MICROSCOPY

Photon-Based Microscopy With 20-nm Spatial Resolution

Weilun Chao, Erik Anderson, Gregory Denbeaux, Bruce Harteneck, J. Alexander Liddle, Angelic Pearson, Deirdre Olynick, Farhad Salmassi, Cheng Yu Song and David Attwood

Spatial resolution is one of the important quantities in microscopy. By use of nanometer-scaled wavelengths and modest numerical apertures, the soft-X-ray transmission microscope, XM-1, has achieved a measured spatial resolution of 20 nm, the highest of all photon-based imaging microscopes.[1]

Situated at the Lawrence Berkeley National Laboratory (LBNL) Advanced Light Source (ALS) synchrotron, the zone plate microscope is depicted in Fig. 1(a). Its setup was described previously.[2] The microscope operates between 0.7- and 4-nm wavelengths (E_{ph} = 300-1800 eV). It provides: large elemental and magnetic sensitivity; penetration depths of up to 10 μm; and in situ imaging under conditions such as cryogenic and elevated temperatures, applied magnetic fields and applied electric currents. The microscope has a throughput of as many as 1,000 images a day. Research areas studied with the microscope include biology, environmental science, magnetism and electromigration.[3]

We define spatial resolution as the half-period of a periodic line and space pattern at which the pattern's image has a 26.5 percent Rayleigh-like modulation. A technique to fabricate high quality resolution test patterns in a sub-30-nm dimension—with high material contrast and transparency in the soft-X-ray region—was developed, for the first time to our knowledge, by use of multilayer coatings. For this experiment we chose Cr/Si multilayers with measured half-periods of 19.5 and 24.3 nm. The cross sections were processed with conventional TEM sample preparation methods to form shallow wedge-shaped thickness profiles that varied from 0 to 200 μm. Scanning electron microscopy and X-ray reflectometry indicated that the layers had uniform thickness and an interface roughness of less than 1 nm. The test patterns were imaged with the XM-1 microscope at λ = 2.07 nm (600 eV). The X-ray image of the 24.3-nm half-period test pattern is shown in Fig. 1(b). The measured modulations of this image and the image of the 19.5-nm half-period test pattern were 75 percent and 20 percent, respectively. A fit to the data indicates that the microscope has a resolution of 20 nm, only 10 percent larger than the diffraction limit. As far as we know, this is the first conclusive measurement result for resolutions below 30 nm in X-ray microscopy.

