Methods xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Methods

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

Sample and substrate preparation for exploring living neurons in culture with quantitative-phase imaging

Sébastien A. Lévesque [a], Jean-Michel Mugnes [a], Erik Bélanger [a,b], Pierre Marquet [a,b,*]

[a] Centre de recherche CERVO, Université Laval, 2601 chemin de la Canardière, Québec, QC G1J 2G3, Canada
[b] Centre d'optique, photonique et laser (COPL), Université Laval, 2375 rue de la Terrasse, Québec, QC G1V 0A6, Canada

ARTICLE INFO

Article history:
Received 24 August 2017
Received in revised form 7 February 2018
Accepted 8 February 2018
Available online xxxx

Keywords:
Quantitative-phase digital holographic microscopy
QP-DHM
Imaging substrate
Label-free live-cell imaging
Neurons

ABSTRACT

Quantitative-phase imaging (QPI) has recently emerged as a powerful new quantitative microscopy technique suitable for the noninvasive exploration of the structure and dynamics of transparent specimens, including living cells in culture. Indeed, the quantitative-phase signal (QPS), induced by transparent living cells, can be detected with a nanometric axial sensitivity, and contains a wealth of information about both cell morphology and content. However, QPS is also sensitive to various sources of experimental noise. In this chapter, we emphasize how to properly and specifically measure the cell-mediated QPS in a wet-lab environment, when measuring with a digital holographic microscope (DHM). First, we present the substrate-requisite characteristics for properly achieving such cell-mediated QPS measurements at single-cell level. Then, we describe how quantitative-phase digital holographic microscopy (QP-DHM) can be used to numerically process holograms and subsequently reshape wavefronts in association with post-processing algorithms, thereby allowing for highly stable QPS obtainable over extended periods of time. Such stable QPS is a prerequisite for exploring the dynamics of specific cellular processes. We also describe experimental procedures that make it possible to extract important biophysical cellular parameters from QPS including absolute cell volume, transmembrane water permeability, and the movements of water in and out of the cell.

To illustrate how QP-DHM can reveal the dynamics of specific cellular processes, we show how the monitoring of transmembrane water movements can be used to resolve the neuronal network dynamics at single-cell level. This is possible because QPS can measure the activity of electroneutral cotransports, including NKCC1 and KCC2, during a neuronal firing mediated by glutamate, the main excitatory neurotransmitter in the brain. Finally, we added a supplemental section, with more technical details, for readers who are interested in troubleshooting live-cell QP-DHM.

Contents

Abbreviations: AMPA, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate; Biph., biphasic; CCD, charge-coupled device; CMOS, complementary metal–oxide–semiconductor; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DHM, digital holographic microscope; DNAse, deoxyribonuclease; D-PBS, Dulbecco's phosphate-buffered saline; DM, dry mass; E17, embryonic day 17; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol or ethyl alcohol; fA, femtoampere; fL, femtoliter; FOV, field of view; GABA, γ-aminobutyric acid; h, hour; GFAP, glial fibrillary acidic protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ID, irreversible decrease; I_{GLUT}, glutamate-mediated current; KCC2, K-Cl cotransporter 2; KK, Kramers-Kronig; OPL, optical path length; MAP-2, microtubule-associated protein 2; MK-801, (5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5-10-imine; MO, microscope objective; mOsm, milliosmole; NA, numerical aperture; NKCC1, Na-K-Cl cotransporter 1; $\overline{n}_c$, mean cell refractive index; n_m, mean medium refractive index; NMDA, N-methyl-D-aspartate; QPI, quantitative-phase imaging; QP-DHM, quantitative-phase digital holographic microscopy; QPS, quantitative-phase signal; RD, reversible decrease; RI, refractive index; ΔT°, temperature difference; μL, microliter; μM, micromolar.

