

Invited Lecture

# Low-Coherence, Common-Path, and Dynamic Holographic Microscopy and Nanoscopy Using Portable Systems

**Natan T. Shaked***Department of Biomedical Engineering Tel Aviv University, Tel Aviv, Israel, [www.eng.tau.ac.il/~omni](http://www.eng.tau.ac.il/~omni)**Author e-mail address: [nshaked@tau.ac.il](mailto:nshaked@tau.ac.il)*

**Abstract:** We present compact, portable and inexpensive interferometric systems for obtaining highly stable, easy-to-align holograms under low-coherence illumination, and use them for quantitative, label-free imaging of live cells and transparent elements with nano-scale thickness.

**OCIS codes:** (090.1995) Digital holography; (090.2880) Holographic interferometry; (180.3170) Interference microscopy.

Digital holographic microscopy is a label-free powerful tool that is utilized for a wide range of applications. This technique is able to capture the amplitude and the quantitative phase profiles of the light interacted with the sample. The quantitative phase profile is proportional to the optical thickness or the optical path delay map of the sample, and thus it enables tracking sub-nanometric changes occurring in it, with acquisition frame rates of up to several thousands of full frames per second.

For biological and medical applications, the ability to record the sample quantitative phase enables the user to see cells and organisms, which are otherwise transparent due to the low absorption and scattering of the light transmitted through the cells. This is done without using contrast agents (such as fluorescent dyes), without contact with the sample, and without the need for scanning. Yet, the map obtained is quantitative, meaning that it is possible to obtain the optical thickness for each point on the sample, and thus using this profile, one can calculate various cell parameters such as dry mass, volume and rigidity map of the cells. Using this technique, it is also possible to quantitatively image transparent elements and structures with micro- or nano-scale thickness, and thus to enable performing quality tests during or after the manufacturing of these elements.

Digital holographic microscopy could be widely used for clinical and industrial applications due to its unique advantages. However, at this point, there are not many options for commercial holographic microscopes compared to other techniques, and this tool is mostly used by optical engineers for research purposes. The main reason for this is the difficulty to obtain high-quality, low-noise and stable interference patterns with modest and portable equipment, and without the need for an expert user.

To solve this problem, our group has suggested the  $\tau$  interferometer [1,2], a compact, portable and inexpensive device that can be connected to the output of a regular microscope and obtain holograms, without the strict stability and the highly coherent illumination that are usually required for conventional holographic setups. The device is built using off-the-shelf optical elements and can easily operate with low-coherence illumination without extensive optical knowledge or the need for meticulous alignment prior to every experiment. Still, due to its common-path, low-coherence design, this device can measure the optical thickness map with temporal and spatial stabilities at the order of 0.2-0.3 nm, which is even better compared to more bulky and expensive conventional holographic setups.

Figure 1 shows the  $\tau$  interferometric setup in on-axis geometry (Fig. 1(a)) [1] and in off-axis geometry (Fig. 1(b)) [6]. In the on-axis  $\tau$  interferometer (Fig. 1(a)), the image plane in the output of the microscope is Fourier transformed by lens L1, while being split into two beams using a beam splitter. One of the beams (defined as the sample beam) is reflected by the mirror M1 and then Fourier transformed back to the camera plane using lens L2, whereas the other beam (defined as the reference beam) is spatially filtered by pinhole P, reflected by mirror M2, and Fourier transformed back to the camera plane by lens L2. The spatial filtering effectively creates a reference beam by erasing the sample information from one of the beams, and thus enables quantitative interference on the camera. Using a pinhole in the reference beam path also increases the beam spatial coherence. In this setup, the two beams are on the same optical axis, causing the beams to propagate in the same direction after L2 lens. This setup generates, in principle, an on-axis interference pattern on the digital camera, which reduces the dynamic capabilities of this setup, since in this case several phase-shifted holograms are required for the reconstruction process.

Figure 1(b) presents the off-axis  $\tau$  interferometer [2], which is able to create a full off-axis interference pattern on the camera and thus enables single-exposure acquisition mode. In order to create an angle between the sample beam and the reference beam, and enable an off-axis hologram, the actual Fourier plane center, described by the continuation of the reflected beam (blue lines in Fig. 1(b)), is shifted using retro-reflector RR. This retro-reflector is built using a pair of mirrors attached to each other in a right angle. Note that only simple optical elements and no

