quality and contrast. The proposed optical configuration, employing a calcite beam displacer, generated two laterally sheared, parallel beams that produced high-density interference fringes, enabling single-shot gradient phase imaging along the shear direction. For complete quantitative phase reconstruction, two orthogonally sheared gradient phase images were acquired and numerically integrated to obtain accurate and artifact-free quantitative phase maps. This approach allowed rapid, high-contrast visualization of cellular morphology and refractive index variations, facilitating detailed analysis of disease-related biophysical properties. Furthermore, the system's geometry enables seamless integration with fluorescence, optical trapping, and microfluidic modules, allowing for versatile multimodal imaging of biological processes.

Next, a novel coverslip-guided total internal reflection fluorescence (Cg-TIRF) microscopy system was developed to provide a simple, cost-effective, and compact solution for surface-selective fluorescence imaging. The system utilizes a conventional glass coverslip as a multimode slab waveguide, where thousands of guided modes collectively produce a spatially averaged evanescent field, ensuring uniform illumination over a large field of view without

the need for mechanical scanning. This innovative configuration eliminates the need for complex optical components, making the system easily adaptable to standard microscopy setups. Furthermore, by introducing temporal modulation of the laser diode, controlled intensity fluctuations were generated electronically through current modulation, providing a straightforward and low-cost means of achieving fluorescence intensity variations. When combined with the intensity fluctuation optical nanoscopy (IFON) algorithm, these modulations enabled artifact-free super-resolution imaging, demonstrating around threefold improvement in resolution using polystyrene beads and fixed human bone osteosarcoma epithelial cells (U2OS cell line). This work establishes a simplified and accessible approach for achieving both high-contrast and super-resolved fluorescence imaging using standard optical substrates.

The laser-based Cg-TIRF system enabled high-contrast, surface-selective fluorescence imaging. When applied to FFPE tissue sections, however, coherent laser excitation enhanced background signals from residual chemical compounds. To mitigate this, an LED-based TIRF platform was developed. Its reduced temporal coherence minimized interference artifacts while preserving shallow evanescent excitation, thereby improving fluorescence image quality in chemically processed tissues.

To expand surface-selective imaging beyond fluorescence labeling, a complementary label-free near-field approach was implemented using the same Cg-TIRF configuration. Under 488 nm excitation, intrinsic photoluminescence of the glass coverslip served as a localized near-field illumination source. In combination with the evanescent field, surface-proximal objects scattered and modified the confined light, producing high-contrast, surface-selective images without labels. Validation using calibrated microspheres and biological samples confirmed accurate feature visualization compared to bright-field microscopy. Temporal modulation of illumination further enabled intensity-fluctuation-based label-free super-resolution imaging.

Collectively, these systems provide versatile platforms for label-free quantitative phase imaging, surface-selective fluorescence, and super-resolution microscopy. The approaches developed in this thesis offer low-cost, compact, and multimodal solutions, opening new avenues for live-cell analysis, histopathology, and high-resolution optical characterization of biological and industrial samples.

सारांश

यह शोधप्रबंध जैविक तथा औद्योगिक नमूनों के उच्च विषमता, मात्रात्मक तथा अतिविभेदित दृश्यांकन हेतु उन्नत लेबल-रहित एवं प्रतिदीप्ति-आधारित प्रकाशीय प्रतिबिंबन तकनीकों के विकास एवं अनुप्रयोग का विस्तृत अध्ययन प्रस्तुत करता है। यह कार्य नवीन सूक्ष्मदर्शी प्रणालियों के अभिकल्प, कार्यान्वयन तथा प्रमाणीकरण पर आधारित है, जिनका उद्देश्य प्रकाशीय प्रतिबिंबन में विद्यमान प्रमुख सीमाओं, जैसे अल्प विषमता, गतिशील घटनाओं के अध्ययन में कालिक अस्थिरता तथा विवर्तन-सीमित विभेदन, को दूर करना है।

प्रथम चरण में अर्धपारदर्शी जैविक नमूनों के सटीक कला मापन एवं उच्च विषमता प्रतिबिंबन हेतु कैल्साइट-आधारित समपथ मात्रात्मक कला सूक्ष्मदर्शी प्रणाली विकसित की गई। इस प्रणाली ने परिवेशीय परिस्थितियों में लगभग ~ 20 mrad कला स्थिरता के साथ लाल रक्त कोशिका झिल्ली उतार-चढ़ाव तथा माउस भ्रूणीय फाइब्रोब्लास्ट कोशिकाओं की गतिकी जैसी कोशिकीय घटनाओं के अध्ययन में उत्कृष्ट कालिक स्थिरता एवं विश्वसनीय प्रदर्शन प्रदर्शित किया।

इसके उपरान्त आंशिक स्थानिक सुसंगत प्रकाश स्रोत आधारित एकल-चित्रण मात्रात्मक विभेदक कला-विषमता सूक्ष्मदर्शी प्रणाली विकसित की गई, जिससे पारंपरिक विभेदक कला-विषमता तथा विभेदक व्यतिकरण-विषमता कला-स्थानांतरण तकनीकों की सीमाओं को दूर किया जा सके। आंशिक सुसंगत स्रोत के उपयोग से लेज़र सुसंगति-जनित शोर तथा स्पेकल विरूपणों में उल्लेखनीय कमी प्राप्त हुई, जिससे प्रतिबिंब गुणवत्ता एवं विषमता में सुधार हुआ। प्रस्तावित प्रकाशीय विन्यास में कैल्साइट किरण-विस्थापक द्वारा पार्श्व-विस्थापित समानांतर किरणें उत्पन्न की गईं, जिनसे उच्च-घनत्व व्यतिकरण फ्रिंज निर्मित हुए तथा शियर दिशा में एकल-चित्रण ग्रेडिएंट कला प्रतिबिंबन संभव हुआ। पूर्ण मात्रात्मक कला पुनर्निर्माण हेतु परस्पर लंबवत शियरित ग्रेडिएंट कला प्रतिबिंब प्राप्त कर उनका संख्यात्मक समाकलन किया गया, जिससे सटीक एवं विरूपण-रहित मात्रात्मक कला मानचित्र प्राप्त हुए। इस तकनीक द्वारा कोशिकीय संरचना तथा अपवर्तनांक परिवर्तनों का तीव्र एवं उच्च विषमता दृश्यांकन संभव हुआ, जिससे रोग-संबंधित जैवभौतिक गुणों के विस्तृत विश्लेषण में सहायता प्राप्त हुई। इसके अतिरिक्त, प्रणाली की ज्यामिति प्रतिदीप्ति, प्रकाशीय पाशन तथा सूक्ष्मद्रविकी मॉड्यूलों के साथ सरल एकीकरण की अनुमति देती है, जिससे जैविक प्रक्रियाओं का बहुआयामी प्रतिबिंबन संभव हुआ।

