LABEL-FREE HYPER-SPECTRAL QUANTITATIVE PHASE MICROSCOPY AND MULTI-MODAL IMAGING TECHNIQUES FOR BIOMEDICAL APPLICATIONS

by

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ABSTRACT

Biomedical procedures are heavily dependent on exogenous labels like chemical stains and fluorophores tagging, to generate visually interpretable contrast. Although these approaches remain indispensable, they are often time-consuming and destructive to the specimen. They can also perturb native structure and function due to fixation, photobleaching, and phototoxicity. They also limit the in-vivo examination of live samples during the procedure. These limitations motivate label-free methods that can extract quantitative and reproducible information directly from intrinsic contrast mechanisms. Additionally, the biological tissue samples used for label-free measurements can be preserved and re-used for downstream characterization.

This thesis develops and applies a coherent toolkit of label-free optical techniques for biological applications, with an emphasis on (i) quantitative phase information, (ii) spectrally enriched measurements, (iii) multimodal integration and (iv) computational translation to pathological environment. The work is organized in a paired structure in which a label-free modality or technique is introduced in a chapter, followed by its applications in the following chapter demonstrating how the introduced technique enables a biologically or clinically relevant task. Across the thesis, Quantitative Phase Microscopy (QPM) serves as a unifying backbone because optical phase encodes nanoscale optical-path length delay variations that relate to morphology and mass density. Spectral and computational extensions are then used to extract richer, more explicit characterization than conventional single-wavelength phase maps.

A first major contribution is the development of a compact Hyper-spectral Quantitative Phase Microscopy (HS-QPM) platform based on a Linnik-type interferometric configuration. Hyper-spectral phase imaging is an important aspect because the phase (optical path delay) of transparent structures depends on illumination wavelength in the form of refractive-index dispersion. This dependence provides the spectral refractive index response of different cellular constituents. However, Hyper-spectral interferometric imaging is challenging in practice which include speckle and coherent noise due to coherent lasers; mechanical wavelength switching can reduce stability; and wavelength changes can induce focus shifts due to dispersion in the optical train. To address these limitations, a HS-QPM

was developed, which uses a supercontinuum source combined with an acousto-optic tunable filter (AOTF) for rapid, non-mechanical wavelength selection with narrow bandwidth.

A second contribution addresses a fundamental limitation of conventional QPM. The measured phase maps contain the coupled information about refractive index and physical thickness, so changes in either property can produce similar phase values. Because refractive index is directly linked to biochemical composition while thickness relates to morphology and hydration. Decoupling these quantities from the phase map is essential for explicit biophysical interpretation and for improving diagnostic specificity. Using the concept of HS-QPM, the thesis blends higher-order Cauchy dispersion fitting of Hyper-spectral phase data to decouple the thickness and refractive index. The results show that Hyper-spectral phase enables label-free extraction of quantitative biophysical biomarkers beyond single-wavelength phase imaging.

While phase imaging provides high-resolution morphology and biophysical proxies for thin specimens, complementary molecular contrast can help to reveal a richer information about the sample. The third contribution of the thesis is the design and evaluation of a multimodal microscopic device for label-free detection of squamous cell carcinoma (SCC) on unstained oral tissue sections. The system integrates bright-field imaging (provides structural context and overall tissue architecture), autofluorescence (AF) microscopy (endogenous fluorophore distributions), AF spectroscopy (molecular composition), and transport-of-intensity-equation (TIE)-based QPM (internal morphology) within a single co-registered platform. Demonstrations on unstained tissue sections from different clinical cases highlight that incorporating multi-modality can provide a richer diagnostic cue than any single modality alone. In addition, it also preserves the specimen for conventional staining or additional molecular assays when required.

A fourth contribution targets the gap between label-free measurements and routine histopathology by developing a multispectral virtual staining framework for reagent-free digital pathology. Virtual staining aims to predict histology-like images (e.g., H&E) from label-free inputs. This helps the pathologists to review the unstained slides in a familiar visual format while benefiting from faster and non-destructive acquisition. In this thesis, tissue autofluorescence is used as the label-free input, and the central hypothesis is that multi-spectral information for training provides richer and less confounded information. These results establish multispectral autofluorescence as an effective pathway for high-quality virtual staining. It offers a time-efficient, reagent-free alternative that can complement conventional histology.

Finally, the thesis extends label-free microscopy beyond static morphology to dynamics by introducing phase correlation spectroscopy (PCS) for diffusion quantification. Fluorescence correlation spectroscopy (FCS) is a standard tool for measuring diffusion via temporal fluorescence fluctuations, but it requires labelling and is best suited to nanoscale diffusers. The samples such as micron-scale

particles and dense suspensions can introduce artifacts and photophysical limitations. PCS replaces fluorescence fluctuations with phase fluctuations. Time-resolved quantitative phase maps are acquired and the phase traces within a defined detection volume are extracted. Then temporal autocorrelation functions are fit with diffusion models to recover diffusion coefficients. The obtained results establish PCS as a practical, label-free alternative for quantifying diffusion in regimes where fluorescence methods are inconvenient, perturbative, or unreliable.

To demonstrate biological applicability, PCS is further applied to live Escherichia coli under controlled stress conditions. Bacteria are prepared in buffered culture media and monitored with time-resolved phase imaging. PCS reveals a systematic shift in phase-autocorrelation decay with changing pH, corresponding to changes in effective diffusivity. The analysis consistently indicates maximal effective diffusivity near neutral pH and reduced effective diffusivity under extreme acidic and alkaline stress. Together with the microsphere validation, this work highlights PCS as a general framework for extracting dynamic parameters from quantitative phase data.

In summary, the thesis shows that quantitative phase, spectral information, multimodal design, and learning-based translation can be combined into a versatile label-free microscopy toolkit for biological applications. The HS-QPM platform enables stable Hyper-spectral phase acquisition; the dispersion-model framework converts Hyper-spectral phase data into explicit refractive-index and thickness maps. The multimodal device provides complementary morpho-molecular contrast for unstained tissue assessment; multispectral virtual staining bridges label-free imaging and familiar histology; and PCS extends label-free phase microscopy to dynamic measurements of diffusion and stress-dependent bacterial behaviour. Collectively, these contributions advance the capability of label-free optics to deliver quantitative and information-rich measurements that are compatible with real-world biomedical workflows.