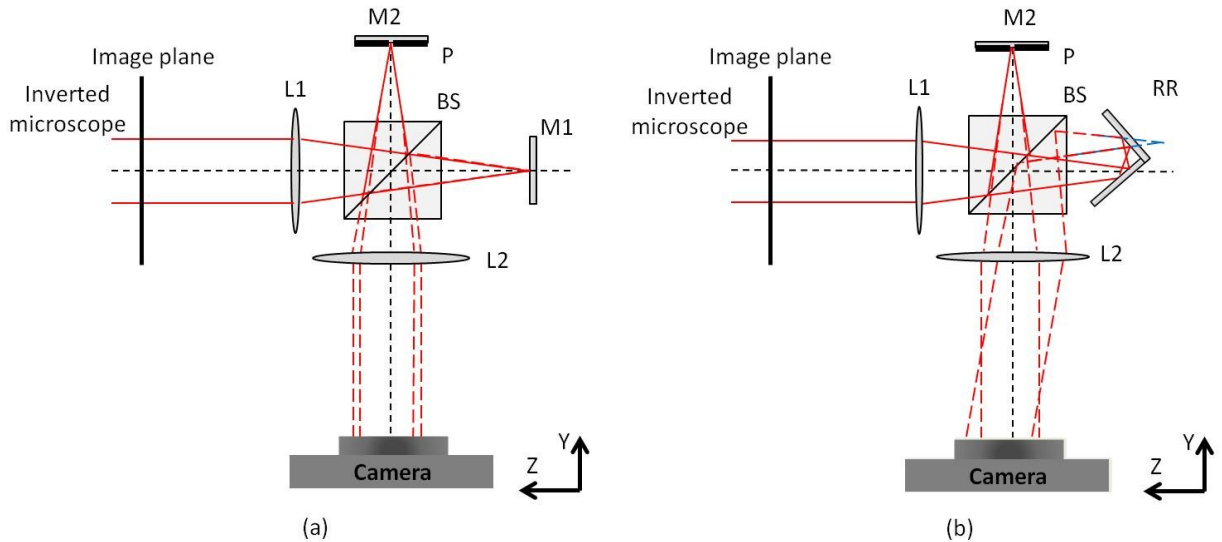


Fig. 1. Schematic system diagrams of: (a) the on-axis  $\tau$  interferometer [1]; (b) the off-axis  $\tau$  interferometer [2]. L1,L2 – lenses in a 4f configuration, BS – beam splitter, M1,M2 – mirrors, P – pinhole, RR – retro-reflector made of a two-mirror construction. Figure is taken from Ref. [2]. OSA, 2013©.

gratings or other diffractive elements are used inside the interferometric system. In addition, there is no limitation on the confluence of the sample (thus, we do not need to use half-empty sample), in contrast to other setups.

Using this device is simple and straightforward: the user is only expected to place the sample on the regular microscope stage, without any preparations or using fluorescent labeling, and without difficult alignment procedures. The user should ensure that the expected varying thickness of the sample does not exceed the temporal coherence length of the light source used. The user is not required considering the thickness of constant or slowly varying elements in the optical path, such as the coverslips and cell media, due to the fact that the optical thicknesses of these elements are still encoded into the reference beam, even after passing through the pinhole [2].

To demonstrate the operation of the  $\tau$  interferometer, we used an inverted microscope with 40 $\times$ , 0.66-numerical-aperture microscope objective, illuminated by a low-coherence source with bandwidth of 6.7 nm. The on-axis or off-axis  $\tau$  interferometers described above were connected between the microscope camera port and the digital camera. Figure 2 [2] shows the optical thickness or optical path delay maps of the words “OMNI Group” with thickness of 20 nm lithographed on glass. These maps were obtained using off-axis holograms of this sample taken in a single camera exposure. As shown in this figure, the results obtained by the new compact design are even better compared to the results obtain by conventional interferometers, which are much larger and harder to align. More results, including biological cell studies, will be presented during the lecture.

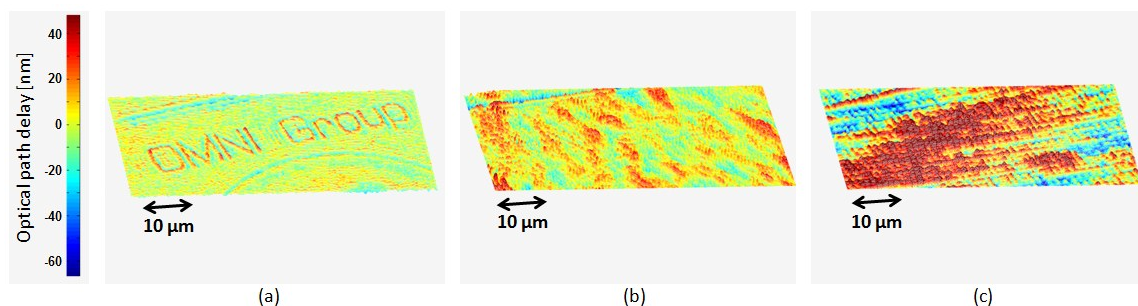


Fig. 2. Optical thickness or optical path delay maps of a 20-nm-thick phase target as obtained by using: (a) the off-axis  $\tau$  interferometer with a low-coherence source; (b) off-axis Mach-Zehnder interferometer with a low-coherence source (difficult alignment process is required); and (c) off-axis Mach-Zehnder interferometer with a high-coherence source (Helium-Neon laser). Figure is taken from Ref. [2]. OSA, 2013©.

## References

- [1] N. T. Shaked, "Quantitative phase microscopy of biological samples using a portable interferometer," *Opt. Lett.* **37**, 2016-2019 (2012).
- [2] P. Girshovitz, P. and N. T. Shaked, "Compact and portable low-coherence interferometer with off-axis geometry for quantitative phase microscopy and nanoscopy," Accepted to *Opt. Express* (2013).