इसके पश्चात् सतह-चयनात्मक प्रतिदीप्ति प्रतिबिंबन हेतु एक नवीन कवर्स्लिप-निर्देशित पूर्ण आंतरिक परावर्तन प्रतिदीप्ति (Cg-TIRF) सूक्ष्मदर्शी प्रणाली विकसित की गई, जो सरल, अल्प-लागत तथा संक्षिप्त संरचना प्रदान करती है। इस प्रणाली में पारंपरिक काँच कवर्स्लिप को बहुरूपी स्लैब तरंगपथ-निर्देशक के रूप में उपयोग किया गया, जिसमें सहस्रों निर्देशित विधाएँ सामूहिक रूप से स्थानिक रूप से औसत क्षीणन क्षेत्र उत्पन्न करती हैं। परिणामस्वरूप यांत्रिक स्कैनिंग की आवश्यकता के बिना विस्तृत दृश्य क्षेत्र में समरूप प्रकाशन प्राप्त हुआ। यह अभिनव विन्यास जटिल प्रकाशीय अवयवों की आवश्यकता को समाप्त करता है तथा प्रणाली को मानक सूक्ष्मदर्शी व्यवस्थाओं के साथ सरलतापूर्वक अनुकूलनीय बनाता है। इसके अतिरिक्त, लेज़र डायोड के कालिक मॉड्यूलेशन द्वारा धारा मॉड्यूलेशन के माध्यम से नियंत्रित तीव्रता उतार-चढ़ाव उत्पन्न किए गए, जिससे प्रतिदीप्ति तीव्रता

परिवर्तनों को सरल एवं अल्प-लागत विधि द्वारा प्राप्त किया जा सका। इंटेंसिटी फ्लक्चुएशन ऑप्टिकल नैनोस्कोपी (IFON) एल्गोरिथ्म के साथ संयोजन करने पर इन मॉड्यूलेशनों द्वारा विरूपण-रहित अतिविभेदन प्रतिबिंबन प्राप्त हुआ तथा पॉलीस्टाइरीन सूक्ष्मकणों एवं स्थिर मानव अस्थि ऑस्टियोसाकोमा उपकला कोशिकाओं (U2OS कोशिका रेखा) में लगभग तीन गुना विभेदन सुधार प्रदर्शित किया गया। यह कार्य मानक प्रकाशीय अधःस्तरों का उपयोग करते हुए उच्च विषमता एवं अतिविभेदन प्रतिदीप्ति प्रतिबिंबन की सरल एवं सुलभ विधि स्थापित करता है।

लेज़र-आधारित Cg-TIRF प्रणाली द्वारा उच्च विषमता एवं सतह-चयनात्मक प्रतिदीप्ति प्रतिबिंबन संभव हुआ। तथापि, FFPE ऊतक अनुभागों पर इसके अनुप्रयोग में सुसंगत लेज़र उद्दीपन के कारण अवशिष्ट रासायनिक यौगिकों से उत्पन्न पृष्ठभूमि संकेतों में वृद्धि देखी गई। इस समस्या के समाधान हेतु एल. ई. डी.-आधारित पूर्ण आंतरिक परावर्तन प्रतिदीप्ति प्लेटफॉर्म विकसित किया गया। इसकी निम्न कालिक सुसंगतता ने व्यतिकरण विरूपणों को न्यूनतम किया, जबकि उथले क्षीणन उद्दीपन को संरक्षित रखा, जिससे रासायनिक रूप से संसाधित ऊतकों में प्रतिदीप्ति प्रतिबिंब गुणवत्ता में उल्लेखनीय सुधार प्राप्त हुआ।

प्रतिदीप्ति लेबलिंग से परे सतह-चयनात्मक प्रतिबिंबन का विस्तार करने हेतु समान Cg-TIRF विन्यास का उपयोग करते हुए एक पूरक लेबल-रहित निकट-क्षेत्र तकनीक विकसित की गई। 488 nm उद्दीपन के अंतर्गत काँच कवर्स्लिप की अंतर्जात प्रकाशदीप्ति ने स्थानीयकृत निकट-क्षेत्र प्रकाश स्रोत के रूप में कार्य किया। क्षीणन क्षेत्र के साथ संयुक्त रूप से सतह-समीप वस्तुओं ने सीमित प्रकाश को प्रकीर्णित एवं परिवर्तित किया, जिससे बिना लेबल के उच्च विषमता एवं सतह-चयनात्मक प्रतिबिंब प्राप्त हुए। प्रमाणित सूक्ष्मकणों तथा जैविक नमूनों के माध्यम से प्रमाणीकरण में ब्राइट-फील्ड सूक्ष्मदर्शिता की तुलना में सटीक संरचनात्मक दृश्यांकन की पुष्टि हुई। प्रकाशन के कालिक मॉड्यूलेशन द्वारा इंटेंसिटी फ्लक्चुएशन-आधारित लेबल-रहित अतिविभेदन प्रतिबिंबन भी संभव हुआ।

