Digital Holographic Microscopy for 3D Imaging of Complex Fluids and Biological Systems

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In the past decade three-dimensional optical imaging has emerged as a leading tool for scientific discovery in many different fields, including condensed matter physics, materials science, and biology. Confocal microscopy and related techniques allow for the quantitative characterization of both structure and dynamics of systems of many interacting microscopic components. In materials science and physics, these real-space imaging techniques have revealed in enormous detail the mechanisms of phase transitions and nonequilibrium phenomena such as glass formation – mechanisms which cannot in general be resolved using ensemble-averaging techniques such as scattering(van Blaaderen and Wiltzius 1995; Weeks, Crocker et al. 2000; Yethiraj and van Blaaderen 2003). At the same time these techniques have led to better quantitative understanding of many biological processes, including cell adhesion (Discher, Janmey et al. 2005), mechanosensing (Ingber 2003), and nucleoid formation in bacteria (Jun and Mulder 2006) (Bates and Kleckner 2005).

But building up a three-dimensional image using optical microscopy requires mechanically scanning the field of view through a relatively thin sample. Thus the techniques are limited to studying processes that occur on time scales slower than the acquisition time, which is on the order of one second. At the same time, confocal microscopes are expensive, costing hundreds of thousands of dollars. Here I discuss a promising interferometric technique, Digital Holographic Microscopy (DHM), that aims to overcome many of these limitations, enabling new experiments and new scientific discoveries.

Digital Holographic Microscopy

Principles

Digital Holographic Microscopy (Schnars and Juptner 2002; Garcia-Sucerquia, Xu et al. 2006) is based on a technique originally outlined by Denis Gabor in 1949 (Gabor 1949). A diagram of a simple, inline holographic microscope is shown in Figure 1. It consists of a laser, camera, and objective lens. Laser light scatters off structures in a microscopic sample, and the camera images the interference pattern between the scattered and unscattered light. The image is called a hologram.

To see how this technique can lead to a three-dimensional image, consider a coherent plane wave scattering off an idealized point particle. This particle represents anything that scatters light within the microscope sample; it could be a nanoparticle in solution, for example, or a subcellular structure. If we place a screen directly in front of the particle we can view a fringe pattern that arises from interference between the scattered wave and the unscattered portion of the plane wave. This interference pattern contains concentric circular fringes of varying intensity. The number and diameter of the fringes varies with the distance of the screen from the

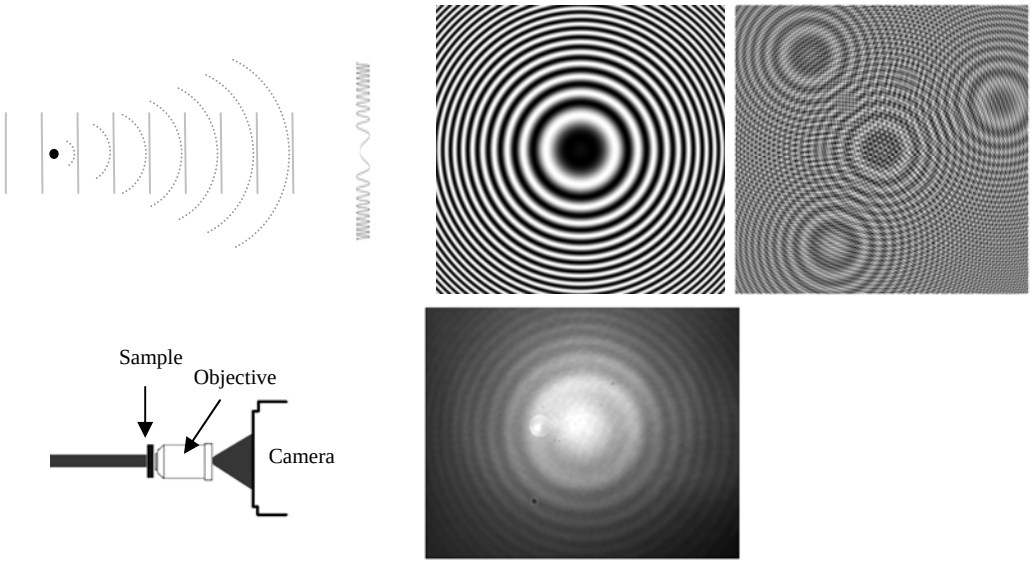

Figure 1: *Hologram formation from a point source, showing a plane reference wave interfering with spherical scattered wave (top left) to produce a Gabor zone plate pattern (center, calculated). A set of point scatterers yields the coherent superposition of zone plates (right, calculated). Diagram of inline holographic instrument (bottom left); image of hologram from 1 μm diameter polystyrene particle in water (bottom center)*

particle, and therefore the interference pattern tells us about the location of the particle in all three dimensions.

