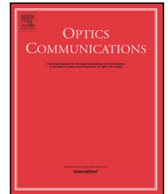
ARTICLE IN PRESS
Optics Communications ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Optics Communications

journal homepage: www.elsevier.com/locate/optcom



Review on methods of solving the refractive index–thickness coupling problem in digital holographic microscopy of biological cells

Gili Dardikman, Natan T. Shaked *

Department of Biomedical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel

ARTICLE INFO

Keywords:
Digital holographic microscopy
Quantitative phase microscopy
Interferometric phase microscopy
Decoupling
Tomographic phase microscopy
Refractive index

ABSTRACT

Digital holographic microscopy is a thriving imaging modality that attracted considerable research interest in quantitative biological cell imaging due to its ability to not only create excellent label-free contrast, but also supply valuable physical information regarding the density and dimensions of the sample with nanometer-scale axial sensitivity. This technique records the interference pattern between a sample beam and a reference beam, and by digitally processing it, one can reconstruct the optical path delay between these beams. Per each spatial point, the optical path delay map is proportional to the product of the sample physical thickness and the integral refractive index of the sample. Since the refractive index of the cell indicates its contents without the need for labeling, it is highly beneficial to decouple the cell physical thickness from its refractive index profile. This manuscript reviews various approaches of extracting the refractive index from digital holographic microscopy measurements of cells. As soon as the refractive index of the cell is available, it can be used for either biological assays or medical diagnosis, as reviewed in this manuscript.

© 2017 Elsevier B.V. All rights reserved.

Contents

1. Introduction 1
2. Theory of the RI–thickness coupling problem 2
3. Extracting the integral RI by thickness evaluation 3
 3.1. Thickness evaluation by approximated modeling 3
 3.2. Thickness evaluation by direct measurement 3
 3.3. Thickness confinement 3
4. Extracting the integral RI by two different interferometric measurements 4
5. Extracting the 3-D RI profile by tomographic phase microscopy 5
 5.1. Optical projection tomography 5
  5.1.1. Radon algorithm 5
  5.1.2. Entropy algorithm 5
 5.2. Optical diffraction tomography 5
6. Medical and biological applications for RI measurements 6
7. Conclusions 6
 Acknowledgments 7
 References 8

1. Introduction

Imaging live biological cells in vitro is of great importance for both biological research and clinical diagnostics. Yet, isolated cells in vitro have very low amplitude modulation, causing standard amplitude-based imaging (bright-field microscopy) to have poor contrast. Cell staining or labeling is often used to obtain better contrast, yet it is time consuming, sometimes suffers from photobleaching, and may disturb the cellular

* Corresponding author.
E-mail address: nshaked@tau.ac.il (N.T. Shaked).

https://doi.org/10.1016/j.optcom.2017.11.084
Received 20 August 2017; Received in revised form 14 November 2017; Accepted 28 November 2017
Available online xxxx
0030-4018/© 2017 Elsevier B.V. All rights reserved.

Please cite this article in press as: G. Dardikman, N.T. Shaked, Review on methods of solving the refractive index–thickness coupling problem in digital holographic microscopy of biological cells, Optics Communications (2018), https://doi.org/10.1016/j.optcom.2017.11.084.

behavior of interest [1]. The phase profile of the sample is the part of the complex wave front that encodes how much light was delayed when interacting with the sample, and is proportional to the product of the cell thickness and the average refractive index (RI), where the latter implies on the cell local density. Since different cellular organelles have different densities and geometries, phase encompasses excellent label-free contrast potential. While in conventional imaging phase cannot be captured due to the lengthy detector integration time relative to the speed of light, digital holographic microscopy (DHM) captures the phase difference between a beam that interacted with the sample (typically by passing through for cells in vitro) and a beam that did not (reference beam); this is done by recording their interference pattern (digital hologram) created on the digital camera, thus converting the phase difference into intensity variations that can be recorded by the camera [2,3]. This method holds great promise, as the phase delay does not only supply good contrast, but also consists of valuable information regarding both the thickness of the sample in the direction of light propagation and the RI distribution of the sample on each spatial point on the sample, and thus is considered a quantitative imaging method (in contrast to Zernike's phase contrast and differential interference contrast (DIC) microscopies) [4]. The cellular RI profile holds great potential for medical diagnosis and for biological research, since it is a physical measurement of the contents of the sample. Nevertheless, after retrieving the quantitative phase profile from the recorded digital hologram, the geometrical thickness and RI information are coupled in a way that makes it difficult to decipher each of these properties separately. For example, when applying hypotonic shock to cells, their swelling is characterized by a thickness increase combined with RI decrease as a result of dilution; in the absence of a decoupling strategy, though, cellular swelling is typically measured as a phase decrease, which may often be inaccurate [5].

Several methods have been suggested for dealing with the refractive index–thickness coupling problem in DHM. The most direct method is not trying to solve the coupling problem at all, but rather directly isolating unique characterizing parameters such as dry mass, cell area or frequency content, based on the quantitative phase values themselves, enabling classification based on the raw phase images [4,6–16]. An equally simple method is relying on existing RI statistics for cell organelles given in the literature to retrieve the cell physical thickness; this is particularly useful for homogeneous cells, such as red blood cells, where the RI is uniform [17–20].