सामूहिक रूप से, इस शोध में विकसित प्रणालियाँ लेबल-रहित मात्रात्मक कला प्रतिबिंबन, सतह-चयनात्मक प्रतिदीप्ति तथा अतिविभेदन सूक्ष्मदर्शिता हेतु बहुउपयोगी प्लेटफॉर्म प्रदान करती हैं। प्रस्तुत विधियाँ अल्प-लागत, संक्षिप्त एवं बहुआयामी समाधान उपलब्ध कराती हैं, जो जीवित कोशिका विश्लेषण, ऊतक रोगविज्ञान तथा जैविक एवं औद्योगिक नमूनों के उच्च-विभेदन प्रकाशीय अभिलक्षणन के लिए नवीन संभावनाएँ प्रस्तुत करती हैं।

Certificate

Acknowledgements

Abstract

Contents

List of Figures

List of Tables

Abbreviations

1	Introduction	1
1.1	Historical overview of conventional microscopy	1
1.1.1	Brightfield and darkfield microscopy	3
1.1.2	Phase contrast and DIC microscope	4
1.2	Imaging parameters	6
1.2.1	Magnification	7
1.2.2	Image field of view	9
1.2.3	Optical resolution	9
1.2.4	Image Contrast	15
1.3	Quantitative phase imaging (QPI)	16

1.3.1	Off-axis interferometry	18
1.3.2	Differential Phase Contrast microscope	23
1.4	Fluorescence microscopy	25
1.4.1	Widefield epi-fluorescence microscopy	27
1.4.2	Total internal reflection fluorescence microscopy	29
1.4.3	Various TIRF modules	32
1.5	Super-resolution optical microscopy	36
1.6	Practical considerations for IFON applications	37
1.7	Thesis overview	40
1.7.1	Challenges and Organization of the Thesis	41
2	Dynamic quantitative phase imaging using calcite crystal-based temporally stable interferometer	45
2.1	Introduction	46
2.2	Objective	49
2.3	Methodology	50
2.3.1	Working principle of calcite crystal as a beam displacer	50
2.3.2	Theory	52
2.3.3	Experimental setup	54
2.4	Results and discussions	57
2.4.1	Temporal Phase Stability	57
2.4.2	Quantitative Phase Imaging	58
2.4.3	Membrane fluctuation of human RBCs	62
2.4.4	Mouse embryonic fibroblast (MEF) cell dynamics	66
2.5	Summary	67
3	Single-shot quantitative differential phase contrast microscope using a single calcite beam displacer	69
3.1	Introduction	70
3.2	Objective	73
3.3	Theory and experimental setup	74
3.3.1	Principle and implementation of Calcite beam displacer-based Q-DPCM configuration	74
3.3.2	Theoretical model for QDPCM imaging	77
3.4	Results and discussion	78
3.4.1	Phase gradient imaging of Polystyrene spheres	78
3.4.2	Measurement of shear	80
3.4.3	Gradient phase imaging of fixed HeLa cell	83
3.4.4	Phase gradient imaging of live U2OS cell	83
3.4.5	Quantitative phase measurement	85
3.5	Conclusion	89

4	Coverslip-guided total internal reflection fluorescence microscopy for super-resolved bio-imaging	91
4.1	Introduction	92
4.2	Methodology	99
4.2.1	Working principle of Cg-TIRF	99
4.2.2	Guided light's power distribution and modes generation	101
4.2.3	Experimental details	103
4.2.4	Principle of resolution enhancement in Cg-TIRF	105
4.2.5	Mathematics of MUSICAL	105
4.2.6	Laser modulation parameters	109
4.3	Results and discussion	113
4.3.1	Sample preparation	113
4.3.2	Cg-TIRF imaging	114
4.3.3	Intensity fluctuations analysis	117
4.3.4	Higher resolution in Cg-TIRF	123
4.4	Summary	133
4.5	Challenges and limitations of the work	134
4.5.1	Alignment Sensitivity and Real-World Variability	135
4.5.2	Scattering and Reflection Losses at the Glass Interface	135
4.5.3	Imaging Limitations in FFPE Tissue Sections Using Cg-TIRF	136
5	LED array-based TIRF microscopic system for histological applications	139
5.1	Introduction	141
5.2	Objectives	145
5.3	Materials & Methods	146
5.3.1	FFPE Lung Tissue Sample Preparation Protocol	146
5.3.2	Methodology	149
5.3.3	Sample Compatibility and Surface Selectivity	153
5.4	LED-TIRF microscopy	154
5.5	Experimental setup	155
5.6	Results and discussions	157
5.6.1	Histology on conventional BF and LED-based TIRF imaging	157
5.6.2	Histology on conventional Epi-fluorescence and LED-based TIRF imaging	159
5.7	Summary	164
5.8	Challenges of the Work	165
5.8.1	Spectral Overlap and Lack of Excitation Filtering	165
5.8.2	Fluorescence Background and Autofluorescence Control	169
5.8.3	Limited Coherence and Mode Control	170
5.8.4	Mechanical Alignment and Coupling Stability	171

6	Evanescent-based Near-field Illumination for Label-free High-Contrast Imaging of Biological Samples	173
6.1	Introduction	174
6.2	Methods and Materials	178
6.2.1	Working principle	178
6.2.2	EXPERIMENTAL SETUP	181
6.2.3	Sample preparation	183
6.3	RESULTS AND DISCUSSION	185
6.3.1	BF and In-TIRM imaging	185
6.3.2	Feature detection and measurement	190
6.4	Summary	192
7	Summary and future scope	195
7.1	Summary	195
7.2	Comparative Benchmarking of Developed Imaging Systems	200
7.3	Future scope	202
	 Bibliography	 205
	 List of Publications	 235
	 Bio-data	 242

