

The generalised phase contrast method and its applications

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Introduction

The imaging and visualisation of optical phase, such as wavefront disturbances or aberrations is a challenging yet often vital requirement in optics. A number of techniques can be applied in fields ranging from optical component testing through to wavefront sensing whenever a qualitative or quantitative analysis of an optical phase disturbance is required. In general, a phase disturbance cannot be directly viewed and a method must therefore be sought to extract information about the wavefront from an indirect measurement. An example of this is the generation of fringe patterns in an interferometer, which gives information about the flatness of an optical component without requiring a physical measurement of the component surface. In this article, we describe a powerful phase-contrast technique that we have developed for the visualisation of phase disturbances, outlining the considerable improvements this method offers over previous analyses and discussing some potential applications.

Background

A number of interferometric phase visualisation techniques can be classed as common path interferometry where the signal and reference beam travel along the same optical axis and interfere at the output of the optical system. Put simply, this means that we perturb a portion of the wavefront we wish to measure to create a reference wavefront and interference between the unperturbed wavefront information and this synthetically generated reference allows the visualisation of the phase information in the original wavefront. A schematic representation of the operation of a common path interferometer (CPI) is shown in Fig. 1. Possibly, the most widely known implementation of the CPI is the Zernike

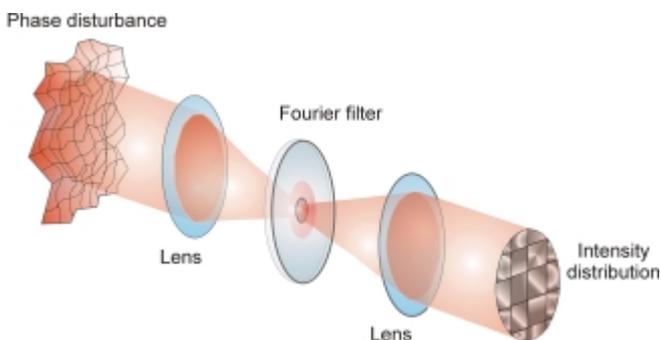


Fig. 1. A schematic representation of a CPI based on a 4-f optical system. A region of a given input phase disturbance is sampled and generates an intensity distribution, in the image plane of the optical system by a filtering operation in the Fourier plane. The values of the filter parameters determine the type of filtering operation. Typically, a versatile and efficient filter might have 100% transmission and a π phase shifting central region.

phase contrast method.¹ They exist however, in many different forms such as the point diffraction, dark central ground filtering, and field absorption methods. Although special cases such as the Zernike method have been previously treated, a comprehensive approach to the analysis of the generic CPI is lacking.

Our recent work has thus concentrated on the establishment of a rigorous analytical framework to describe the operation, design and optimisation of this class of interferometers. The approach we use is based on a generalisation of the Zernike technique. The Generalised Phase Contrast (GPC) method²⁻⁴ is not limited by the operational constraints of the Zernike technique and by careful choice of the parameters for the Fourier filter (see Fig. 1) to match the phase disturbance, it is possible to convert the phase information into a high contrast intensity distribution with a minimal loss of photons.

Based on the theoretical framework of the GPC method we can design CPI systems for a range of applications and achieve optimal performance in terms of fringe accuracy, visibility and peak irradiance. The GPC approach is an extension of the Zernike method and in the following sections; we therefore use this as the starting point in our explanation of the requirements for a generalised description. In the remainder of this article, we give an overview of some potential applications that exploit the GPC method. These include phase-visualisation and wavefront sensing, programmable optical tweezers and optical encryption systems.

Zernike phase contrast

The Zernike phase contrast technique allows the visualisation of phase perturbations by the use of a Fourier plane phase shifting filter. The Dutch physicist Fritz Zernike received the Nobel Prize in 1953 for inventing this method, which led to a break-through in medicine and biology by making essentially transparent cell or bacteria samples clearly visible under a microscope. Its successful operation, however, requires that the spatial phase distribution, $\phi(x, y)$, at the input is limited to a “small-scale” phase approximation where the largest phase is typically taken to be significantly less than $\pi/3$. If the phase distribution at the input is thus restricted, then a Taylor expansion to first order is sufficient for the mathematical treatment and the input wavefront can be written as,

$$\exp \{i\phi(x, y)\} \approx 1 + i\phi(x, y). \quad (1)$$

The light corresponding to the two terms in this “small-scale” phase approximation can be separated spatially by use of a single lens, where the phase distribution is located in the front focal plane and the corresponding spatial Fourier transformation is generated in the back focal plane of the lens. In this first-order approximation, the constant term represents the amplitude of on-

