DIGITAL HOLOGRAPHIC MICROSCOPY, A NEW 3D IMAGING TECHNIQUE

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Digital Holographic Microscopy (DHM) is a new imaging technique, which is developing rapidly, offering both sub-wavelength resolution and real time observation capabilities. The method is based on the acquisition of a hologram formed by an object beam passing through a microscope objective and interfering with a reference beam. The object field is recovered when the hologram is re-illuminated by a digitally computed replica of the reference wave, allowing quantitative measurement of both phase and amplitude [1]. The transverse resolution is diffraction limited, as with classical microscopes, but phase measurements are performed with a precision of about 1° (corresponding to 2nm of free wave propagation distance) and provide high accuracy optical path length measurements through the specimen. Compared to classical phase shifting interferometry, DHM offers similar performances in terms of resolution, precision, repeatability and field of view, and has in addition two main advantages. First measurements are performed much faster, as the complete description of the complex wavefront is obtained from a single hologram capture (few microseconds integration time), resulting in a reduced sensitivity to external perturbations (vibrations, ambient light). Secondly, an original numerical procedure of DHM for automatic wavefront corrections enables a simplification of the optical design. A variety of applications of this new type of optical microscopy are described. Among others, we can mention: DH applications in microlenses metrology [2]; live cell imaging [3] where DHM quantitative phase distribution contains information concerning both morphology and refractive index of the observed specimen [4]; tomography of biological specimen based on quantitative phase data acquired with DHM [5]; polarization and birefringence imaging [6].

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