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Volume 146

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PETER W. HAWKES

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PREFACE

The three subjects examined in this volume are taken from phase-contrast microscopy, the mathematics of vision, and the new field of information geometry.

The need to image phase objects in microscopes has given rise to many ingenious suggestions; the best known is that of Frits Zernike in the 1930s. A recent addition involves the use of a spiral phase filter in the Fourier plane of the instrument; this is the subject of the first chapter by S. Fürhapter, A. Jesacher, C. Maurer, S. Bernet, and M. Ritsch-Marte, who have explored the technique in detail. Here, they explain how such a phase filter works and give numerous examples of its value in practice. The presentation is extremely clear and helpful and will, I am confident, help to bring the technique into wider use.

This is followed by a chapter inspired by the theory of vision, by C.H. Rohrer and M. Wild. This begins with a presentation of "LULU" theory, where L and U are operators made up of the max and min operators. The second part is concerned with stack filters and their design, and includes sections on mathematical morphology and lattice stack filters. This is a broad-ranging article, and contains many speculations, as well as the formal theory.

The volume concludes with an account by H. Snoussi of information geometry, a relatively new field. The attempts to employ it in information theory and physics were made in the mid-1980s. Here, this information geometry is used for selecting the best priors in Bayesian learning structures. The author shows how this problem can be solved and makes a convincing case for using this new tool in related areas. This lucid presentation of a new subject will surely be much appreciated.

As always, I thank all the authors for contributing to the series and for the trouble they have taken to make their material accessible to a wide readership. Forthcoming contributions are listed in the following pages.

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Spiral Phase Microscopy

SEVERIN FÜRHAPTER, ALEXANDER JESACHER, CHRISTIAN MAURER, STEFAN BERNET, AND MONIKA RITSCH-MARTE

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I. Introduction

The invention of the light microscope allowed a first glimpse into the world of micron- and smaller-sized objects that are otherwise not resolvable with the human eye. First microscopes used the brightfield mode, where a specimen is illuminated and the transmitted or reflected light is imaged by a microscope

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objective. This method still plays an important role in microscopy. Because the human eye is unable to recognize phase changes, a brightfield microscope is only suitable for specimens that show an amplitude contrast. An object is called an *amplitude object* if it absorbs parts of the incoming light due to pigments within the sample. The fact that the majority of the examined biological samples consist largely of water leads to poor contrast from the surrounding medium.

In fluorescence microscopy, biological cells are stained so that specific parts can be examined. The labeling of cells is a complex process that needs extensive preparation. The examiner must know in advance which parts of a sample are to be imaged, and on this basis, a marker must be selected. In many cases, the dyes used are harmful and destroy the sample. These shortcomings led to the development of a variety of microscopy methods whose aim was to enhance the contrast and to unveil parts of transparent specimens that are not visible in brightfield mode. Established methods in optical microscopy that solve this problem are, for instance, darkfield, phase contrast, differential interference contrast, Hoffman contrast, or Dodt contrast imaging.

In order to enhance contrast in light microscopy, the origin of the contrast must be understood (Born and Wolf, 1980). An excellent compendium that describes the principles of contrast in microscopy is, for example, given in Microscopy Primer (2006), http://micro.magnet.fsu.edu/primer/techniques/contrast.html and a general overview of imaging methods for living samples is given in Tadrous (2002) and Stephens and Allan (2003). When a microscopic sample is illuminated (e.g., by a white light source), some of the light passes through the sample without being absorbed or scattered. The remaining part of the light is diffracted from the sample and acquires a phase shift in comparison to the undiffracted light. The microscope objective projects all light beams into the image plane, where the undiffracted light evolves to a plane wave. The diffracted light focuses at different positions in the image plane, and there interference with the plane wave occurs, resulting in an intensity image of the sample.

Darkfield microscopy is one method to increase image contrast. There the zeroth order of the illumination beam is blocked such that only light diffracted, refracted, or reflected at the specimen is coupled into the microscope objective, where it can contribute to the formation of the image. The result is an illuminated object in front of a dark background. The sample is illuminated by a hollow cone of light, which is blocked by a ring in the darkfield objective, or the illumination light completely misses the collecting lens of the objective (ultra-darkfield method). This method works well for objects with low contrast and is suitable to enhance edges and contours. Since the direct illumination beam is blocked, and thus intensity is lost, this microscopy technique requires a bright light source.

An object is called a *phase object* if it does not absorb light and only modifies the phase of the incoming light field. The following microscope techniques are based on the fact that they convert phase differences (Barone-Nugent *et al.*, 2002) into amplitude variations that are visible to the human eye.

Phase contrast microscopy (Zernike, 1934; Zernike, 1935; Zernike, 1955; Noda and Kawata, 1992; Barty *et al.*, 1998; Paganin and Nugent, 1998; Liang *et al.*, 2000; Bellair *et al.*, 2004; Paganin *et al.*, 2004), which was first introduced by *Frits Zernike*, images small differences in *refractive index* or *thickness* variations between several parts of the cell. The original central phase contrast technique is based on a filter that is placed in a Fourier plane of the imaging pathway, creating a phase difference between the diffracted and undiffracted wavefront. For small phase variations, Zernike could show (Zernike, 1942a; Zernike, 1942b) that there exists a difference of a quarter wavelength between the diffracted and the undiffracted light field in a phase sample. This phase variation cannot be seen by the human eye, which is sensitive only to intensities. By shifting the phase of the undiffracted light by another quarter wavelength, these phase variations on the sample can be transformed into amplitude variations in the image plane. If the resulting phase shift between the diffracted and the undiffracted light is half a wavelength, both light fields interfere destructively, and the method is called *positive phase contrast*. The specimen appears dark against a bright background. Conversely, if the diffracted and undiffracted light are in phase after the phase filter, the method is called *negative phase contrast*. The resulting images have bright specimen details on a dark background. The success of his method earned Zernike the Nobel Prize in Physics in 1953. An advantage of this method is that living samples can be examined. As a disadvantage, "halo effects" (i.e., bright areas around dark objects or dark areas around bright objects) appear when thicker probes are analyzed.

Differential interference contrast (DIC) was introduced by Georges Nomarski (Nomarski, 1955; Padawer, 1968; Allen *et al.*, 1969; Pluta, 1989; Cogswell *et al.*, 1997; Van Munster *et al.*, 1997; Preza, 2000; Franz and Kross, 2001; Arnison *et al.*, 2004) and utilizes *phase gradients* in the sample for contrast. Linearly polarized light is passed through a first modified Wollaston (or Nomarski) prism, which splits the light into two parts with a 90-degree difference between their polarizations. Behind the Wollaston prism, the two rays have a small shear in their directions, less than the optical resolution of the microscope. After passing the condenser, the light traverses the sample, and differences in refractive index or thickness affect each beam differently. Subsequently, the two beams are collected by the objective, recombined by a second Wollaston prism, and finally interfere behind a second polarizer. This procedure detects the phase difference between the sheared image