Abstract

Lensless in-line holography is a simple, portable, and cost-effective method of imaging, especially for the biomedical microscopy applications. Digital refocussing, multi-depth imaging and phase imaging are some advantages which makes digital holography an invaluable imaging method. In this thesis, a multiplicative gradient descent optimization based method to obtain multi-depth imaging and phase imaging from a single hologram has been proposed. The negative-log-likelihood functional with the assumption of Poisson noise has been used as the cost function to be minimized. The ill-posed nature of the problem has been handled by the sparse regularization and the upper-bound constraint in this expectation-maximization framework. In microscopy of non-fluorescent objects, the pixel-level upper bound is previously known from the reference illumination image. The gradient descent optimization requires calculation of the partial derivative of the cost function with respect to a given estimate of the object at every iteration. A method of obtaining this quantity for holography, for both the cases of real object and complex object has been shown. The reconstruction method has been validated using extensive simulation and experimental studies. The comparison with the previously established iterative shrinkage/thresholding algorithm based compressive in-line holography shows that the proposed method has following advantages: faster convergence rate ($\sim$4 times less iterations), better reconstructed image quality (average structural similarity index improved from 0.59 to 0.65), the ability to perform phase imaging and an improved axial resolution (from 59 microns to <40 microns).

Apart from these reconstruction frameworks, the effects of different illumination strategies on lensless in-line holographic microscopy have also been studied in this thesis. These studies have been primarily focussed on the imaging resolution and the ringing artifacts. In one study, the advantages of using a high numerical aperture single mode optical fiber (photonic crystal fiber) have been studied.

In the established holography methods, both the spatial and temporal coherence of light play a crucial role in determining the resolution of reconstructed object. This strongly restricts this imaging method to use light sources of high spatio-temporal coherence. In this thesis, lensless in-line holographic microscopy with a spatially extended white LED, a light source of low spatial and very low temporal coherence has been presented next. The wave-field propagation between two parallel planes can be obtained

using a convolution operation, where the convolution kernel depends on the object-sensor distance and the characteristics of the light source. For a light source of unknown characteristics, this kernel is an unknown function. Two different approaches of reconstruction has been investigated and presented in this thesis. First reconstruction method is based on the constrained and regularized reconstruction with the monochromatic optical transfer function corresponding to the center wavelength. The resolution reduced due to decreased coherence is regained (from $\sim$4 microns to $\sim$1 microns)using this method of object estimation. In the second imaging approach, the concept of the co-design of the physical optics and the reconstruction framework has been adapted, to develop the final computational imaging system. In this second method, the above mentioned unknown convolution kernel of very large size (128×128) is decomposed into a small unknown kernel (size 9×9) and a large but known kernel (size 128×128). This second kernel is the monochromatic optical transfer function. This drastically reduces the number of unknown parameters to be estimated at the system identification step (parameters to b estimated reduced from 16384 to 81), which has been performed in this thesis by one time imaging of known microscopic objects. A lateral resolution of ~ 1 μm has been demonstrated for sensor-object distance of ~ 0.3 mm. Around four fold improvement in the resolution has been demonstrated, with the imaging of static red blood cells of diameter $\sim 5 - 7$ μm.

High resolution holographic microscopy with a low coherence light source eliminates the requirements of optical spatial filtering and monochromatic sources. This contribution is important for speckle free imaging and in the development of more compact holographic imaging systems.

Keywords: Holography, Microscopy, Reconstruction, Compressed sensing, Diffraction, Coherence.