List of Figures

1.1	(Left) A labeled diagram of a standard BF microscope, highlighting key components such as the objective lenses, specimen stage, condenser lens, and light source. (Right) A comparison of the illumination and imaging principles of brightfield (a) and DF (b) microscopy. In brightfield microscopy, light passes directly through the specimen, and the image is formed from the transmitted light. In DF, an opaque disc blocks the central light, so only scattered or refracted light from the specimen is captured against a dark background. Adapted from [1]	4
1.2	(Left) An illustration of the optical pathway for PC microscopy, showing how an annular diaphragm at the condenser and a phase plate in the objective lens work together to convert phase shifts from the specimen into visible changes in brightness, creating contrast for transparent samples. (Right) A diagram of DIC microscopy, also known as Nomarski microscopy. This technique uses two Nomarski prisms (Wollaston prisms) and polarizers to split the light beam into two orthogonally polarized beams that pass through the specimen. The phase differences between these beams are then converted into intensity variations, creating a three-dimensional-like relief image of the specimen [2, 3].	6
1.3	Schematic illustration of a microscope FOV. While in traditional optical microscopes the FOV appears circular, in modern systems using digital cameras, it is defined by the rectangular dimensions of the camera sensor, under which every box shows a pixel of the camera chip. Adapted from [4]	8
1.4	Diffraction limit and resolution. The left plot shows two light-emitting sources separated by a distance large enough to be distinguished as individual points. The right plot depicts two sources placed closer than the diffraction limit, causing their signals to overlap and preventing them from being resolved [5].	11
1.5	Symmetrical visualization of the PSF in the XY-, YZ-, and XZ-planes. The simulation, performed using the PSF generator in FIJI [34], highlights the lateral Airy pattern and the elongated axial profile. Simulated with a PSF Generator plugin in FIJI.	12

1.6	Schematic of NA. Low-NA objectives have a long working distance with a narrow light cone, while high-NA objectives have a short working distance with a wide light cone.	13
1.7	Schematic of the off-axis quantitative phase imaging system. A 660 nm laser is passed through a rotating diffuser (RD), collimated by an objective lens (MO_1), and coupled into a multimode fiber (MF) to generate pseudo-spatial coherence (PSC). The output is relayed through lenses (L_1 , L_2) and directed to a BS, which splits the beam into sample and reference arms. The sample arm is imaged using MO_2 , while the reference beam is reflected by a mirror (M) and relayed by MO_3 . Interference fringes are captured by a CMOS camera through lens L_3 . (a) Raw interference fringes recorded using direct laser illumination, showing strong speckle and non-uniformity, (b) Interference fringes obtained with a pseudo-spatially coherent laser (PSCL), exhibiting reduced speckle and improved fringe uniformity. Adapted from [6].	20
1.8	Schematic and working principle of Differential Phase Contrast (DPC) microscopy using asymmetric LED illumination. (a) The system uses a programmable LED array to provide asymmetric illumination from two directions—LED 1 (left) and LED 2 (right)—in alternating time frames t_i and t_{i+1} . The sample, placed above the microscope objective, introduces phase gradients that modify the light path. The resulting intensity-modulated beam is imaged onto a camera via a tube lens and processed to generate a DPC image. (b) Comparison of image contrast under simultaneous (top) and differential (bottom) illumination using a sample composed of two thin metal plates. Differential illumination reveals edge contrast enhanced by phase gradient information, while simultaneous illumination lacks phase sensitivity. (c) Timing diagram for differential illumination control, where LED 1 and LED 2 are alternately turned on and off at times t_i and t_{i+1} , enabling directional phase gradient extraction. (b & c are adapted from [7])	24
1.9	Simplified Jablonski diagram. Upon excitation by a photon, the fluorescent molecule transitions from the ground state level to an excited state. After a short relaxation time of combined vibrational and heat losses, the molecule transitions back to the ground state, emitting light with a less energetic wavelength compared to the excitation light. Adapted from ref. [8].	26
1.10	Fluorescence imaging using an epifluorescence microscopy configuration. (a) Schematic of the optical setup, where excitation light is directed through an excitation filter and reflected by a dichroic mirror into the objective lens to illuminate the sample. The emitted fluorescence is collected by the same objective, transmitted through the dichroic and emission filter, and detected by the camera. (b) Fluorescence image of stained tissue showing fibrous structures (scale bar: 50 μm). (c) Wide-field fluorescence image of tissue section highlighting structural heterogeneity (scale bar: 50 μm). (d) Magnified region from (c), providing higher-resolution detail of cellular and fibrous components (scale bar: 25 μm).	28