If the interference pattern is exposed onto photographic film, it produces an amplitude pattern called a Gabor zone plate. The film containing the zone plate now functions as a type of diffractive lens; if we shine a plane wave back through the developed film, it will come to a focus at a point exactly where our point particle was. If the object consists of many point particles distributed through space, then the interference pattern is simply the coherent superposition of Gabor zone plates. As Gabor first noted, when the scattering from the particles is weak, one can shine a plane wave back through the hologram (the photograph of the interference pattern) and recover an accurate, three-dimensional reconstructed image of the object.

In DHM the hologram is captured on a digital camera and the reconstruction is done by numerically solving the Fresnel-Kirchhoff diffraction equation – in effect, digitally "shining light" back through the hologram. Today nearly all holography is done digitally. Because the hologram of a set of point sources is mathematically equivalent to the Fourier convolution of zone plate patterns, one can use Fourier-transform methods to obtain the reconstruction (Kreis 2002).

Holography therefore allows us to obtain a volume rendering of a three-dimensional object or particle distribution from a single two-dimensional image. This means DHM is, in principle, a fast real-space, three-dimensional imaging technique. Compared to other 3D techniques such as tomography (Xu, Jericho et al. 2001) and confocal microscopy (Weeks, Crocker et al. 2000), DHM is potentially 3 orders of magnitude faster in acquisition time (see data in section **Error! Reference source not found.**). Moreover, DHM, especially in the inline configuration, is much simpler and cheaper to build, requiring only a laser, objective, and camera. The main price one pays is the huge increase in processing time required to reconstruct the 3D image.

DHM is not a new technique, but until recently its applications have been limited due to the computational cost of processing holograms. The availability of fast computers for image processing, inexpensive semiconductor lasers, and high-speed CMOS cameras – all made

possible by advances in semiconductor technology – have made it possible to cheaply and easily build holographic microscopes in the laboratory.

Quantitative holographic microscopy

The particles that form the structure of the sample and which are imaged in the hologram can be tracked through space and time with high spatial precision, allowing one to use DHM as a quantitative imaging technique. As with many particle tracking techniques (Crocker and Grier 1996), there is some confusion over the word "resolution." In holography, as with all optical techniques, the spatial resolution is limited by diffraction; at best, one can resolve two point sources about a wavelength apart, but not much closer than that. But it is possible to resolve the center of brightness of a *single spherical particle* to a precision on the order of tens of nanometers, well below the diffraction limit.

For example, using a DHM built with a diode laser and a high-speed CMOS camera, my research group has captured holograms of a 1 μm polystyrene sphere diffusing in water as a function of time. Reconstructing these images and tracking the centroid of the particle allows us to measure the diffusion coefficient in all three dimensions to within 5% of the value expected from the Stokes-Einstein relation. We find that DHM can accurately measure particle dynamics in real space, in three-dimensions, with spatial precision of about 10 nm and temporal resolution of milliseconds. It is possible to go to even smaller time scales (tens of microseconds) with faster cameras.

Holographic microscopy for studying self-assembly in complex fluids

We use DHM primarily for studying the self-assembly of complex fluids, and in particular colloidal and nanoparticle suspensions. The particles in these systems have diameters between 10 and 1000 nm. The interactions between these particles can drive them to self-assemble into ordered structures at equilibrium such as colloidal crystals, which can serve as the basis for advanced functional materials like photonic crystals (Vlasov, Bo et al. 2001). At least in principle, self-assembly therefore represents a cheap and easy way to fabricate advanced materials (Dinsmore, Crocker et al. 1998; Dinsmore, Hsu et al. 2002; Manoharan and Pine 2004;

Klein, Manoharan et al. 2005). The main problem is that we do not yet understand the dynamic processes involved in self-assembly. Digital holographic microscopy is one of the few experimental tools able to resolve the particle positions and dynamics in these small, rapidly changing and isolated systems.

