Spiral phase contrast imaging in microscopy

Severin Fürhapter, Alexander Jesacher, Stefan Bernet, and Monika Ritsch-Marte
Division for Biomedical Physics, Innsbruck Medical University, Müllerstr. 44
A-6020 Innsbruck, Austria
Stefan.Bernet@uibk.ac.at

Abstract: We demonstrate an optical method for edge contrast enhancement in light microscopy. The method is based on holographic Fourier plane filtering of the microscopic image with a spiral phase element (also called vortex phase or helical phase filter) displayed as an off-axis hologram at a computer controlled high resolution spatial light modulator (SLM) in the optical imaging pathway. The phase hologram imprints a helical phase term of the form $\exp(i\varphi)$ on the diffracted light field in its Fourier plane. In the image plane, this results in a strong and isotropic edge contrast enhancement for both amplitude and phase objects.

OCIS codes: (070.6110) Spatial filtering, (090.1970) Diffractive optics, (100.5090) Phase-only filters.

References and links

1. N. R. Heckenberg, R. McDuff, C. P. Smith, and A. G. White, "Generation of optical phase singularities by computer-generated holograms," Opt. Lett. **17,** 221–223 (1992).
2. R. Oron, N. Davidson, A. A. Friesem, and E. Hasman, "Transverse mode shaping and selection in laser resonators," Progress in Optics **42,** 325–386 (2001).
3. S. S. R. Oemrawsingh, J. A. W. van Houwelingen, E. R. Eliel, J. P. Woerdman, E. J. K. Verstegen, J. G. Kloosterboer, and G. W. 't Hooft, "Production and characterization of spiral phase plates for optical wavelengths," Appl. Opt. **43,** 688–694 (2004).
4. A. Y. M. NG, C. W. See, and M. G. Somekh, "Quantitative optical microscope with enhanced resolution using a pixelated liquid crystal spatial light modulator," J. Microscopy **214,** 334–340 (2004).
5. J. A. Davis, D. E. McNamara, D. M. Cottrell, and J. Campos, "Image processing with the radial Hilbert transform: theory and experiments," Opt. Lett. **25,** 99–101 (2000).
6. K. Crabtree, J. A. Davis, and I. Moreno, "Optical processing with vortex-producing lenses," Appl. Opt. **43,** 1360–1367 (2004).
7. G. A. Swartzlander, Jr., "Peering into darkness with a vortex spatial filter," Opt. Lett. **26,** 497–499 (2001).
8. M. R. Arnison, K. G. Larkin, C. J. R. Sheppard, N. I. Smith, and C. J. Cogswell, "Linear phase imaging using differential interference contrast microscopy," J. Microscopy **214,** 7–12 (2004).
9. G. O. Reynolds, J. B. DeVelis, G. B. Parrent, Jr., and B. J. Thompson, *The new physical optics notebook: Tutorials in Fourier optics* (SPIE Optical Engineering Press, Bellingham, Washington, 1989).
10. K. G. Larkin, D. J. Bone, and M. A. Oldfield, "Natural demodulation of two-dimensional fringe patterns. I. General background of the spiral phase quadrature transform," J. Opt. Soc. Am. A, **18,** 1862–1870 (2001).
11. A. Jesacher, S. Fürhapter, S. Bernet, and M. Ritsch-Marte , "Diffractive optical tweezers in the Fresnel regime," Opt. Express **12**, 2243-2250 (2004), http://www.opticsexpress.org/abstract.cfm?URI=OPEX-12-10-2243.

---

1. Introduction

In optical microscopy, methods like dark-field and phase contrast imaging have been developed to increase the contrast of observed amplitude and phase objects. These methods are based on filtering of the image information in its Fourier plane.

#5940 - $15.00 US Received 1 December 2004; revised 10 January 2005; accepted 19 January 2005

Our proposed method is related to these spatial Fourier filtering methods, but using a different phase filtering function in the Fourier plane. The spiral phase operation manipulates the phase of the whole light field in the Fourier plane in the form of a spiral-shaped phase profile of the form $\exp(i\varphi)$, where φ is the polar angle in a plane transverse to the light propagation direction. In our case we obtain such a spatially varying helical phase offset by diffraction of the Fourier image at a specially designed blazed phase hologram, which is displayed at a high resolution spatial light modulator. Alternatively, it would be possible to replace the SLM with a static phase hologram [1], or a spiral phase plate [2, 3]. Related Fourier plane filtering [4] with so-called vortex lenses has been investigated in a few publications [5, 6, 7]. So far, only macroscopic amplitude objects with 100% transmission contrast (macroscopic circular apertures and slits) have been investigated and an edge enhancement has been reported.

