INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

An optical microscope is an imaging tool that provides images of a specimen with finer details relative to the image provided by an unaided eye. Owing to the far field imaging capability the optical microscopes have become very important in diverse application areas. They are in particular useful for imaging of living biological samples which is not possible with other high resolution microscopes such as an electron microscope. Based on the illumination mechanism the optical microscopes are divided into two broad categories, namely, the widefield microscope and the point scanning microscope. In comparison with the widefield microscope, the point scanning microscope facilitates the use of laser as the illumination beam and implementation of various user defined beam profiles. The amount of finer details in the image that can be provided by an optical microscope is decided by the parameter called resolution, which has an upper limit due to the diffraction phenomenon. A point scanning microscope with a pinhole placed in front of the detector and positioned conjugate to the illumination spot in the sample plane is known as confocal microscope. The confocal microscope provides significantly better axial resolution and marginally better lateral resolution relative to a widefield microscope. However to achieve the best resolution performance the pinhole of the microscope should be as small as possible. Unfortunately as the pinhole size decreases the amount of signal reaching the detector also decreases resulting in a drop in the signal-to-noise ratio. On the other hand the use of an array detector instead of a point detector in a scanning optical microscope enables pixel reassignment in the detector plane during image formation, in an imaging scheme called image scanning microscopy. Image scanning microscopy is shown to provide superior lateral resolution relative to a confocal microscope without compromising on the signal level. Moreover the detector array available for each scan position enables implementation of state-of-the-art imaging schemes for the enhancement of both lateral and axial resolution. However the array detection microscope requires a stable and accurate scanning mechanism and the synchronization of the same with the array detector often slows down the imaging process. In addition to the point scanning microscope, resolution beyond the diffraction limit can also be achieved in the widefield microscope working in the fluorescence mode. The imaging system called stochastic optical reconstruction microscope (STORM) implemented in a widefield microscope can provide super-resolved images of biological samples. In this thesis we develop two types of array detection microscopes, one using galvo mirror based scanning and the other using holographic beam scanning. We configure the beam scanning mechanisms and synchronize them with the array detector in such a way that the imaging can be achieved without compromising on the other performance parameters. We employ the two array detection microscopes to implement different imaging schemes to achieve enhancement in both lateral and axial resolutions. We also develop a STORM system and combine the same with our array detection microscope so as to be able to obtain both super-resolved imaging and reflected light imaging of the same part of the specimen.