High-numerical-aperture optical microscopy and modern applications: introduction to the feature issue

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This feature issue comprises a collection of papers from groups in the forefront of the research of high-numerical-aperture optical systems and their applications. We have assembled 13 papers from a wide subject area within optical microscopy. Contributions include surface-plasmon microscopy, interference microscopy, optical coherence tomography, polarized-light microscopy, and the optical theories of these techniques. © 2000 Optical Society of America

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1. Introduction

Optical microscopy has come a long way since its invention in 1590. Interestingly, apart from the Nobel Prize winning contribution of Zernike[1] in 1953 and the invention of the Nomarski[2] differential-interference contrast technique in 1955, not much practical development seems to have occurred in the 20th century until the late 1970's. It is quite safe to say that the invention of lasers in 1961 triggered the development of modern confocal scanning microscopes whose principle was proposed by Minsky[3] in 1957 in a U.S. patent application.

Practical realization of modern confocal microscopes[4] was first demonstrated by Kompfner and Sheppard[5] in 1977 and subsequently by Brakenhoff[6] and Brakenhoff *et al.*[7] in 1979. The first theoretical treatment that appeared in the literature was by Sheppard and Choudhury[8] in 1978. The appearance of confocal scanning microscopy was the likely reason for the second renaissance of optical microscopy methods. Perhaps somewhat surprisingly, Minsky[3] thought of the depth-sectioning property of his invention as disadvantageous. The depth-sectioning property of confocal microscopes became the main reason for their extensive use, as this permits one to cut thin sections of a thick specimen in a nondestructive manner, hence to build up a three-dimensional reconstruction of the specimen with the help of a computer.

It is especially pleasing to see the wealth of scientific papers that have been published in the literature on novel developments in optical microscopy. Probably the lion's share of these publications involves the use of some form of high-numerical-aperture optics, as scientists strive to obtain ever-improved optical resolution. Scanning-microscopy principles have been used in optical data storage in which the increasing demand for higher data densities has now pushed the numerical apertures of lenses in modern digital versatile disk (DVD) technology to as high as 0.8. Solid immersion lenses with effective numerical apertures of more than 1.5 are being experimented with for mass use.

The major use of high-numerical-aperture optical microscopy is in the biological sciences, primarily for fluorescence microscopy. Two-photon confocal microscopes are sold as off-the-shelf instruments, and three-photon excitation and second- and third-harmonic-generation optical microscopes are currently being developed as commercial products. Coherence gating is used in conjunction with high-numerical-aperture optical microscopy to produce optical coherence microscopy. Such microscopes can provide clear optical sections in turbid media in which light scattering was thought to be prohibitive for obtaining clear images.

This feature issue of *Applied Optics* is intended to provide reports from groups at the frontiers of optical microscopy research on the current state of this exciting field. Topics of papers selected for this feature

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issue describe new microscopy techniques, novel or improved applications of existing methods, and the latest theoretical developments in the field.

The first paper by Somekh *et al*. describes high-resolution scanning surface-plasmon microscopy. In this study, the authors report on a method that improves the resolution of this technique by an order of magnitude over those results previously published. In the second paper, Holzwarth *et al*. report on a modification of a differential-interference contrast microscope that uses a variable retarder. Images obtained with this setup exhibit doubled contrast and reduced noise. Next, Totzeck *et al*. demonstrate a phase-shifting common-path interference microscope that is capable of measuring linewidths as small as 0.5λ. Both experimental results and theoretical treatment are presented. Schoenle *et al*. present a method for fluorescence-lifetime imaging in multiphoton fluorescence-excitation microscopy. Their method provides a temporal resolution of tens of picoseconds.

Xu *et al*. investigate the effects of handling and fixation on specimens prepared for two-photon spectroscopy purposes. They suggest a method to maximize the emission spectra from muscle tissue. In the next paper, Jiao *et al*. use a polarization-sensitive optical coherence tomograph to investigate depth-dependent polarization properties of turbid media, such as biological tissues. They obtain two-dimensional depth-resolved images of both the Stokes vectors of the backscattered light and the full Mueller matrix of a biological sample. Oldenbourg and Török use a polarized-light optical microscope with a universal compensator mounted on it to obtain experimental point-spread functions by imaging small birefringent crystals. They describe the results of a theoretical model that confirms their experimental findings.

Dhayalan and Stamnes compare exact and approximate results for the focusing of electromagnetic waves through a dielectric interface—a problem that has great implications in optical microscopy theory. They establish a method to speed up their previous numerical computations by 3 orders of magnitude without degradation of the accuracy of more than 7.8% even for subwavelength aperture-interface distances. The next paper by Grochmalicki and Pike describes a theory, based on the inversion of the Debye–Wolf integral, to obtain superresolution in DVD readers. They present the design of a superresolving mask that can be inserted into the reading unit of a DVD player to obtain a resolution improvement of a factor of 2.

Cronin and Cogswell discuss the problem of the minimum number of optical beams needed to obtain unambiguous velocity measurement in three-dimensional scanning-laser Doppler anemometry. They find that such data can be obtained only by the simultaneous use of four laser beams. Varga develops a novel electromagnetic, high-numerical-aperture theory that can be used in ellipsometry and conoscopy. Varga shows that the field in the focal region of a high-numerical-aperture lens is related by a simple algebraic expression to the field in the back focal plane. This relation also holds for a thin object placed at an oblique angle in the focal plane of the lens. Next, Sheppard investigates the effects of spherical aberration that is caused by weak refractive-index mismatch. Sheppard finds that spherical aberration can be balanced by a change in the tube length of the objective of moderately high-numerical-aperture objectives. Finally, Inami and Kawata investigate the imaging properties of confocal polarized-light microscopes by using Mie scattering theory. They also discuss the three-dimensional resolution of such microscopes.

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References and Note

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