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Enhancing the performance of digital holographic microscopy

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The use of digital optics to compensate for aberrations simplifies the digital holographic microscope while increasing the accuracy and resolution of full-field phase measurements.

High-resolution digital imaging techniques have a great potential for material and life science applications. We have recently developed a digital holographic microscope (DHM) suitable for the quantitative study of dynamic processes with subnanometer resolution. Our DHM allows the reconstruction of full-field optical waves out of a single digital hologram that can be acquired in a few microseconds. Nanometric axial resolution can be achieved for the topography (reflection mode) or for the optical path length (transmission mode), which depends on the sample's refractive index and thickness. However, since the DHM is a precise wavefront sensor, it is also very sensitive to optical aberrations produced by the microscope objective (MO), the specimen holder, and other optics. In our design, digital optics (DO) interfaced with automatic procedures allow complete compensation for aberrations while significantly increasing performance.

The operating principle of our DHM is illustrated in Figure 1. A digital camera is used to digitize the hologram formed by the interference between a reference (R) and an object wave (O) in an off-axis geometry. The digital wavefront is then numerically reconstructed out of this single hologram to compensate for aberrations.[1] Different techniques have been traditionally used to minimize aberrations, such as calibrating in the reconstruction plane with a reference hologram, using mathematical models to describe specific aberrations, or placing optical components in the optical path. In our design, we use numerical lenses (NLs) that mimic the aberration compensations usually performed by optical components in conventional microscopy.[1]

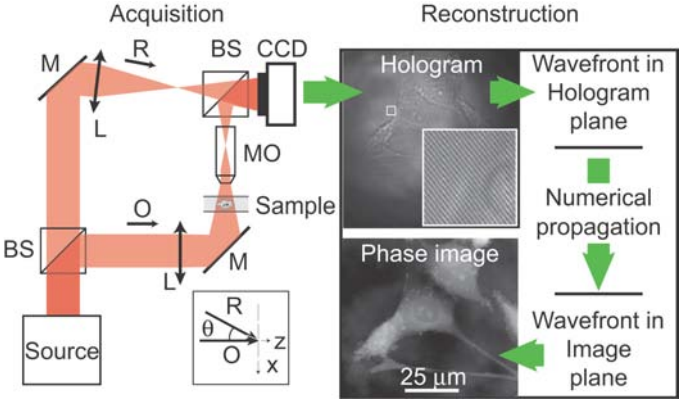

Figure 1. *In the acquisition of a prostate cell phase image, light from the source is split into two paths: a reference wave (R) and an object wave (O), which passes through the sample and the microscope objective (MO). The two waves converge to the charge-coupled device (CCD) camera with a small angle between them (off-axis geometry) and interfere to produce the hologram. BS: beamsplitter; M: mirror; L: lens. During image reconstruction, the digital wavefront recorded in the hologram is propagated numerically in the image plane, from which the phase image is extracted.*

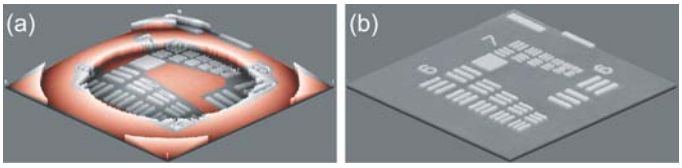

Figure 2. *The computed aberration coefficients on the assumed flat areas, red in (a), define the numerical lens that provides the unaberrated topography (b).*

Compensation for aberrations and image distortion

Because digital holography allows direct access to the quantitative phase of the wavefront, the ideal shape of the NLs can be computed directly from the acquired holograms. This can be

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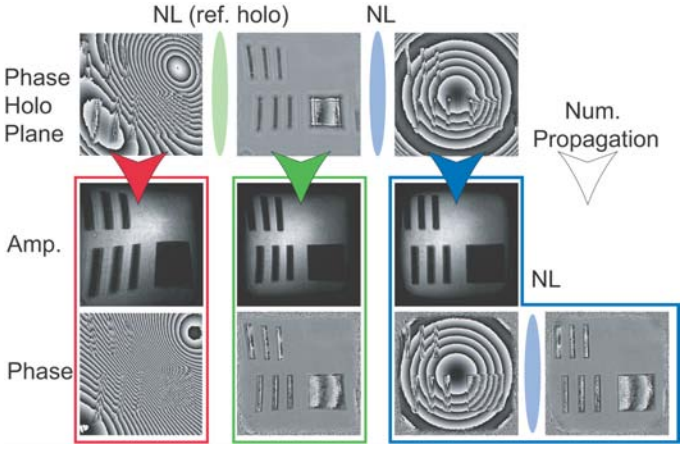

Figure 3. *Complete numerical compensation for image distortion and phase aberration induced by a lens ball used as microscope objective. Red arrow: image reconstruction without compensation; green arrow: image corrected with the reference hologram correction method; blue arrow: manual compensation for residual image distortion. NL: numerical lens.*

achieved with a calibration procedure using a reference hologram (without sample)[2] or with a least-square fitting procedure using aberration model parameters[1] (e.g Zernike polynomials) in assumed constant phase areas of the field of view, shown as red regions in Figure 2(a). The addition of the NL to the the measured phase image then provides the corrected phase reconstruction, as shown in Figure 2(b).