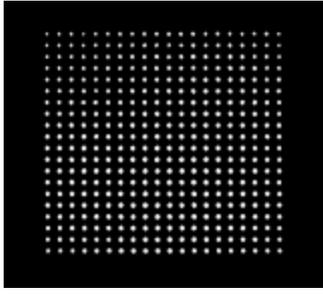


Fig. 2. A 20x20 spot array generated using the GPC method in combination with a fixed phase mask. A 60 micron diameter PCF was used and the array dimensions are approximately 3x3mm.

axis light focused by the lens in the back focal plane and the second spatially varying term represents the off-axis light. Zernike realised that a small phase shifting quarter wave plate acting on the focused light makes it possible to obtain an approximately linear visualisation of small phase structures by generating interference between the two phase quadrature terms in Eq. (1):

$$I(x', y') \approx 1 + 2\phi(x', y'). \quad (2)$$

Generalised phase contrast

In the general case, we do not wish to be restricted to a limited phase range and we therefore cannot make the first order approximation of the Zernike technique. Higher order terms in the expansion need to be taken into account, so the expansion takes the form:

$$\exp\{i\phi(x, y)\} = 1 + i\phi(x, y) - \frac{1}{2}\phi^2(x, y) - \frac{1}{6}i\phi^3(x, y) + \frac{1}{24}\phi^4(x, y) + \dots \quad (3)$$

In this expression however, the contribution of spatially varying terms cannot be separated from the supposedly focussed light represented by the first term in this Taylor series expansion. In fact, all of these spatially varying terms can contribute to the strength of the on-axis focused light. For a significant modulation in the input phase, this contribution from the spatially varying terms can result in a significant modulation of the focal spot amplitude in the back focal plane of the lens. These terms can thus result in either constructive or destructive interference with the on-axis light, the net result of which will be an attenuation of

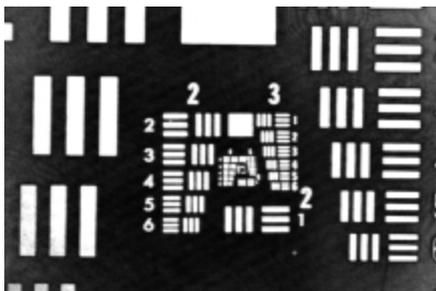


Fig. 3. The GPC method with a phase-only SLM generating the input phase object, in this case a phase version of the USAF resolution target.

the focussed light amplitude which can only have a maximum value for a perfectly plane wave at the input.

Thus for phase objects or wavefronts breaking the Zernike approximation we must find an alternative mathematical approach to that of the Taylor expansion given in Eq. (3). We have chosen a Fourier analysis as a more suitable technique for completely separating the on-axis and higher spatial frequency components. This gives the following form for $\exp(i\phi(x, y))$, where $(x, y) \in \Omega$:

$$\exp\{i\phi(x, y)\} = \left(\iint_{\Omega} dx dy \right)^{-1} \iint_{\Omega} \exp\{i\phi(x, y)\} dx dy + \text{"higher frequency terms"}. \quad (4)$$

In this Fourier decomposition, the first term is a complex valued constant linked to the on-axis focused light from a phase disturbance defined within the spatial region, Ω . The second term describes light scattered by spatially varying structures in the phase object. Comparing Eq. (3) and Eq. (4) it is apparent that the first term of Eq. (3) is a poor approximation to that of Eq. (4) when operating beyond the Zernike small-scale phase regime.

A key point in the Generalised Phase Contrast method is the identification of the operating regime where a match can be achieved between the strength of the focused light (first term of Eq. 4) and the Fourier filter parameters (see Fig. 1). If the system is applied to wavefront sensing or the visualisation of unknown phase objects the GPC method specifies the filter parameters for achieving optimal performance in extracting and displaying the phase information carried by the incoming wavefront.⁴ In the case where we have control over the incoming wavefront or phase modulation the method provides extra means of optimisation by encoding the phase distribution itself²⁻³ in addition to the filter parameters. This approach is particularly useful when the filter parameters have a restricted dynamic range or are fixed. The rigorous derivation of the equations for choosing these parameters can be found in Ref. 4 and the references therein.