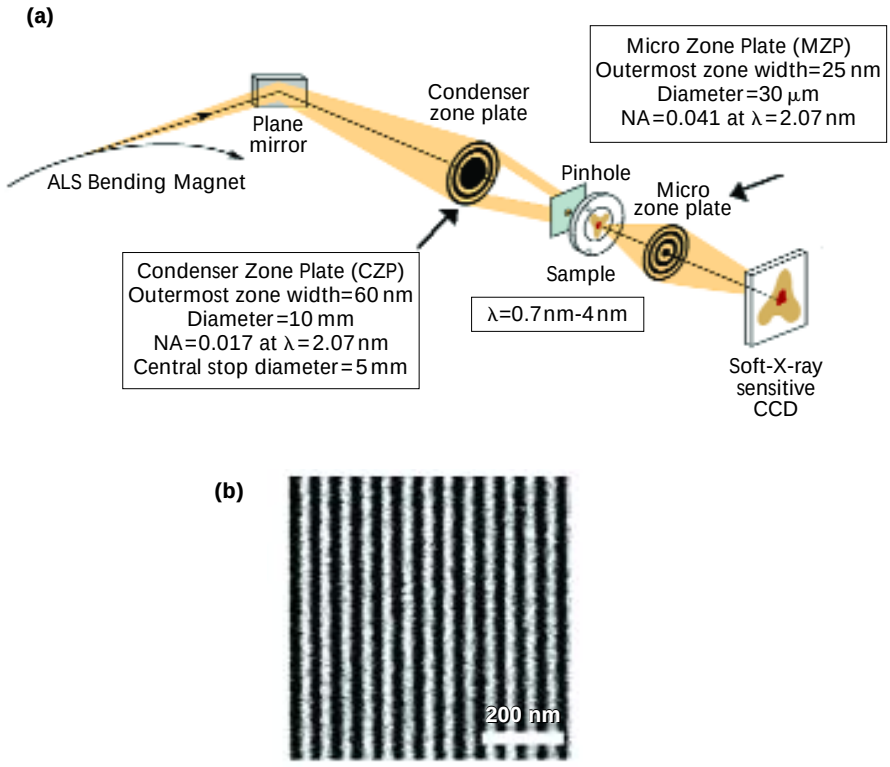

Figure 1. (a) Schematic layout of the soft-X-ray XM-1 microscope. A condenser zone plate and its central stop form a hollow-cone illumination. (b) A soft-X-ray image of the 24.3-nm half-period Cr/Si multilayer test pattern.

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Weilun Chao (wlchao@lbl.gov), Erik Anderson, Bruce Harteneck, J. Alexander Liddle, Angelic Pearson, Deirdre Olynick, Farhad Salmassi and David Attwood are all with the Center for X-Ray Optics, Lawrence Berkeley National Laboratory, Berkeley, Calif. Weilun Chao and David Attwood are with the Department of Electrical Engineering and Computer Sciences, University of California, Berkeley. Cheng Yu Song is with the National Center of Electron Microscopy, Lawrence Berkeley National Laboratory. Gregory Denbeaux is presently at the University at Albany, State University of New York.

Dispersion-Free Ultrahigh-Resolution Optical Coherence Tomography

Yimin Wang and Zhongping Chen

Optical coherence tomography (OCT) is a noninvasive technology used to perform in vivo high-resolution, cross-sectional imaging of microstructures in biological tissues.[1] It was first used clinically in ophthalmology for the imaging and diagnosis of retinal disease.[1] Recently it has been applied to imaging subsurface structures in skin, vessels and oral cavities, as well as in respiratory, urogenital and gastrointestinal tracts.[2] Technology that increases spatial resolution will have a significant effect on clinical applications of OCT. The axial resolution of OCT is determined by the coherence length of the light source, which could be improved by development of a broadband light source. [3]

With the improvements in OCT resolution made possible by an increase in source bandwidths, dispersion has become a critical limiting factor. For ultrahigh-resolution OCT, any unbalanced dispersion in the interferometer will degrade axial resolution. Depth-dependent dispersion is an especially critical limiting factor in high-resolution OCT imaging of the eye, for which a long depth of view is required.

We have investigated the influence on the resolution of OCT of depth-dependent dispersion by water, the main component of biological tissues such as the eye. Our studies showed that by choosing a light source with a center wavelength near 1.0 μm it is possible to eliminate the influence of depth-dependent dispersion by water.[4] Using broadband continuum generation from a photonic crystal fiber, we developed an ultrabroadband light source near this wavelength with a bandwidth of 370 nm.[5] We obtained—to the best of our knowledge for the first time—a dispersion-free ultrahigh-resolution image of an eye with an axial resolution of 1.3 μm (Fig. 1). Figure 1(a) shows an OCT image of the anterior chamber at the limbus of the eye. To investigate the evolution of OCT resolution with increasing imaging depths inside the eye, we derived the envelope of the interference peaks at line A-A in Fig. 1(a). The envelope of the interference peaks which we derived is shown in Fig 1(b). The width of the interference fringe at the interface in the front surface is similar to the width at the interface of the aqueous humor and lens, indicating that the resolution of OCT is not degraded after light passes through a 1.64-mm thickness of cornea, aqueous humor and iris inside the eye. This demonstrates that high resolution is maintained within a great range of depths. In summary, a dispersion-free ultrabroadband light source with a center wavelength near 1.0 μm has been developed, and it has the potential for ultrahigh resolution OCT in biomedical imaging, especially for ophthalmic imaging.