The spiral phase contrast method is also related to the Nomarski (or DIC) method by their common property of being sensitive to phase gradients. The Nomarski method is a kind of shearing interferometry, based on the interferometric superposition of two slightly shifted images of the object. However, the obtained Nomarski image is not isotropic, i.e., it amplifies only edges with a certain local direction, depending on the the orientation of the Nomarski prisms. Furthermore, the Nomarski method employs two light waves with orthogonal polarizations, thus being disturbed by a possible birefringence of the sample [8]. The disadvantages of the Nomarski method are avoided in spiral phase imaging since the method isotropically highlights all edges within a sample simultaneously.

Here we focus on the application of spiral phase filtering to optical microscopy, particularly for imaging phase objects. We observe strong edge detection for objects with small phase jumps. Numerical comparison of the spiral phase method with a ”normal” phase contrast method predicts that the resolution for phase jumps is enhanced by orders of magnitude, suggesting that optical thickness gradients of phase objects can be imaged on a nanometer scale.

2. Basic properties of a spiral-phase filter in the Fourier plane

Spatial filtering of an image-carrying light wave with an amplitude or phase filter located in a Fourier plane of the image wave corresponds to a convolution of the image field amplitude with a kernel which is the Fourier transform of the filter [9]. In our case, we use a phase filter in the Fourier plane, which imprints a ”spiral staircase” on the phase of the incoming light wave, of the form $\exp(i\varphi)$, where φ is the polar angle in a plane transverse to the light propagation direction, measured from the center of the Fourier image. Such a spiral filtering function has recently been proposed as a two-dimensional generalization of the one-dimensional Hilbert transform [10]. It has been shown that this kind of phase shifting in the Fourier space corresponds to a convolution of the image amplitude with a kernel function $r^{-2}\exp(i\varphi)$ in real space.

Obviously, convolution with such a kernel function replaces each image point in a point-symmetric environment or an uniform area with a zero. This is the result of the dependence of the kernel on the angular coordinate φ, which guarantees that any contribution to the convolution integral by one image point is cancelled by a negative contribution of a central-symmetric opposite point. The same reasoning suggests that the convolution produces intensity maxima at amplitude or phase edges of an image. Due to the spherical symmetry of the kernel, this applies for all directions of an edge within the object plane, i.e., the method is isotropic. The dependence on r^{-2} concentrates the convolution process to the nearby surrounding of each point, such that the intensity enhancement at edges within an image falls off rapidly with increasing distance from the edge. Image filtering with such a kernel therefore results in an intensity distribution which is proportional to the intensity gradient of the original image, conserving the total image intensity. This promises extremely sensitive detection of phase jumps or edges, which are orders of magnitude smaller than those detectable with the phase-contrast method.

#5940 - $15.00 US Received 1 December 2004; revised 10 January 2005; accepted 19 January 2005

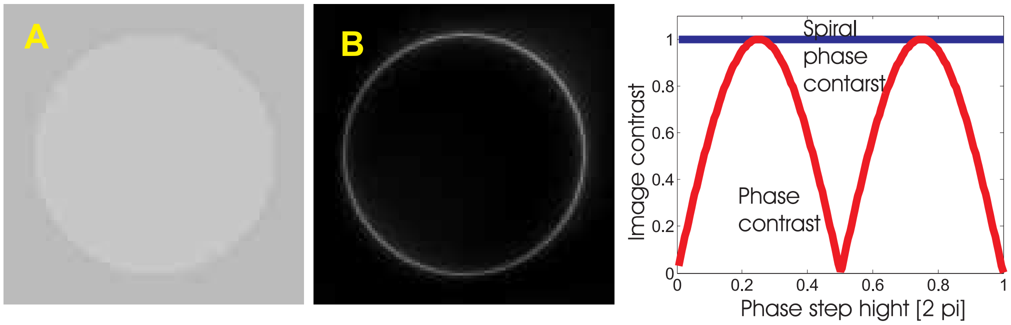

Fig. 1. Simulation of the imaging of a circular phase step for the phase contrast and for the spiral phase contrast method. Image A shows the result for phase contrast imaging of a phase step of 0.25% of the optical wavelength. The image contrast is 6%. In B the same simulation is performed for spiral phase imaging. There the image contrast is 100%. The graph at the right shows the resulting image contrast of the phase contrast method (oscillating) and the spiral phase contrast method (equals 1 at any point).

