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.

Following the development of the Cg-TIRF system and the LED-based TIRF platform, the work was extended toward a label-free near-field imaging approach utilizing the intrinsic photoluminescence (PL) of coverslips under total internal reflection. The resulting images arose from the combined PL emission of the glass and scattering from objects within the evanescent field, where near-field coupling perturbed guided modes for enhanced sensitivity. The system's performance and feature measurement accuracy were validated using standard polystyrene beads and bull sperm cells, with the results compared to those obtained with a conventional bright-field microscope. The approach demonstrated high-contrast, surface-selective imaging with improved dimensional accuracy, highlighting its potential for compact, label-free, near-field super-resolution applications. Temporal modulation of illumination further enabled intensity fluctuation-based, label-free super-resolution imaging.

Finally, a label-free near-field imaging approach was developed, leveraging the intrinsic photoluminescence of coverslips under total internal reflection. The final images combined PL emission from the glass and scattering from objects in the evanescent field, with near-field coupling perturbing guided modes for enhanced sensitivity. Temporal modulation of illumination paves the way for intensity fluctuation-based, label-free super-resolution imaging. Demonstrations on biological and industrial samples showed high-contrast, surface-selective, and fine-feature visualization.

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.