* Corresponding author at: Centre de recherche CERVO, Université Laval, 2601 chemin de la Canardière, Québec, QC G1J 2G3, Canada.
E-mail address: pierre.marquet@neuro.ulaval.ca (P. Marquet).

https://doi.org/10.1016/j.ymeth.2018.02.001
1046-2023/

1. Introduction

Quantitative-phase imaging (QPI) is a powerful quantitative noninvasive contrast-generating approach for visualizing transparent specimens. Similar to phase contrast (PhC) microscopy, and differential interference contrast (DIC) microscopy, which are widely used in biology, QPI allows for the noninvasive high-resolution visualization of living cells in culture. However, unlike PhC and DIC, QPI can quantitatively measure the minute phase shift, namely, the quantitative-phase signal (QPS) that a transparent microscopic specimen, differing from the surroundings only by a slight difference of refractive index (RI), induces on the transmitted wavefront. Such data, obtained via QPS, encompasses information on both the morphology and content of the observed specimen.

Examining living cells with such a promising quantitative and noninvasive contrast-generating approach has already been conducted within the framework of interference microscopy in the 1950s [1]. However, few studies attempted to perform live-cell imaging because interferometric techniques, at that time, required demanding and costly opto-mechanical designs to accurately measure QPS, which is sensitive to experimental noise [2,3]. In the fields of holography and interferometry, scientific advances, lowering the cost of lasers and data acquisition equipment, as well as the development of computing facilities and digital signal processors, have drastically changed perspectives. Several new approaches for optical QPI have recently emerged; these approaches are simpler to implement than interference microscopy, and represent label-free live-cell imaging that can be used to explore cell structure and dynamics at high resolution [4–6].

In live-cell imaging, these different approaches to accurately measure QPS have been driving the development of algorithms, and allowed the automated counting [7–12], recognition, and classification of cells [13–20]. QPS is highly sensitive to the intracellular RI, reflecting the cellular dry mass (DM) in particular; therefore, applications based on its analysis can discriminate between physiological and pathophysiological cellular states. This is especially useful in the fields of assisted reproduction [12,21,22] and cancer research [23–26]. DM is mainly dependent on cellular proteins, according to a relation established over 60 years ago by Barer [1]. Several groups have used various QPI techniques to successfully exploit this phase-DM relationship to study the dynamics of cell growth and characterize the cell cycle [4,27–31]. Because interferometric QPS measurements allow the detection of the minute phase shift [4–6], various QPI techniques can be used to efficiently monitor cell membrane fluctuations at the nanometric scale, especially with respect to red blood cells [4,31–37].

Although these novel QPI techniques suitable for efficiently measuring QPS, to explore cellular structure and dynamics in wet laboratories, QPS itself remains highly sensitive to experimental noise. Additionally, because QPS depends on both the intracellular RI and cellular thickness, its interpretation in terms of biophysical cell parameters, for characterizing specific cell processes, remains a major issue.

In this chapter, we describe how to explore the structure and dynamics of living cells in culture using quantitative-phase digital holographic microscopy (QP-DHM), a technique to the development of which we have significantly contributed. In the Methodology section, we pay particular attention to technical details and substrates required to perform adequate QP-DHM of living cells. In particular, we describe how the numerical hologram reconstruction offered by QP-DHM allows for shaping QPS and control its stability. We also explain the experimental procedures for extracting specific cellular parameters from QPS, including intracellular RI, absolute cell volume, and transmembrane water permeability and movements. As an illustration, we show how specific mechanisms, underlying neuronal dynamics in response to stimulation with glutamate, can be explored with QP-DHM. Finally, in Section 5, we provide concrete suggestions for troubleshooting issues arising from imaging substrate (cells, coating, imaging chamber, etc.), perfusion and temperature control, digital holographic microscope (DHM) hardware, acquisition of QPS to perform live-cell imaging, and treatment of the acquired data.

1.1. Theoretical basis and framework for the technique

1.1.1. QP-DHM

The experimental setup of QP-DHM is presented in Fig. 1A. The figure shows a Mach-Zehnder interferometer, and a detailed