Digital holographic microscopy: a new coherent imaging modality to quantitatively explore living cell dynamics with nanometer sensitivity: toward resolving local neural network activity

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General Considerations

Digital holographic microscopy (DHM) that we have developed is an interferometric microscopy technique which has the advantage of providing in real-time, the full-field complex wavefront diffracted by the observed specimen. The first idea of reconstructing the wavefront by digitally processing an hologram recorded with a camera dates back to the sixties[1]. The propagation of the wavefront was discovered to be precisely simulated by the numerical computation of the Huyghens Fresnel expression of a diffracted wave [2]. Further on, the basic approach developed for Digital holography has been applied to Microscopy [3, 4 5]. One of the relevant interests of DHM resides in its capacity to provide the quantitative phase measurement of the reconstructed wavefront with a very high accuracy (sub-nanometer). Practically, DHM allows to perform measurements in a very short time interval (acquisition time), as a single hologram is required to achieve the numerical reconstruction. In addition, the acquisition rate can be also very fast (camera limited). Consequently, a large immunity to external perturbations (vibration and ambient light) can be achieved. Otherwise, numerical processing of holograms presents the unique advantage of offering not only the means to reconstruct an exact replica of the wavefront diffracted by the specimen but also the means to reshape it. Such a numerical reshaping allows correcting experimental artifacts including lens defects and aberrations (6, 7, 8, 9,10 ,11).

Quantitative phase imaging of living cells

As far as transparent specimen are considered, the reconstructed quantitative phase images provide accurate measurement of the phase retardation (PR) or optical path difference (OPD) induced by the observed specimen on the transmitted wave front (12). PR which arises from a mismatch Δn between the intracellular integral refractive index (RI) n_c, defined as the mean cellular RI along the optical axis, and the RI of the extracellular medium n_s. PR can be regarded as a powerful endogenous contrast agent, as it contains information about both the thickness and the RI of the transparent sample (13). As far as biological cell are considered the origin of the "phase signal" resides in the refractive index difference generated by the presence of organic molecules: proteins, DNA, organelles, nuclei present in cells. Practically, important biophysical cell parameters can be calculated from the quantitative phase signal, including cell shape and absolute volume (13, 14) dry mass concentration (15), membrane mechanical properties [16], permeability (14), transmembrane water movements (17).

Resolving local neural network activity

Practically, experiments on primary cultures of mouse cortical neurons with a set-up combining electrophysiology and digital holography quantitative phase microscopy (DH-QPM), has allowed to reveal that one of the mechanisms inducing the activity-related modifications of the neuronal intrinsic optical properties are transmembrane water movements, related to transmembrane currents. This result, allowing to perform simultaneous multiple site optical recording of transmembrane currents, opens thus the possibility to non-invasively resolve local neuronal network activity with DH-QPM (18).

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