1.11	Evanescent field excitation. (a) Schematic of evanescent field generation in TIRF microscopy, with the dotted curve indicating penetration depth. (b) Variation of penetration depth with refractive index contrast for glass, Si_3N_4 , and TiO_2 cores, showing shallower fields at higher contrast. Adapted from [4].	31
1.12	Glass-based TIRF microscopy configurations: (a) Objective-based TIRF, where the same high-NA objective lens is used for both excitation and fluorescence collection, offering strong confinement but a limited field of view. (b) Prism-based TIRF, where excitation is introduced through a prism, decouples illumination and detection, thus allowing fluorescence collection with an arbitrary objective lens.	33
1.13	Conventional waveguide-based TIRF: (a) opaque configuration, (b) transparent configuration of the photonic chip.. Image adapted from Ref. [9].	35
1.14	Working principle of waveguide-based TIRF microscopy. (a) Schematic of lateral edge coupling into multimode waveguides, where guided modes establish evanescent fields at the waveguide-sample interface. (b) Experimental demonstration: lateral scanning of the coupling objective produces uniform illumination of cells, with the averaged image (right) showing enhanced structural contrast under waveguide-TIRF. Image adapted from Ref. [10].	36
1.15	Schematic illustration of the IFON approach. After acquiring an image stack, pixel-wise intensity fluctuations over time are subjected to statistical analysis by the IFON algorithm, enabling the extraction of sub-diffraction information from the sample. Adapted from ref. [11].	38
2.1	(a) The schematic of the calcite crystal with its optic axis plane, (b) represents the normal beam incident on the calcite beam displacer and splits the incident beam into o-ray and e-ray.	51
2.2	The schematic of the transmission-mode common-path off-axis quantitative phase microscope for biomedical imaging. MO: Microscope objective; and IP: Image plane. The amplitude mask has a through-hole (dia. 6mm) and pinhole (dia. 20 μm) situated at the Fourier transform plane. L_2 : 60mm, L_3 : 150mm and L_4 : 120mm.	56
2.3	Temporal phase stability measurement: (a) Recorded interferogram of reference and object beam without sample, (b) Shows phase variation as a function of time at four different locations: (20,20), (20,420), (420,20), and (420,420), and the calculated temporal stability is around 20 mrad. FOV= 512 $\times$ 512 pixels or (1391.92 $\times$ 1391.92) μm for MO: 60 $\times$ of 0.9 NA	58
2.4	(a) An interferometric image of polystyrene spheres (dia. $\sim 12.01 \pm 0.07 \mu\text{m}$) with the zoomed interference fringes recorded using the present nearly common-path QPM. (b-d) 2D view, 3D view, and corresponding line profile (along the yellow dotted line) of the reconstructed phase map obtained using the Fourier transform method. (e-g) 2D view, 3D view, and corresponding line profile (along the yellow dotted line) of the height map of polystyrene spheres obtained from the phase map. FOV = 512 $\times$ 512 pixels or (1391.92 $\times$ 1391.92) μm^2	60

2.5	(a) A full interferogram of human RBCs recorded using the present QPM system, (b) shows a single RBC and fringe bending due to cell shape. (c, d) 2D and 3D views of the reconstructed phase image of human RBCs obtained using the Fourier transform algorithm and the color bar showing the phase in radians.	61
2.6	Measurement of membrane fluctuation of live healthy RBC. (a) One of the recovered phase maps of healthy human RBCs corresponding to the time-lapsed interferometric movie, point A, and B represent the location at the cover glass and at the RBC's membrane, respectively. (b) Phase fluctuations at point A and B. and (c) Respective height fluctuations at point A, B for 30 seconds.	64
2.7	(a-i) Reconstructed phase maps of MEF cells over 2 hours and recorded using a 40x MO of 0.7 NA objective lens. The reconstructed phase maps present the cell functioning and morphological changes at different time points. . . .	67
3.1	depicts a schematic of a single-shot off-axis Q-DPCM setup. A partially spatially coherent light (PSCL) source illuminates the sample, effectively reducing speckle noise compared to direct laser [see (a, b)] and enhancing phase accuracy measurement. The panels (a) and (b) represent the fringe pattern obtained with a highly coherent laser and PSCL, respectively. (c) Illustrates object beam splitting and generated shear between OB and EB. (d) Represents the polarization direction of light at different places in color-coded form. (e) Zoomed view of the camera position from IP_2 and shear directions along the x-direction. Abbreviations: L_1 , L_2, L_3 and L_4 : Lenses; RD: Rotating Diffuser; MMF: multimode fiber and MO: Microscope Objective, IP: Image Plane.	75
3.2	(a) Recorded polystyrene beads differential interferogram (inset shows magnified fringes of the dashed boxed area); (b) Fourier transform of the differential interferogram: the red square shows the frequency component used for obtaining differential phase. (c) Differential phase map of the PS spheres, while the dashed arrow shows the respective sheared direction. The scale bar is $8 \mu\text{m}$	80
3.3	(a) depicts the line profile of gradient phase along a PS bead [across a dashed line in Fig. 3.2c]. (b, c) Corresponding phase and height profiles after performing 1D integration. (d) represents the plot of height variation vs shear. The measured shear value ($dx = 134 \text{ nm}$) corresponds to a $5.2 \mu\text{m}$ size of PS bead, shown by green dashed lines.	81
3.4	(a) depicts a graph between the measured shear values and the number of shear measurements; the dashed red line shows the measured average value, i.e., $\sim 134 \text{ nm}$, and (b) represents the shear data at different points in the tabular form.	82
3.5	(a) Represents the recorded digital DIC interferogram of the fixed HeLa cell with an enlarged view of the interference fringes. Interferogram is recorded with $40\times/0.7\text{NA}$ MO. (b) Corresponding numerically retrieved gradient phase by using FT method; scale bar, $10 \mu\text{m}$	84