The first approach for solving the RI–thickness coupling problem reviewed in this paper is evaluating the thickness of the sample at each spatial location, which allows the isolation of the average RI (also called integral RI) in that location. The simplest and fastest method for performing this is approximating the local thickness based on the premise that cells in suspension assume a spherical shape [21–28], yielding the integral RI 2-D profile of the cell from its quantitative phase profile with no prior knowledge other than the RI of the suspension medium. Another option is using a different imaging method to directly measure the geometrical thickness [29–32]. A third option is not measuring the native thickness of the sample, but rather constraining the cell into a known dimensional microstructure that confines the cell in the vertical direction such that its thickness is known [21].

The second approach reviewed here is performing two interferometric measurements, each with either a different surrounding medium [5,33,34] or a different wavelength [35–37]. This results in retrieving two phase profiles, yielding two equations with two unknowns for each spatial location, enabling decoupling the integral RI from the thickness.

The third and final approach reviewed here is tomographic phase microscopy (TPM); a method that enables not only to decouple the cell thickness from the integral 2-D RI profile, but rather to obtain the 3-D distribution of the RI of the cell. This is achieved by capturing phase images of the sample from multiple viewing angles, and digitally processing all of them to yield the 3-D RI index distribution [38–56].

This manuscript is constructed as follows. First, in Section 2, we explain the theory of the RI–thickness coupling problem. Then, in Section 3 we review decoupling methods involving the extraction of the integral 2-D RI by thickness evaluation, either by approximation, direct measurement, or confinement. In Section 4, we analyze methods solving the coupling problem by preforming two different interferometric measurements, yielding two equations with two unknowns. In Section 5, we review setups and algorithms for reconstructing the 3-D RI. Afterwards, in Section 6, we review medical and biological applications for which the RI measurement is useful. Finally, Section 7 concludes this review.

2. Theory of the RI–thickness coupling problem

The phase difference, φ, between the sample and reference waves, is proportional to the optical path difference (OPD) between these beams, as following:

$$\varphi(x,y) = \frac{2\pi}{\lambda} \cdot \mathrm{OPD}(x,y), \tag{1}$$

where λ is the illumination wavelength. Neglecting diffraction for simplicity, the OPD can be written as:

$$\mathrm{OPD}(x,y) = \int_{h_1(x,y)}^{h_2(x,y)} \left[n(x,y,z) - n_m\right] dz, \tag{2}$$

where z is the direction of light propagation, $h(x,y) = h_2(x,y) - h_1(x,y)$ is the thickness of the sample in the z dimension, $n(x,y,z)$ is the RI distribution of the sample, and n_m is the RI of the medium.

In a discrete representation, the OPD can be described as a finite sum:

$$\mathrm{OPD}(p,q) = \sum_{l=N_1(p,q)}^{N_2(p,q)} \left[n(p,q,l) - n_m\right] \cdot \Delta l, \tag{3}$$

where $N(p,q) = N_2(p,q) - N_1(p,q)$ is the number of discrete increments of the sample in the l dimension for pixel (p,q), and Δl is the discrete increment length in the l dimension, given by:

$$\Delta l = \frac{\Delta_{CCD}}{M}, \tag{4}$$

where Δ_{CCD} is the pixel size in the digital camera and M is the total optical magnification used in the setup. In Eq. (4), we assume that the l dimension increment size is the same as the p and q dimensions increment size.

Since Δl and n_m are constants and do not depend on l, we can take them out of the sum. We can also multiply and divide by $N(p,q)$. Altogether, we get:

$$\mathrm{OPD}(p,q) = \Delta l \cdot N(p,q) \cdot \left[\frac{\sum_{l=N_1(p,q)}^{N_2(p,q)} n(p,q,l) - N(p,q) \cdot n_m}{N(p,q)}\right], \tag{5}$$

which is equivalent to:

$$\mathrm{OPD}(p,q) = h(p,q) \cdot \left[\frac{\sum_{l=N_1(p,q)}^{N_2(p,q)} n(p,q,l)}{N(p,q)} - n_m\right], \tag{6}$$

where $h(p,q)$ is the thickness of the sample for pixel (p,q). Thus, the OPD in each pixel is the product of the thickness of the sample at that point with the difference between the l axial dimension average (integral) RI of the sample in that point and the medium:

$$\mathrm{OPD}(p,q) = h(p,q) \cdot \left[n_{cell}(p,q) - n_m\right], \tag{7}$$

where $n_{cell}(p,q)$ is the integral RI distribution of the cell. The OPD by itself is not a conventional physical quantity, thus for many biological and medical assays, one first needs to decouple the thickness of the sample, $h(p,q)$, and the integral RI of the cell, $n_{cell}(p,q)$. This constitutes the RI–thickness coupling problem, for which the next sections present solutions.

Please cite this article in press as: G. Dardikman, N.T. Shaked, Review on methods of solving the refractive index–thickness coupling problem in digital holographic microscopy of biological cells, Optics Communications (2018), https://doi.org/10.1016/j.optcom.2017.11.084.

Download English Version:

https://daneshyari.com/en/article/7925075

Download Persian Version:

https://daneshyari.com/article/7925075

Daneshyari.com