INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
PhD-17 SHORT ABSTRACT OF THESIS

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Thesis Title: Development of an affordable and state-of-art optical super-resolution imaging system utilizing stochastic optical reconstruction microscopy

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Thesis Submitted to the Academic Division	:	Physics
Date of completion of Thesis Viva-Voce Exam	:	02/07/2026
Keywords for the description of Thesis Work	:	Optics, Microscopy, Super-Resolution, Stochastic Optical Reconstruction Microcopy, Affordable, Cancer, Confocal, Image Scanning, Holography

SHORT ABSTRACT

Optical microscopy plays a crucial role in biological and biomedical sciences, although its resolving power is fundamentally restricted to ≈ 200 nm due to diffraction. Optical super-resolution microscopy (OSRM) techniques, such as SIM, STED, and STORM, can overcome this barrier and achieve resolutions from about 100 nm down to a few tens of nanometers. However, the high cost and complexity of commercial OSRM systems limit their accessibility in low resource organizations. Besides, in regions such as Asia and the Indian subcontinent, hot and humid climates can significantly impact the performance of OSRM, further exacerbating access. Since STORM can be implemented relatively easily and in an affordable way in a widefield microscope, in this thesis, we develop a modular STORM system based on an open-source microscopy concept. The modular platform, referred to as openFrame, is built using industry-grade components and open-source control software, enabling brightfield, widefield epi-fluorescence, and super-resolution imaging with STORM at a much lower cost compared to the commercial systems. We present sample preparation and preservation protocols for STORM and demonstrate that the developed system operates reliably in sub-tropical environmental conditions. We additionally address a few major challenges in STORM, using this modular platform. First, we introduce a UV-violet assisted photoinduced recovery mechanism that significantly extends the blinking duration of fluorophores in an imaging buffer, namely, Mowiol with β-ME, thereby increasing the number of localizations and improving the image resolution in STORM. We then implement a holographic user-defined illumination scheme that allows excitation of arbitrary regions of interest on the sample plane, leaving other regions unilluminated except the targeted ones and thus enabling spatial time-lapse STORM while minimizing the photodamage of the unilluminated regions. We combine STORM with confocal and image scanning microscopes to get high-resolution and super-resolution images of the same sample region using a single microscope platform. This integration ensures that if STORM fails to reconstruct a super-resolved image, at least a high-resolution image of the beam scanning microscope will be available. Overall, the developments of advanced microscopy demonstrated here expand the reach of state-of-art optical super-resolution imaging to resource limited organizations and provide valuable tools and schemes for biological and biomedical research.