A numerical simulation of the contrast enhancement is shown in Fig. 1. The first image (A) shows the outcome of standard phase contrast imaging of a circular phase step with a step hight of 0.005 π, corresponding to 0.25% of an optical wavelength (i.e., 2 nm in the case of 780 nm light). It turns out that the contrast, defined as the intensity amplitude (maximal minus minimal intensity) normalized by the maximal intensity is in this case 6%, i.e., the intensity modulation within the image is hardly recognizable. In (B) an analogous simulation is performed for the spiral method. In this case, the contrast is 100% even for the used small modulation amplitude.

The simulation was performed by first Fourier transforming the sample phase image, then multiplying pixel by pixel with the respective filter function, and then performing the reverse Fourier transform. In the case of phase contrast imaging the filter mask consists of an array of ”ones”, with only one changed pixel in the center of the mask, consisting of the imaginary unit ”i”, corresponding to a relative phase shift of $\pi/2$ between the center and the other parts of the filter. The spiral-phase filtering mask consists of the spiral complex phase function $\exp(i\varphi)$, where the central pixel ($r=0$) is set to 0. A calculation of the obtained image contrast as a function of the phase step hight is shown in the right graph of Fig. 1. It turns out, that the contrast of the spiral phase method always equals one, i.e., the background is completely suppressed. The contrast of the phase contrast method oscillates, i.e., it first increases with increasing phase step hight and it reaches full modulation at a phase step of $\pi/2$, but then it decreases to zero at a phase step hight of π, since in this case the zeroth order Fourier component vanishes. In the interval between π and 2π the behavior is repeated. The detailed behavior of the phase contrast method depends on the phase distribution within the chosen sample object, which determines the magnitude of the zeroth order Fourier component. On the other hand, the contrast of the spiral-phase method is not influenced by this effect. The numerical results suggest that, depending on the quality of the experimental setup (i.e., resolution and diffraction efficiency of the SLM, and centering of SLM with respect to the object Fourier plane) phase jumps on the order of 1% of the light wavelength or less should be detectable.

3. Experiment

A sketch of our optical setup is displayed in Fig. 2. In the object plane of an inverted microscope (Zeiss Axiovert 125) a transparent amplitude or phase sample is illuminated with an expanded plane light wave emitted from a single-mode 780 nm laser diode. In the figure, the propagation

#5940 - $15.00 US Received 1 December 2004; revised 10 January 2005; accepted 19 January 2005

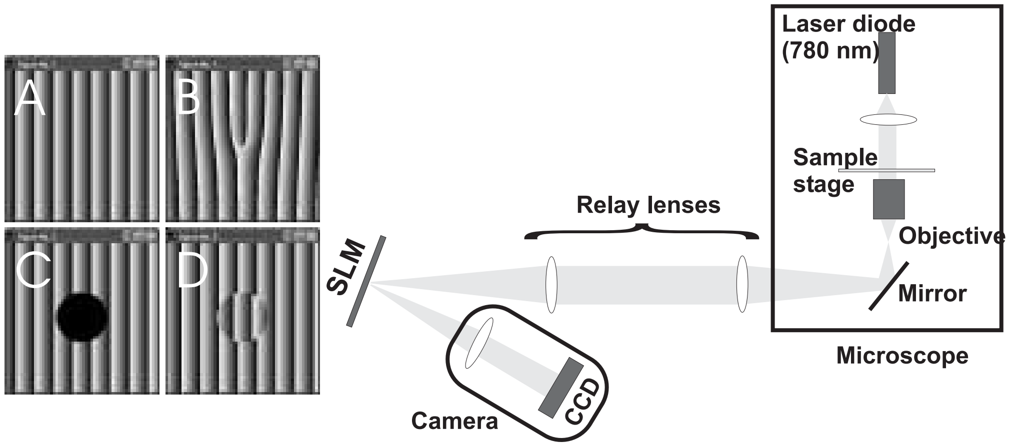

Fig. 2. Sketch of our experimental setup. The displayed holograms A-D used for certain spatial filtering purposes in the Fourier plane are not to scale. Details of the experiment are explained in the text.

of the illuminating laser light is indicated. The transmitted light is collected by a microscope objective (magnification 10x or 20x). Behind the objective a further set of two relay lenses is arranged such that the rear focal plane of the microscope objective is imaged in a plane outside of the microscope. This plane is therefore conjugate with respect to the image plane, i.e., it contains a Fourier transform of the image amplitude information. There, a reflective spatial light modulator (Holoeye 3000 system, resolution 1920 x 1200 square pixels with an edge size of 10 microns [11]) is located, which can display blazed phase holograms which perform the selected filtering tasks. The SLM is driven by a second graphics output of a computer, and it just displays a copy of the computer monitor screen. Therefore holograms can be displayed in ”normal” image windows and dragged under mouse-control across the screen.