3.6	(a,b to l) representing time-lapse differential phase images of U2OS cells at different times (marked at each image). The U2OS cell functioning was recorded for 1 hr. at 2 fps, and the scale bar is at 10 μm	85
3.7	The flowchart represents the workflow of acquiring quantitative phase value from two orthogonally sheared gradient phase images.	87
3.8	The Q-DPCM experiment was performed on human RBCs, and the quantitative phase value is reconstructed by the 2D integration algorithm using two orthogonal PG images. (a,d) Differential interferograms of RBCs recorded at two orthogonal directions, with zoomed views of fringe patterns (at bottom right), (b,e) Fourier spectrums, and (c,f) corresponding reconstructed differential phase maps, (g) retrieved integrated quantitative phase map, and scale bar is 8 μm	88
4.1	(a) illustrates energy levels of molecules and shows molecule excitation and various emission types (shown in the black box). (b) The molecule absorbs light energy and reaches the excited state; the transition from S_1 to T_2 to S_0 shows the dark state, i.e., T_2 , where the molecule remains for a long time. These dark states help to generate spatio-temporal sparsity in the sample.	96
4.2	illustrates blinking density of different fluorescent emitters. (a) Each frame shows 'ON' and 'OFF' conditions, i.e., excited and dark states, respectively, of emitters, most of the fluorophores in the OFF state, which generates high spatio-temporal sparsity and enables precise localization of emitters. (b) Most of the fluorophores are in the ON state, and these fluctuations enable differentiation of the information.	96
4.3	represents local field distribution of the laser on the surface of the glass coverslip substrate.	100
4.4	schematic illustrates the optical configuration of the Cg-TIRF microscope and depicts the evanescent field generation, which illuminates the sample. A diode laser is coupled into Cg using a cylindrical lens. Abbreviations: Cg: glass coverslip; MO: microscope objective.	104
4.5	Block diagram of the IFON algorithm and MUSICAL workflow.	108
4.6	Represents the pulse parameters and laser pulse synchronization with the camera.	110
4.7	(a-c) reconstructed MUSICAL images of different acquisition times for $T_{on} = 35$ ms laser modulation; the scale bar is 0.5 μm . (d) Intensity line profiles along the dashed line marked in images (a-c).	111
4.8	(a-c) are reconstructed MUSICAL images for different laser pulse widths, $T_{on} = 40, 60, \text{ and } 80$ ms, of the same sample and position, and (d) presents the intensity profile along the red dashed lines in images (a-c).	112

4.9	(a) Cg-TIRF image of $7.3\ \mu\text{m}$ PS beads using $20\times$ of $0.4\ \text{NA}$ objective, scale bar: $20\ \mu\text{m}$. (a_1 - a_4) Zoomed area indicated in (a); scale bar: $10\ \mu\text{m}$. (b, c, d, & e) Intensity line profile of dashed line under (a_1 - a_4). (f-h) represents the line profile across the dashed lines f, g, and h drawn in image (a); these lines illustrate the signal and background noise along the line in three parts of the recorded image.	115
4.10	(a) Cg-TIRF image of $0.955\ \mu\text{m}$ PS beads using $20\times$ of $0.4\ \text{NA}$ objective, scale bar: $20\ \mu\text{m}$. (b, c, & d) represents the line profile across the dashed lines b, c, and d drawn in image (a); these lines illustrate the signal and background noise along the line in three parts of the recorded image.	116
4.11	(a) Cg-TIRF image of actin-tagged U2OS cell line, the scale bar is $30\ \mu\text{m}$. (a_1 and a_2) are the zoomed selected areas under the red boxes in image (a), and scale bars are $15\ \mu\text{m}$	117
4.12	(a & b) represent the temporal autocorrelation of fluorescence fluctuations across a signal for 350 and 320 frames, respectively, at two different pixel locations.	119
4.13	(a & c) mean Cg-TIRF images of $0.955\ \mu\text{m}$ PS beads recorded by MO_1 and MO_2 , respectively, (b) is the FOV selected in yellow dotted box in image (a), and (b & c) are the same FOV, and scale bars are $50\ \mu\text{m}$ for images a, b, c. (d & f) are the zoomed common area under the green box in image (a) and blue box in image (b), respectively. (e) shows the MUSICAL image after IFON application on image (d). (c_1, c_2, c_3), (d_1, d_2, d_3), (e_1, e_2, e_3) are some selected common areas for the comparative study, and scale bars are $10\ \mu\text{m}$. MO_1 : $10\times$ of 0.25NA and MO_2 : $20\times$ of 0.4NA	121
4.14	illustrates a comparative study of resolution enhancement with the ground truth: (c_1, c_2, c_3) represent mean Cg-TIRF images recorded by $10\times$ of 0.25NA , and (d_1, d_2, d_3) are corresponding MUSICAL images with improved resolution, (e_1, e_2, e_3) are the mean Cg-TIRF images recorded by and $20\times$ of 0.4NA , for the ground truth, and scale bars are $2\ \mu\text{m}$. (f, g, and h) represent the line profile across the yellow dashed lines in the images (c_1, c_2, c_3), (d_1, d_2, d_3), and (e_1, e_2, e_3).	122
4.15	Represents the workflow to generate mean Cg-TIRF and super-resolved MUSICAL images from fluorescence intensity-fluctuated images at different time points.	125
4.16	(a) mean Cg-TIRF images of $0.303\ \mu\text{m}$ PS beads recorded by MO ($20\times$ of 0.4NA), (b) shows the MUSICAL image after IFON application on image (a). For the comparative study, some selected common areas are illustrated in yellow boxes (a_1 - a_5), and corresponding MUSICAL images (b_1 - b_5). Scale bars are $20\ \mu\text{m}$	125