A small self-assembling system consisting of colloidal particles on the surface of a spherical oil droplet is shown in Figure 2 (McGorty, Fung et al. 2008). There are 10 polymer particles total, each with a diameter of about 800 nm. They interact on the surface of the droplet to form an ordered polyhedron. Such small self-assembled structures are common in industrial fluids and personal care products; they are also useful as building blocks for new materials. Such systems are nearly impossible to probe in three dimensions with confocal microscopy, because of droplet rotation and particle motion. With DHM, however, it is possible to capture the full three-dimensional positions of all the particles at a given time in one hologram. Figure 2 shows how the instantaneous positions of all the particles in three-dimensions can be captured using DHM. We gather these fully three-dimensional data sets at a rate of 30 per second. Because the system is at equilibrium, we can use Boltzmann statistics to extract parameters such as the potential of mean force (also shown in the figure), which is the interparticle pair potential averaged over the configurations of all the other particles in the system.

The power of this technique is that it gives us (in theory) *all the information* about the system at equilibrium. With each hologram we take we obtain a 3D snapshot of the system at a particular spot in its configuration space. The statistics of these configurations can be used to derive the interactions and energetics through the Boltzmann distribution (Crocker and Grier 1994; Lee and Grier 2007). The measurements are beginning to reveal the mechanism of how these interactions lead to the structure.

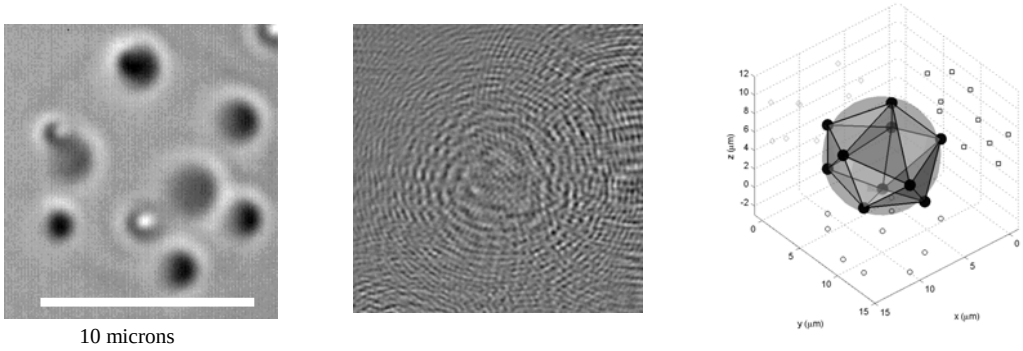

Figure 2*: Left, bright field microscope image of 10 particles at the interface of an emulsion droplet (index matched) Middle, hologram of same droplet taken with our DHM instrument. Right, reconstruction of hologram showing positions of particles on the surface of the droplet and projections of positions onto coordinate planes. Reconstruction is drawn to scale.*

Challenges and prospects

There are still some challenges to using holography as a general-purpose 3D imaging technique. The most severe drawback is the enormous processing power, memory, and storage required for computing holographic reconstructions. Today, with fast Fourier transform algorithms, we can reconstruct a hologram for a single z-section within a few tenths of a second on a common PC. But we generally work with thousands of holograms taken at different times, and for each hologram we reconstruct a volume consisting of about a thousand different z-sections. Processing a typical time series of holograms takes several days on a personal workstation. And for each 1 gigabyte holographic "movie" we can generate nearly a TB of volumetric reconstructions. Some work therefore remains to be done before DHM can replace confocal microscopy as a general tool, but in the meantime there are specific systems and important scientific questions that can be probed using DHM.

An intriguing possibility is biological imaging. Several studies have demonstrated that holographic microscopy can be used to image subcellular structures in three dimensions in near-real time (Xu, Jericho et al. 2001; Marquet, Rappaz et al. 2005; Rappaz, Marquet et al. 2005; Choi, Fang-Yen et al. 2007). Holographic microscopes could offer huge reductions in cost and sample preparation time over confocal microscopy. In fact, the most expensive part of a DHM is the computer that is used to reconstruct the images. It therefore becomes possible to use a microscope as an "add-on" to an existing biological apparatus, such as a cell culture chamber,

rather than as a central facility. With this goal in mind we have started to build inexpensive holographic microscopes from diode lasers and consumer-grade digital cameras. Such instruments can be built for less than $1000, and might prove useful for studying traction forces (Dembo, Oliver et al. 1996), cell rheology (Bursac, Lenormand et al. 2005; Hoffman, Massiera et al. 2006) and for obtaining a more physical understanding of certain pathologies such as sickle cell anemia (Chien 1987).

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