The holograms are calculated as off-axis holograms such that the light diffracted into the first order can be spatially separated and used for imaging. For spiral phase filtering, a hologram is displayed which has a vortex discontinuity in its center (see B), which coincides with the focused zero-order Fourier spot of the image light wave. The incident light wave is then diffracted from the blazed phase hologram displayed at the SLM with an absolute diffraction efficiency of approximately 35%, and only the light diffracted at the first diffraction order is used for further imaging, after blocking undesired other diffraction orders (not indicated in the Figure). Imaging is then performed with a third lens located symmetrically (at a focal distance) between the SLM and a CCD chip.

For recording an image in ”normal” bright-field mode, the SLM displays just a blazed grating (A), which diffracts the incoming light wave to the CCD camera. The system can be used to simulate other phase and amplitude Fourier filters, as e.g., a dark-field filter (C - by replacing the overall blazed grating in a small circular area in its center with a non-diffracting unstructured area, thus discarding the center of the Fourier transform), or a phase contrast filter (D - by replacing the central area of the blazed grating with a $\pi/2$-phase shifted grating).

The spiral phase contrast method has already been shown to produce an edge-enhanced image of a macroscopic circular aperture [6]. For completeness, we present here a related experiment, demonstrating the imaging of a microscopic absorptive object with a non-circular symmetry. As a sample object we used a standard transmissive resolution target. Figure 3 displays at the left a bright-field image of a letter within the sample (size 250 microns), which was

#5940 - $15.00 US Received 1 December 2004; revised 10 January 2005; accepted 19 January 2005

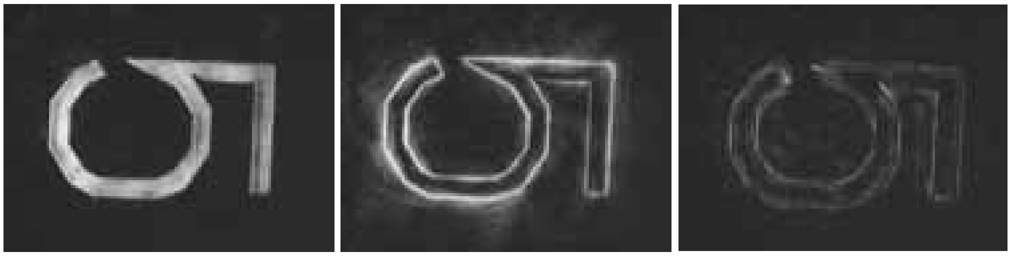

Fig. 3. From the left: Bright-field, spiral phase contrast and dark-field image of an absorptive sample (resolution target, size of the total image area is 400 x 300 microns), imaged in transmission geometry. The three images were taken under identical illumination and camera settings.

recorded by just displaying a blazed grating (see Fig. 2(a)) as a phase hologram at the SLM. The next image shows an edge contrast enhanced version of the letter, obtained by switching the SLM display to present a spiral phase hologram (see Fig. 2(b)), keeping all other settings of the imaging setup constant. A numerical comparison of the integrated intensities of the two images shows that they are equal, although strongly redistributed. For comparison we also recorded a dark-field image of the sample (right side of the figure), by switching the SLM display to a blazed grating with a small non-diffracting sphere in its center, resulting in a suppression of light diffracted from the center of the hologram (see Fig. 2(c)). The result resembles the spiral-phase filtered image in the middle of Fig. 3, however the brightness and the contrast are strongly decreased. This is a result from throwing away the zeroth Fourier component, which carries a large amount of the overall light intensity.

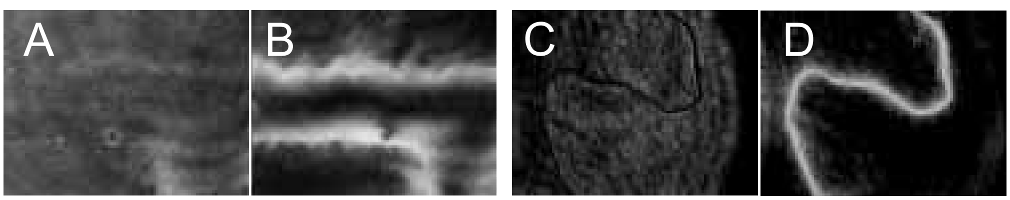

Fig. 4. (1.04 MBytes) Contrast enhancement of phase objects. Two pairs (A-B, and C-D) of bright-field images (A, C) and spiral phase filtered images (B, D) of the same sample areas of a phase object. In A-B the sample consisted of a scratch in a glass coverslip, coated with water. The attached movie shows an extended scan over a larger sample area, comparing the two imaging modes. In (C) and (D) another sample is imaged, consisting of a sharp oil-water boundary.