4.17	illustrates a comparative study of resolution enhancement between Cg-TIRF and IFON image reconstruction: (a_1 - a_5) selected FOV of mean Cg-TIRF images under Figure 4.16a, recorded by $20\times$ of 0.4NA, and (b_1 - b_5) are corresponding MUSICAL images with improved resolution, scale bars are $0.5\ \mu\text{m}$. (c-g) represent the line profile across the dashed lines in the images (a_1 - a_5) and (b_1 - b_5).	126
4.18	Comparison between Cg-TIRF and MUSICAL for fixed U2OS cells: (a & b) show the mean Cg-TIRF and reconstructed MUSICAL image of recorded U2OS cells by NA 0.4 of $20\times$ MO, respectively. (a_1 , a_2 , a_3 , and a_4) There are four selected regions of interest (ROIs) in the image (a); similarly, (b_1 , b_2 , b_3 , and b_4) are four corresponding areas selected in the MUSICAL image (b). (c-f) compares the intensity profiles of mean Cg-TIRF and MUSICAL images along the yellow dashed lines c, d, e, and f, respectively. Scale bars: $2\ \mu\text{m}$ for (a & b); and $0.5\ \mu\text{m}$ for ($a_1 - a_4$ and $b_1 - b_4$).	128
4.19	Table 1 and Table 2 present the FWHM for Cg-TIRF and MUSICAL images of resolved PS beads of dia. $0.303\ \mu\text{m}$ and actins in Figs. 4.17 and 4.18, respectively. For the quantification of resolution enhancement of the present system, a resolution enhancement factor (R) was also calculated.	130
4.20	represents the plots of resolution enhancement factor for different sets of PS beads and U2OS cells' actins and their standard deviation.	130
4.21	(a) is an average projection Cg-TIRF image of a fixed U2OS cell, and (b and c) are reconstructed SOFI4 and MUSICAL images of the same data. Scale bar: $50\ \mu\text{m}$. (d, e, and f) DFT was applied to the results obtained for average projection, SOFI, and the MUSICAL of the raw data. The spectrum was padded to match in size and normalized by their respective maximum value. Here, the log-scale of the normalized spectrum's magnitude is used.	131
4.22	DFT applied to the results obtained for mean Cg-TIRF, SOFI, and MUSICAL of the raw data. The spectrum was padded to match in size and normalized by their respective maximum value.	132
4.23	Comparison between Cg-TIRF and bright-field imaging of FFPE kidney tissue sections. (a) Cg-TIRF fluorescence image of a formalin-fixed paraffin-embedded (FFPE) kidney section showing enhanced contrast and surface-specific features. Regions of interest (a_1 - a_3) highlight a clear visualization of glomerular and tubular architectures. (b) BF image of the same section under identical magnification reveals limited structural contrast and poor delineation of fine tissue features; the Scale bars are $20\ \mu\text{m}$ for images a and b. Magnified insets (a_1 - a_3 , b_1 - b_3) demonstrate the superior visibility of epithelial outlines and cellular morphology in TIRF mode. Arrowheads indicate fine surface structures resolved by Cg-TIRF; the Scale bars are $5\ \mu\text{m}$ for images (a_1 - a_3 , b_1 - b_3). Imaging was performed using a $20\times/0.4$ NA objective.	137
5.1	Standard histopathology workflow for FFPE tissue preparation, including fixation, embedding, sectioning, deparaffinization, fluorescent labeling, and final mounting for fluorescence imaging. Adapted from [4]	146

5.2	Schematic of Total Internal Reflection (TIR) at the boundary between medium η_1 and η_2 ($\eta_1 > \eta_2$). The incident wave undergoes TIR, resulting in an evanescent wave in the lower-index medium. The decay of the field into medium η_2 is characterized by the exponential term $e^{-\kappa z}$, and the penetration depth d is defined as the distance at which the field amplitude falls to $1/e$ of its value at the interface.	151
5.3	Schematic of total internal reflection (TIR) at the glass-sample interface. Light introduced from flat-surface LEDs into the edges of a standard microscope glass slide (refractive index η_1) is confined within the slide via TIR. Each reflection generates an evanescent field extending a short distance into the sample medium (η_2), enabling surface-specific excitation within ~ 100 – 200 nm of the interface. This confined illumination suppresses background signal and enables high-contrast imaging.	154
5.4	The schematic of the conventional microscope consists of three modalities: the BF module, epi-fluorescence, and the LED-TIRF module for biomedical imaging. BF: Bright field, MO: Microscope objective.	156
5.5	(a) The LED-TIRF method allowed for higher contrast and resolution on the FFPE lung section compared to the BF method. This enabled the better visualization of basal cells, luminal cells, collagen fibers, and smooth muscles under a common ROIs; the scale bar is $50\ \mu\text{m}$. (b_1, c_1, d_1, e_1) are zoomed BF images of some selected ROIs in image (a), and (b_2, c_2, d_2, e_2) are corresponding LED-TIRF images with a sharper visualization of subcellular features; the scale bar is $10\ \mu\text{m}$	158
5.6	(a) Epifluorescence image of FFPE lung tissue acquired using laser illumination with a rotating diffuser to suppress speckle noise. Despite the smoother illumination, bulk excitation causes reduced sharpness and lower contrast in structural features. (b) Corresponding image of the same tissue region acquired using the developed LED-based TIRF system, which restricts excitation to a shallow region near the tissue surface. Scale bars are $50\ \mu\text{m}$ for (a & b). (a_1 – a_3) and (b_1 – b_3) are magnified views of selected regions (green dashed boxes) highlighting cellular structures. The LED-TIRF images show enhanced sharpness, membrane clarity, and reduced background due to evanescent field excitation, which is particularly beneficial for surface-resolved histological assessment. These results demonstrate the LED-TIRF system's advantage in providing high-contrast, surface-specific visualization of FFPE tissue samples; the scale bars are $20\ \mu\text{m}$	160
5.7	(a) LED-based TIRF image of an FFPE lung tissue section showing distinct epithelial and connective tissue regions; the scale bar is $50\ \mu\text{m}$. (b) highlights epithelial cells, crypt structures, lumen, goblet cells, stromal cells, and stroma with high contrast. Areas of disorganized cellular architecture suggest potential cancerous abnormalities; scale bar is $20\ \mu\text{m}$	163

