Title: HIGH CONTRAST LABEL-FREE SINGLE-SHOT QUANTITATIVE PHASE IMAGING AND SPECTROSCOPIC TECHNIQUES FOR BIOLOGICALAPPLICATIONS

ABSTRACT

Quantitative phase imaging (QPI) is the most widely used label-free optical imaging technique to visualize the cellular and subcellular structures of a sample with high contrast and quantitative information. In the past, advanced QPI techniques were developed to improve the contrast, acquisition speed, resolution, sensitivity, and specificity. In addition, label-free spectroscopic techniques are essential to investigate molecular fingerprint information about the sample. Therefore, label-free optical imaging and spectroscopic techniques are introduced to extract the microscopic, nanoscopic, and molecular fingerprint information about the sample and thus gained popularity in various applications. The aim of the present work is to develop label-free optical imaging and spectroscopic techniques for industrial and biological applications.

First, we present a single-shot phase-shifting interferometric technique for accurate phase measurement using white light phase-shifting interference microscopy (WL-PSIM) and deep neural network (DNN). WL-PSIM is a prominent technique for high-resolution QPI of industrial and biological samples. However, multiple interferograms with accurate phase shifts are essentially required in WL-PSIM for measuring the accurate phase of the object, which is time-consuming. Thus, we trained a DNN for the generation of intermediate multiple equal phase-shifted interferograms that are finally used in a traditional phase-shifting interferometry (PSI) algorithm for phase reconstruction. In a similar manner, the network is further trained on the same dataset for the direct phase map from a single low fringe density interferogram without any intermediate multiple phase-shifted interferograms in PSI. The network was trained and validated on two different samples: the optical waveguide and MG63 osteosarcoma cells. The phase map comparison and structure similarity index measure (SSIM) value between the experimental and network-generated data results validates the proposed approach for high-resolution single-shot phase reconstruction in WL-PSIM.

In QPI, the phase (basically refractive index) of cells and tissues are wavelength-dependent properties and accurate measurement with spectral dependence is required to increase the accuracy of diagnostic applications. However, multispectral quantitative phase imaging (MS-QPI) is an imaging platform used for the quantitative analysis of phase maps with spectral dependence. But, to perform MS-QPI, we require a multiwavelength light source with multiple optical components to avoid dispersion, and a multi-chip camera or multiple single-chip camera, which makes the system bulky and time-consuming. In addition, the color cross-talk from a multichip camera detector is another limitation in MS-QPI. Therefore, we aim to extract spectral-dependent quantitative phase information in a single shot using a deep learning (DL) -based QPI system. The network was trained and validated on two different samples:

the optical waveguide and MG63 osteosarcoma cells. For training of the DNN, we acquire a series of multispectral datasets by scanning the multiwavelength light source and their corresponding phase maps. After successful training, the trained DL-based model is used to generate multispectral quantitative phase map generation corresponding to a single wavelength interferogram. The phase map comparison and image quality assessment between the experimentally reconstructed and network-generated MS-QPI images provide the validation for the proposed study. The proposed DL-based MS-QPI system overcomes the limitations of traditional MS-QPI approaches and provides significant time, performance, resources, and cost reduction management.

So far, we performed the study to ease the complexity of the QPI technique in less time. Now, we showed the application of QPI for nanoscale motion tracing of moving samples. Filtration of male semen samples and selection of healthy spermatozoa (sperm cells) is an important step in assisted reproduction technologies. The motion of the spermatozoa indicates their motility and is therefore used as a visual cue for selecting high-quality sperms during intracytoplasmic sperm injection and in-vitro fertilization. For this, we combine an optical imaging platform with an intensity fluctuation-based optical nanoscopy method (primarily used in a labelled technique for super-resolution) for the motion trace analysis of the specimen. We used a multiple signal classification algorithm (MUSICAL) with QPI sequences to perform nanoscale motion trace analysis; refer to this technique as MusiQ, where 'Musi' stands for MUSICAL, and 'Q' stands for QPI. For the proof of concept (POC) of the proposed approach, a 1-μm polystyrene bead particle is trapped and propelled in the evanescent field of the waveguide and traced its motion using MusiQ with finer details although the object was diffraction limited in each frame. Further, we trace the motion of human sperm cells in a straight path with helical motion trace with finer features. We achieved motion precision in the range of 340 nm using a 10×, 0.25 NA lens, whereas the diffraction-limited resolution at this setting was 1320 nm. Our technique presents nanoscale motion features beyond the conventional features used for motility and progressive motility analysis, allowing further distinction among the progressively motile sperms in terms of the regularity of their motion patterns.

Further, the application of QPI is combined with multimodal imaging and spectroscopic techniques to investigate the morphological and molecular fingerprints of the Penguin brain sample. The aim of the current work is to study the Penguin brain and provide different parameters in order to ultimately create a comparative digital brain atlas. Here, we used multiple label-free optical techniques, such as QPI, autofluorescence spectroscopy, and Raman spectroscopy, along with cresyl violet (CV)-staining to extract the optical parameters of the Penguin brain for their morphological and molecular study. All these techniques provide the microscopic and molecular fingerprints of the Penguin brain, which can be useful for understanding the anatomical, physiological, and social behaviour of the Penguins.

Although the QPI technique provides the morphological information of the thin biological samples with high resolution and contrast, it still has limitations when we apply it to thick biological samples. In the case of thick biological samples, the scattered light has the sample information from the multiple layers or out-of-focus planes, which results a noisy image. So, we introduced the concept of longitudinal spatial coherence (LSC) gated full-field optical coherence microscopy (FF-OCM) for the cellular and subcellular imaging of thick multilayered biological samples. We synthesized a partially spatially coherent (PSC) light from a monochromatic light source and utilized its LSC property for the high-resolution sectioning of multilayered thick biological specimens. First, we determined the LSC length of the light source and performed topography of a standard gauge block with a height difference of 20μm. Further, we performed the tomography of a multilayered biological and an oral cancer tissue sample. The PSC-FFOCM system provides ±20 mrad spatial phase sensitivity and ±2 mrad temporal phase sensitivity with a high axial resolution of 6μm - 8μm.

The multimodal and multispectral techniques discussed in this thesis provide morphological and molecular fingerprints of biological samples. The present thesis discusses motivation, challenges, advantages, results, and opportunities associated with the methods utilized during various biological studies.