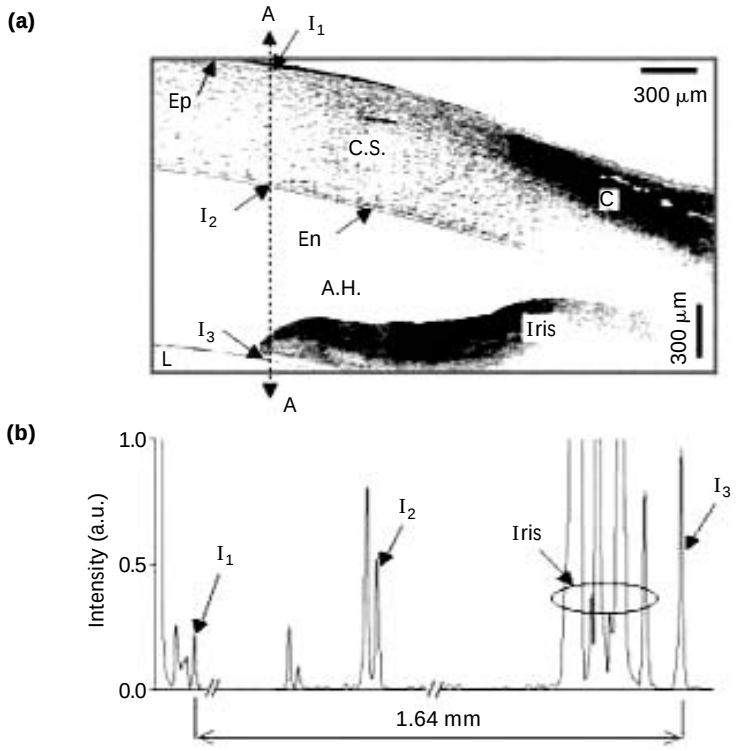

Figure 1. (a) High-resolution OCT image of the anterior chamber of the eye with an image size of 3.0 mm x 2.0 mm. Ep, epithelium; C.S., corneal stroma; En, endothelium; A.H., aqueous humor; C, conjunctiva; L, lens; I_1, the interface between Ep and C.S.; I_2, the interface between En and A.H.; I_3, the interface between the aqueous humor and the lens. (b) Envelope of the interference peaks at line A-A in the above image.

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Yimin Wang and Zhongping Chen (Zchen@laser.bli.uci.edu) are with the Department of Biomedical Engineering, Beckman Laser Institute, University of California, Irvine.

Multiple Dynamic Optical Traps Facilitate Active Microscopy

Peter John Rodrigo, Vincent Ricardo Daria and Jesper Glückstad

In 1970, Arthur Ashkin conducted pioneering experiments that showed that light-induced forces are strong enough to trap and manipulate microscopic particles. Since then, optical micromanipulation has continued to capture the attention of researchers in physical and biological fields. In microscopy, it has supplemented the functionality of optical microscopes, bolstering the simple imaging instrument to one that allows piconewton forces to be applied to and measured from a microscopic specimen. Increasing the number of light handles built into a microscope opens the door to many interesting studies on biological systems as well as to applications in materials engineering, transforming a passive microscope to an active one.

