Digital Fourier Holography Enables Wide-Field, Superresolved, Microscopic Characterization

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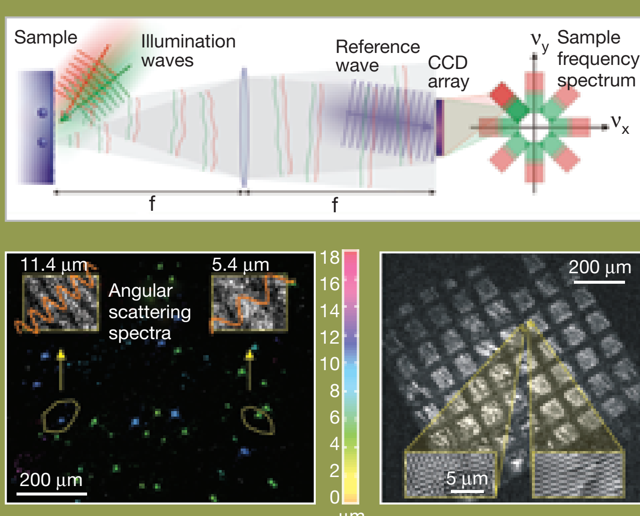
At high magnifications, a microscope's field of view is much smaller than the region of interest. Pathologists examining suspected tumor sections overcome this limitation by translating the section. Biologists assessing cell division in a cell population undertake a more quantitative analysis, examining multiple images that cover a region of interest.

Currently, these tasks cannot be performed using a single digital wide-field image. We have been investigating two approaches that use digital Fourier holography to accomplish them more efficiently—ultimately automatically.[1,2,3]



(Top) The magnitudes of the captured 2D sample spatial frequencies depend on the illumination-wave polar angle, indicated by the red/green scheme. The spatial frequency spectrum is filled in using multiple azimuthal angles of illumination. (Bottom left) Reconstruction of a water suspension of two sizes of polystyrene microspheres. Sphere size is inferred from the spatially resolved angular scattering spectra over a scattering angle range of 13°, due to the correspondence between diameter and spectral ripple frequency. Thus, sphere diameter is indicated with a false-color scale. (Bottom right) Single-hologram reconstruction of a transmission electron microscopy calibration target consisting of a 28,800-lines/in. diffraction grating mounted on a scattering grid with 125-µm pitch. Insets: Reconstructed phase images of the synthesis of four holograms at azimuthal angles separated by 90°. The scattering region (grid) shows high-spatial-frequency structure in two perpendicular orientations, whereas the grating region is dominated by a single orientation.

Common features of our approaches include the use of simple, low-numerical-aperture optics, giving a wide field of view and long working distance, and super-resolution to extract the microscopic information.

Computational image reconstruction from a digital hologram has many advantages,[4] including amplitude and phase imaging, digital wavefront manipulation and 3D imaging. Fourier holography naturally captures part of a sample's 3D spatial frequency spectrum by recording a hologram in the Fourier plane of an objective lens. Each scattered ray angle, corresponding to a particular frequency component, maps to a unique location in the Fourier plane. Such selective access to a sample's spatial frequency spectrum can dramatically reduce the required optical system aperture. When one uses off-axis plane illumination, high-spatial-frequency microscopic information remains accessible well beyond the optical system's conventional cutoff frequency.

We have recorded portions of the complex angular scattering spectra of samples over wide fields of view.[1,2] For mixtures of microspheres, we have used a Mie-theory inversion routine to automatically determine the local scatterer size in the images. We have shown that such a routine works well even on discocyte red blood cells. This spatially resolved particle characterization is obtained from a single wide-field capture without resolving the individual scatterers—in a sense, a form of superresolution. The product of spatial and angular resolution is invariant, but resolving the Mie-theoretical oscillations leaves sufficient spatial resolution for many applications.

We have also exploited Fourier holography's direct access to a sample's spatial frequency spectrum.[3] Instead of the conventional wide-field imaging approach of recording a large spatial frequency range over a narrow field of view, we record a small spatial frequency range over a wide field of view. In both cases, a set of images is required to build up the wide-field, high-resolution image. However, our synthetic-aperture approach has the intrinsic advantages of a low-numerical aperture objective: no immersion and centimeter working distances. With a collection objective of 0.13 NA, we have demonstrated superresolution equivalent to a synthetic aperture of 0.72 NA.

Challenges remain in how to perform particle characterization when Mie theory breaks down, and how to correct for offsets and complex scaling factors when combining holograms. Even so, there are excellent prospects for efficient, flexible wide-field microscopic characterization based on Fourier holography. ▲

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