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Introduction

Biomedical imaging technologies promise opportunities for effective diagnostics and treatments as well as new economic perspectives for the medical technology industry. Increasing demand for accurate early diagnosis and the growth of an aging population are major forces driving the biomedical imaging market. An increase in the number of patients suffering from chronic diseases, such as cancer, and a demand for early diagnosis and treatment are also major factors fueling the growth of medical diagnostics and medical therapeutics.

Cellular imaging remains one of the most important techniques to solve major challenges in life sciences and medicine. Optical microscopy is one of the most productive scientific tools in cell imaging. The identification of microorganisms and cells was explored for the first time in the nineteenth century, which could be considered the beginning of the emergence of modern biology and medicine. However, optical microscopy still faces two major problems. One is resolution limitation due to Abbe's law. Another is the lack of quantitative information due to the inherent limits of conventional optical microscopes. Conventional intensity-based imaging techniques are not robust enough to provide detailed quantitative information for cell morphology. They provide low-contrast images, especially when investigating cells with transparent or semi-transparent features, which makes it difficult to analyze cells.

Consequently, several optical imaging modalities based on contrast mechanisms were developed to overcome these limitations. Among many contrast-generating modes, the Zernike phase contrast (PhC) mode and Nomarski differential interference contrast (DIC) are widely used for live-cell imaging. Unlike fluorescence-based imaging techniques, PhC and DIC can visualize transparent specimens, particularly subcellular structures of living cells, without using a specific staining contrast agent. However, these two non-invasive modes cannot provide a direct or quantitative measure of phase shift or the optical path length over a cell area. Since

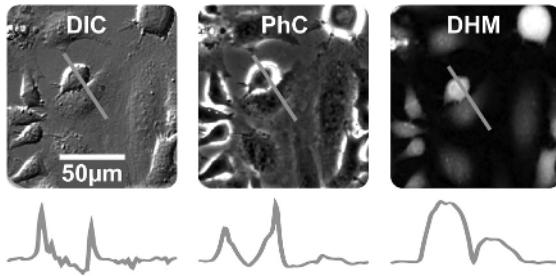


Figure 1.1 Quantification comparison of digital holographic microscopy and other optical imaging modalities.

PhC or DIC signals contain only qualitative information, it is difficult to further perform quantitative analysis of the biophysical properties of live cells with reconstructed cell images (see Figure 1.1).

On the other hand, interference microscopy can provide a direct quantitative measurement of the optical path length based on interference between the reference wave and the object wave that passes through the specimen. Interference microscopy was introduced in the 1950s. Gabor proposed the concept of holography in 1948, which enabled lens-less imaging by reproducing the exact wavefront emerging from the observed specimen. However, due to the non-availability of coherence light sources such as lasers and the high cost of opto-mechanical designs, only a few studies of imaging of live cells are reported in the literature.

In recent years, fluorescence microscopy in a confocal configuration, and its extension into multi-photon fluorescent excitation, have been widely used for cell imaging in biology among various contrast-generating modes. However, they cannot provide any information about dielectric properties in terms of the underlying biological functions of live cells. Fortunately, these dielectric properties can be measured using new digital holographic imaging approaches that have recently emerged as promising techniques for the accurate, quantitative visualization of cell structure and dynamics in a non-invasive manner. To accurately observe live cells without disturbing them is tremendously important. For example, when the aim is to assess drug-mediated cellular effects.

The rapid development of computing technologies and scientific advances in light sources has opened up a new opportunity in the field of holography and interferometry. Integrating techniques in holography with numerical processing has led to the development of digital holographic microscopy (DHM) with a nanometric axial sensitivity that provides a reliable and quantitative phase signal observed in live cells [1–5]. Therefore, the fusion of DHM and information technologies offers an automatic, low-cost, and reliable tool to identify various cell types, including protozoa, bacteria, plant cells, blood cells, nerve cells, stem cells, and cardiomyocytes. DHM allows scientists [2] to observe the growing process of

the cell as close to natural conditions as possible, whether in a cell-culture flask or the tissue environment. Moreover, by numerical reconstruction, DHM offers unique possibilities including an extended depth of focus and a posteriori numerical refocusing, which allow quantitative and non-invasive analysis of cell structure, contents, and dynamics with different time scales that vary from a few milliseconds to several days. Another advantage of DHM is that images of both single cells and populations can be obtained. However, application of DHM to the field of cell biology is still in its beginning stages. There are few studies on the automated quantitative analysis of large-scale holographic cell datasets. Therefore, systems and methods for performing intelligent analyses of large-scale holographic images are becoming more important as digital holographic information rapidly increases with the development of DHM technology. The development of automated procedures to study various cell types using DHM can significantly benefit cell biology studies. Until now, most experiments using DHM in its single mode were performed to prove that the technique is useful. Practically, DHM can measure relevant biophysical cell parameters including absolute volume, dry mass density, nanoscale membrane fluctuations, and biomechanical properties. Furthermore, the development of DHM in a multimodal platform with the fusion of DHM and other cell imaging methods such as fluorescence confocal imaging opens up the possibility to simultaneously measure a large number of relevant and specific cell parameters, which can help scientists understand cellular processes including cell differentiation, cell cycle, apoptosis, and cell migration. For example, DHM systems integrated with fluorescence microscopy can add valuable information about cell morphology and motility with a broad variety of fluorescence labeling tools to study cell function. Measurements of multiple cell parameters resulting from multimodal imaging could even enable high throughput cellular screening assays to identify cell biomarkers for various diseases. The time has come to develop intelligent DHM in a multimodal platform and apply multimodal holographic cell imaging informatics to medical and biological research.

To summarize, the DHM enables label-free, quantitative assessment of biological specimens. There is recent growth in the study of techniques and applications of DHM to address important biomedical questions that cannot be solved with conventional optical imaging techniques. This rapidly emerging field enables scientists to investigate cells and tissues in terms of their morphology and dynamics at a nanoscale resolution over temporal scales ranging from milliseconds to days. Quantitative measurements of intrinsic optical, chemical, and mechanical properties are likely to yield a new understanding of cell and tissue pathophysiology.

Before explaining key concepts of DHM and its success in biomedical imaging and applications, Chapter 2 provides an introductory background on DHM with a quick review and summary of scalar diffraction theory, coherent imaging, and

diffraction-limited imaging. In Chapter 3, fundamental definitions and concepts in lateral and depth resolutions in optical imaging are described. Phase information obtained by DHM provides principal values wrapped in a range of $-\pi$ to π , which can cause 2π phase jumps due to phase periodicity of trigonometric functions. A phase unwrapping process must be conducted to remove 2π phase discontinuities in the image and obtain an estimate of the true continuous phase image. Chapter 4 provides a review of phase unwrapping algorithms to solve challenging problems such as phase discontinuities. Advanced unwrapping algorithms can be categorized into three types: global algorithms, region algorithms, and path-following algorithms. This chapter further gives a detail explanation of the key concepts of quality-guided path-following algorithms. Chapter 5 introduces off-axis DHM in an example transmission of the quantitative visualization of phase objects such as living cells. This chapter also presents a detailed description of the numerical reconstruction procedure in DHM. In addition, this chapter shows that the transverse resolution is equal to the diffraction limit of the imaging system. Chapter 6 introduces Gabor DHM with a simple optical setup as a promising tool for measuring the distribution(s) of particles in a liquid solution with large depths. It demonstrates that the Gabor DHM can resolve the locations of several thousand particles and measure their motions and trajectories with time-lapse imaging.

References

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