Correction of system aberration for a holographic phase microscope with a long working distance and high numerical aperture

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High-resolution optical images can be acquired by using high numerical aperture (NA) objective lenses. Although there are a number of commercially available high NA objective lenses, their working distance (WD) is limited due to the limited diameter of the lenses. This hinders researchers from capturing fine details of structures within a thick specimen.

As a potential solution for this issue, microscope condensers can be implemented as alternatives for the objectives. Because of the relatively larger diameter, condensers provide high NA and an order of magnitude longer WD than objective lenses. However, they deteriorate the system’s resolution by presenting strong aberration. We applied a method to measure and numerically correct the aberration of an arbitrary system [1]. By using this method, we measured aberrations of condensers. This process allows the system to capture diffraction-limited coherent images.

We set up a holographic phase microscope in transmission geometry by using a laser diode with 785 nm wavelength and two oil-immersion type microscope condensers (Nikon MBL78700) for illumination and detection [2-3]. Galvanometer mirrors were used to scan the direction of illumination. The transmission matrix of the optical system is computed from the holographic images acquired from angle scanning. With these data, we estimated aberration by maximizing the intensity of synthetic aperture images. The spatial resolution of the coherent images is enhanced by numerically compensating input and output aberrations. To demonstrate longer WD of the optical system, we measured images of a resolution target which is sandwiched between two slide glasses.

References