A major advantage of the spiral phase filter function becomes obvious by imaging phase-sample objects with small phase jumps. An example is shown in Fig. 4. In the upper row, two images of the same object section of a phase object, consisting of a scratch in a glass coverslip coated with water, are displayed. In (A) a transmission bright-field image was recorded by displaying a ”normal” blazed grating at the SLM display in the Fourier plane of the setup, recording only the first order diffracted beam. In (B) the blazed grating was substituted by a hologram with a spiral-phase discontinuity in its center, and again the first order diffracted light was used for imaging. The two images were recorded with identical settings of illumination and image integration time. Obviously, the edge enhanced version in B obtained by spiral phase filtering shows much more details of the object plane section. An attached mpeg-movie shows

#5940 - $15.00 US Received 1 December 2004; revised 10 January 2005; accepted 19 January 2005

a larger area of the same sample, obtained by scanning the object coverslip with a motorized microscope translation stage.

In (C) and (D) a bright-field image and a spiral phase filtered image of the same region of an oil-water boundary are displayed. Again the two images were taken under the same circumstances, i.e., the same illumination and camera settings. In this case, the boundary is barely visible with the bright-field method, but clearly recognizable in the spiral phase filtered image. It can be seen that the intensity of the edge of the oil-water boundary does not depend on its local direction, demonstrating that edge detection is isotropic. Quantitative analysis of the image pair reveals that the total light intensity, integrated over the whole imaged area is again almost identical for the bright-field and the spiral-phase methods. This means, that edge contrast enhancement of a spiral phase filtered image is the result of a redistribution of the image background intensity to phase edges of the imaged area.

Compared to on-axis spiral phase elements (like a spiral wave plate, or on-axis diffractive optics), the off-axis holographic method has the advantage that filtering is almost perfect, i.e., all parts of the first-order diffracted image wave have automatically acquired the desired phase shift. Limitations of the SLM, like electronic noise, undesired additional polarization rotation, or non-linear phase shifting properties are only influencing the diffraction efficiency, but not the purity of the phase filtering function in the first diffraction order. A more crucial point is the resolution of the SLM. The filtered image is the convolution of the actual image with the point spread function of an infinitely small point in the object plane, which corresponds to the ”plane wave response” of the SLM hologram. Therefore, in order to conserve the optical resolution of the setup, the resolution of the SLM should be on the same order (or higher) than that of the optical setup. As an empirical estimate the number of actually illuminated SLM pixels (which can be controlled by the optical magnification of the relay lens system) should be larger than the number of optically resolvable object pixels in a corresponding ”normal” microscopic setup. In our case we use a high resolution SLM, effectively illuminating approximately 1000×1000 SLM pixels. There we could not observe any reduction in optical resolution as compared to ”normal” microscopy (replacing the SLM by a mirror).

4. Discussion

We have investigated a novel Fourier filtering method for applications in microscopic imaging of amplitude and phase objects. Using a high resolution SLM for displaying the desired filter function, it is possible to switch between different microscopic methods, like ”normal” bright-field imaging, dark-field imaging, phase contrast imaging, and the new spiral phase contrast method. Compared to the dark-field and the phase contrast method, we find that the edge contrast enhancement is much more pronounced, since the spiral phase contrast method produces output images with an intensity distribution proportional to the gradient of the original sample, while conserving the total image intensity. We expect that the spiral phase contrast method should allow the imaging of phase jumps, which are on the order of 1% or less of the optical wavelength. On the other hand, if a phase sample has a smooth, continuous topography extending over multiple optical wavelength, the method produces an interferogram of the sample. This will be the subject of future investigations. Additionally, it will be interesting to examine the effects of spiral phase filters with higher helicities which are easily produced with the SLM setup. First observations already show that the image changes drastically as a function of the spiral helical index, and it may be assumed that this can be used to assemble even more detailed image information. Overall, we think that the setup itself, allowing dynamic arbitrary Fourier filtering operations, and its particular application of spiral phase filtering can be an enrichment for microscopy in the optical regime.

#5940 - $15.00 US Received 1 December 2004; revised 10 January 2005; accepted 19 January 2005