The novelty of the proposed DO resides in placing the NLs not only in the image plane as previously done,[3] but also in the hologram plane. The advantage is that phase aberrations are compensated as well as image distortion (Figure 3). Chromatic aberrations can also be compensated for,[1] thus enhancing multi-wavelength DHM applications.[4,5] Finally, DO also allow the aberration characterization of the amplitude point spread function of MOs[6,7] and of micro-optics.[1,8]

The DHM does not require mechanical calibration. It is calibrated by its wavelength and a numerical calibration procedure using the fitting or reference hologram approach. The real-time compensation for small aberrations orders (offset, tilt produced by external vibrations) associated with the fitting procedure also enhances its stability and axial resolution.

Material and life sciences DHM applications

In material sciences, the optical measurement of the movements of micro-systems is commonly used to investigate their mechanical properties. Recently, the vibration insensitivity and the short acquisition times of the DHM were exploited to characterize the

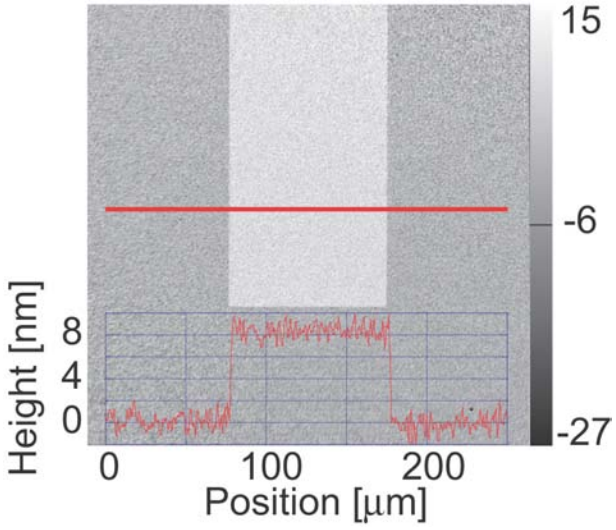

Figure 4. *(a) Topography and extracted profile of a* 8.9 ± 0.5*nm calibrated high step obtained with a 20x microscope objective. The digital optics (here reference hologram) allow subnanometer resolution.*

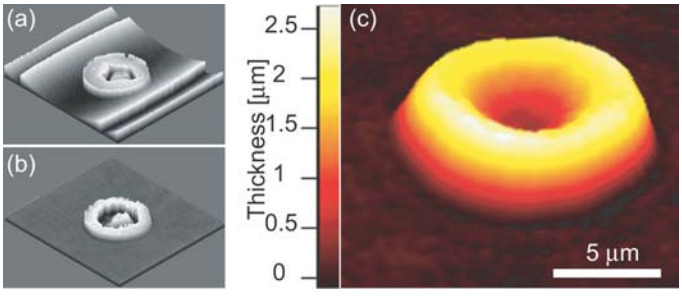

Figure 5. *3D phase representations of a yew pollen grain before (a) and after (b) compensation for aberrations introduced by the micropipette sample holder and topographic representation of an erythrocyte (c). The mean refractive index and the measured volume of the cell are: n=1.401 and v=109*μm^3*, respectively.*

in-plane and out-of-plane movements of a microelectromechanical capacitor. High-frequency stroboscopic measurements up to 20MHz could be performed using 10ns laser pulses.[9] The capacity of the DO to compensate for aberrations resulted in subnanometer resolution, as shown in Figure 4.

In the life sciences, the use of DO allows compensating for aberrations introduced by the medium used to keep cells alive or by the specimen holder. Figure 5(a, b) provides an example of the compensation achieved for aberrations introduced by the use of a micropipette to deliver a sample to a tomographic DHM.[10] It

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can be seen that the measurement is clearly enhanced by the DO. Figure 5(c) shows that the decoupling procedure allows measuring the volume and the refractive index of a red blood cell, two useful clinical parameters that are altered in many blood diseases, as for example anemia.[11]

Digital holographic microscopy coupled with digital optics achieves the full-field, real-time and very precise measurement of a wavefront. The ability of the DHM to image with subnanometer resolution opens up numerous applications in material and life sciences.

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