5.8	Simulated spectra demonstrating overlap in the LED-TIRF system. The blue curve represents the LED excitation spectrum (centered at ~ 470 nm), the green curve shows the fluorophore emission spectrum (peaking around 510 nm), and the black dashed line indicates a typical emission filter with a 500 nm long-pass cutoff. The red-shaded area highlights the overlap between the LED emission and the detection window, representing undesired LED leakage.	166
5.9	Measured emission spectrum of the blue LED used for excitation, showing photon counts (a.u.) as a function of wavelength. The spectrum exhibits a dominant peak near ~ 480 nm with a low-intensity tail extending into longer wavelengths. A small residual intensity (on the order of ~ 300 – 500 counts) is observed within the fluorescence detection range, indicating limited spectral leakage into the detection band. This observation is consistent with the experimentally obtained high signal-to-background contrast in the imaging results.	168
6.1	Shows the contrast comparison of sperm cells: (a) BF imaging [12], (b) PC imaging [13], (c) DIC imaging [14], and (d) reconstructed quantitative phase map from interferometry-based QPM [15].	175
6.2	Shows the coverglass as a slab waveguide with light coupling direction and the waveguide's dimension (x, y) coordinates.	179
6.3	(a) Represents coverglass-based In-TIRM, utilizing the PL of coverglass for near-field illumination.	182
6.4	A detailed process for preparing polystyrene bead samples.	183
6.5	Flowchart of the sperm sample preparation.	185
6.6	shows the comparison between the BF and In-TIRM imaging: (a & b) are the BF and In-TIRM images of ~ 5.2 μm diameter PS beads, respectively; (c & d) are the line plot along the yellow line across the same PS (shown in the zoomed area under the dotted box in figs.'a' and 'b') and the measured diameters of 4.68 and 5.2 μm , respectively.	186
6.7	(a) Shows the averaged intensity image from the recorded conventional chip-based incoherent image stack on the coverslip by laser scanning (or spatial modulation) similar to pc-TIRF; scale bar is 10 μm , (b) is a zoomed image of the selected region under the dotted red box in the image (a); scale bar is 5 μm .	187
6.8	Comparison of label-free near-field imaging without (a) and with (b) emission filtering. The unfiltered image (a) captures both intrinsic photoluminescence (PL) and evanescent-field-assisted scattering, while the filtered image (b) isolates the PL contribution by suppressing scattered excitation light. Although the filtered condition exhibits higher contrast, the unfiltered image retains clear structural features, indicating that both PL and scattering contribute to image formation.	188

6.9	(a & b) shows a comparison between the BF and In-TIRM images of label-free bull sperm cells. The morphology of sperm represents the head, middle, and principal pieces; scale bar is $10\ \mu\text{m}$. (a_1 & b_1 , a_2 & b_2 , a_3 & b_3 , and a_4 & b_4) are selected as some common regions for contrast and feature comparison; scale bar is $2\ \mu\text{m}$	189
6.10	Comparison of bull sperm cell measurements obtained using the proposed label-free In-TIRM based near-field illumination system (blue bars) with reported literature values (orange bars) [16]. The parameters measured include head length, head width, and tail length. Error bars denote standard deviation across 20 cells. The measurements demonstrate strong agreement with previously reported biological data, validating the system's suitability for quantitative sperm morphology analysis.	191

List of Tables

1.1	Key imaging parameters and their impact on microscopy.	7
2.1	Beam splitting condition with optic axis orientation:	52
4.1	Temporal autocorrelation function and its features:	120
6.1	Comparison of conventional label-free optical microscopy techniques:	176
6.3	Comparison of measured morphological features of bull sperm cells obtained using the In-TIRM system with values reported in literature.	190
7.1	Benchmark comparison of quantitative phase imaging techniques.	200
7.2	Benchmark comparison of fluorescence and super-resolution imaging techniques.	201

Abbreviations

BF	B right F ield
DF	D ark F ield
PC	P hase C ontrast
DIC	D ifferential I nterference C ontrast
FOV	F ield O f V iew
PSF	P oint S pread F unction
NA	N umerical A perture
MO	M icroscope O bjective
FWHM	F ull W idth H alf M aximum
OPD	O ptical P ath D ifference
QPI	Q uantitative P hase I maging
QPM	Q uantitative P hase M icroscopy
RD	R otating D iffuser
MF	M ultimode F iber
BS	B eam S plitter
CCD	C harge- C oupled D evice
FT	F ourier T ransform
TIE	T ransport of I ntensity E quation
nm	N anometer
PSCL	P artially S patially C oherent L ight
Q-DPCM	Q uantitative D ifferential P hase C ontrast M icroscopy
°C	D egree C elsius
DPC	D ifferential P hase C ontrast
TIR	T otal I nternal R eflection
TIRF	T otal I nternal R eflection F luorescence
SBR	S ignal-to- B ackground R atio
SRM	S uper- R esolution M icroscopy

SIM	Structured Illumination Microscopy
SMLM	Single-Molecule Localization Microscopy
FF-SRM	Fluorescence Fluctuation-based SRM
IFON	Intensity Fluctuation-based Optical Nanoscopy
MUSICAL	Multiple Signal Classification Algorithm
SOFI	Super-Resolution Optical Fluctuation Imaging
Cg-TIRF	Coverslip-guided Total Internal Reflection Fluorescence
FFPE	Formalin-Fixed Paraffin Embedded
DPM	Diffraction Phase Microscopy
LS-DHM	Lateral-Shearing Digital Holographic Microscopy
BS-DHM	Beam-Splitter-based Digital Holographic Microscopy
FB-DHM	Fresnel Biprism-based Digital Holographic Microscopy
CBD	Calcite Beam Displacer
PH	Pinhole
OPLD	Optical Path Length Difference
PS	Polystyrene
CC	Calcite Crystal
FDTD	Finite Difference Time Domain
PDMS	Polydimethylsiloxane
PBS	Phosphate-Buffered Saline
PLL	Poly-L-Lysine
WP	Wollaston Prisms
OB	Ordinary Beam
CW	Continuous Wave
EB	Extraordinary Beam
ROIs	Regions of Interest
QDIC	Quantitative DIC
a.u.	Arbitrary Unit
s	Second
μm	micron
μl	microliter
PSI	Phase Shifting Interferometry
PG	Phase Gradient
H&E	Hematoxylin and Eosin
GLIM	Gradient Light Interference Microscopy
DFT	Discrete Fourier Transform
o-TIRF	Objective-based TIRF
pc-TIRF	Photonic-Chip-based TIRF
MMI	MultiMode Interference
CMAG	CellMask Actin Green