By use of a virtually lossless phase-to-intensity converter based on the generalized phase contrast method, user-configured arrays of dynamic optical traps have recently been demonstrated.[1,2] Reports in the literature of this interactive micromanipulation system have been described in news articles.[3,4] A recent cover story in *Optics Express*[1] described the versatility of this system for the simultaneous trapping and active control of a plurality of microstructures with different geometric profiles. In the approach described, the generation of arbitrarily shaped trapping beams is the result of a direct conversion of phase patterns into corresponding intensity distributions at the microscope's sample plane. Two-dimensional phase patterns encoded on a programmable spatial light modulator (SLM) serve as input to the phase-to-intensity converter. The converter is composed of a 4-*f* lens imaging system and a spatial phase filter centered on the optical axis at the optical Fourier plane. The resulting output intensity distributions are obtained as direct mappings of the phase patterns encoded on the SLM. The interactive programmability of the SLM enables direct and simultaneous position control on an arbitrary number of trapping beams and permits individual specification of trap geometry and intensity. Compared with light pattern synthesis, which uses a SLM for encoding computer-generated holograms, the phase-to-intensity conversion approach offers a significant reduction in computational power requirements and does not suffer from the limited space-bandwidth product and relatively poor modulation transfer function that characterize currently available phase-encoding devices.[5]

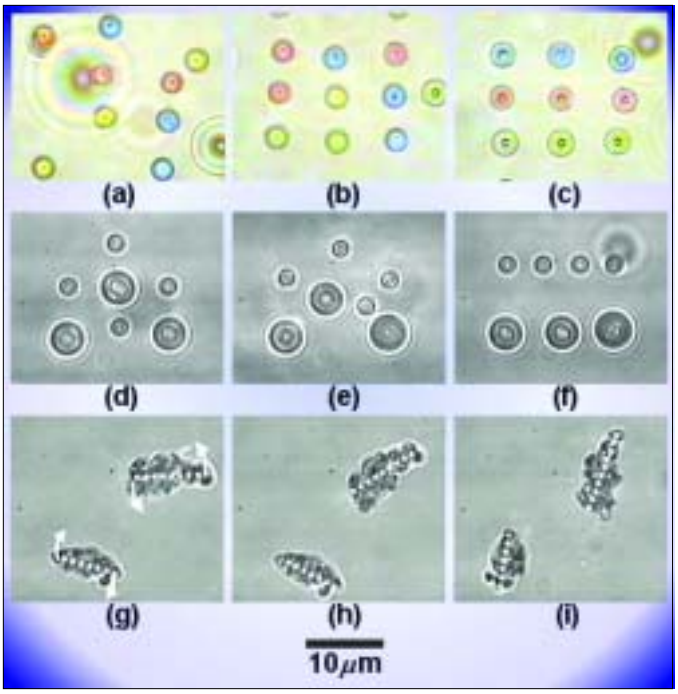

Figure 1. Optical manipulation of various assemblies of microscopic particles. Multiple optical traps with arbitrary spatial profiles are created for simultaneous trapping and dynamic manipulation of inhomogeneous colloidal mixtures.

The experimental results in Fig. 1 illustrate versatile optical manipulation schemes of various clusters of microstructures by use of multiple traps.[1,6] Figures 1(a)-1(c) show commercially dyed polystyrene spheres of 3-μm diameter trapped in unison and sorted according to color. Figures 1(d)-1(f) show simultaneous trapping and rearrangement of microspheres of different sizes. Figures 1(g)-1(i) demonstrate the angular rotation or orientation of irregularly shaped particles by use of optical traps with rectangular symmetry.

The compact 200-mW laser diode source used to create the multiple traps operates at a wavelength of 830 nm, which is suitable for noninvasive manipulation of biological microstructures. Moreover, the profile of each trapping beam can be configured to incorporate an intensity void at its center.[1] For cells, the optical trap geometry avoids illumination of cell nuclei, which potentially reduces the risk of damage to or mutation of the specimen. Thanks to the functionalities of the multiple, dynamic optical traps described here, studies of cell biomechanics that involve adhesion, growth, interactions and signaling can now be performed with superior flexibility and control.

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Peter John Rodrigo, Vincent Ricardo Daria and Jesper Glückstad (jesper.gluckstad@risoe.dk) are with the Optics and Fluid Dynamics Department, Risø National Laboratory, DK-4000 Roskilde, Denmark.