Phase imaging by the Generalised Phase Contrast Method

By controlling the input phase distribution, a system for visualising phase objects becomes a highly effective system for the generation of intensity distributions, where interference is used to

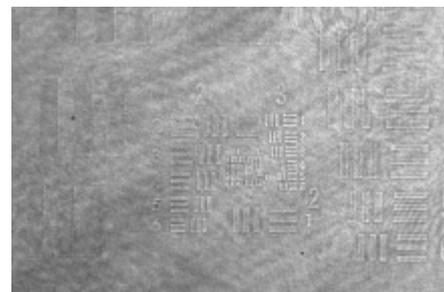


Fig. 4. With the PCF removed from the optical system, contrast is lost and the input phase object is not visualised. Residual amplitude modulation in the phase-only SLM accounts for the slight visibility of the pattern.

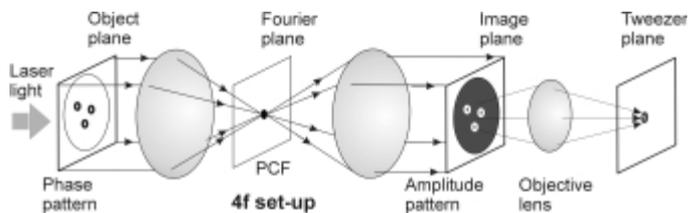


Fig. 5. The proposed system layout for a phase contrast based optical tweezer system. A phase-only modulation in the object plane of a 4-f imaging system can produce a high-contrast intensity distribution in the image plane when a suitably matched phase contrast filter (PCF) is placed in the Fourier plane. An objective lens reduces and focuses this image in the desired tweezer plane.

generate high contrast light patterns. One application for the GPC technique is array generation, the splitting of a light beam into a given number of secondary beams. An example of this is shown in Fig. 2. In this case, a fixed phase mask generates a controlled “phase disturbance” (see Fig. 1) and from this filtered imaging operation a high contrast intensity pattern is obtained, the structure of which is determined by that of the phase mask.

It is also possible to use a phase-only spatial light modulator (SLM) as a controllable dynamic phase disturbance. An example of an irregular array (a USAF resolution target) generated with an SLM as input is shown in Fig. 3. Such irregular arrays are extremely challenging to produce with alternative techniques such as computer-generated holography demonstrating a significant advantage of the GPC method. The role of the phase contrast filter (PCF) in generating the high contrast is emphasised by Fig. 4. In this example, the PCF is not present and the resulting image shows an almost complete loss of contrast when compared to Fig. 3 in which the same input phase disturbance is used.

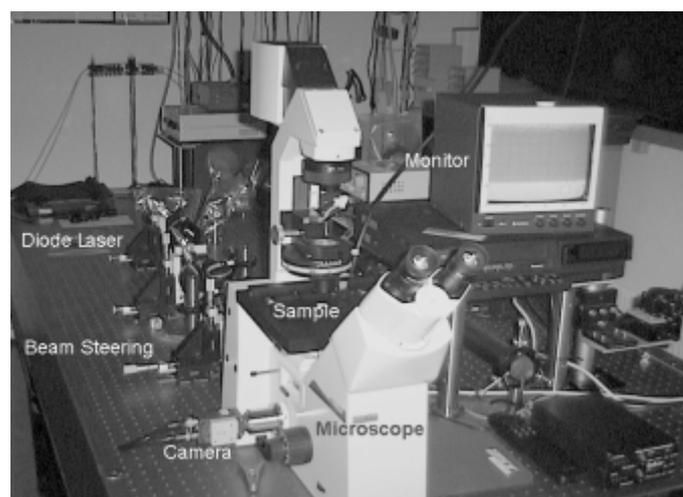


Fig. 6. Photograph of the Optical tweezer system showing the inverted microscope with the objective beneath the sample stage. The laser system and focussing optics can be seen directly behind the microscope and the trapping and manipulation of samples is observed with a CCD camera.

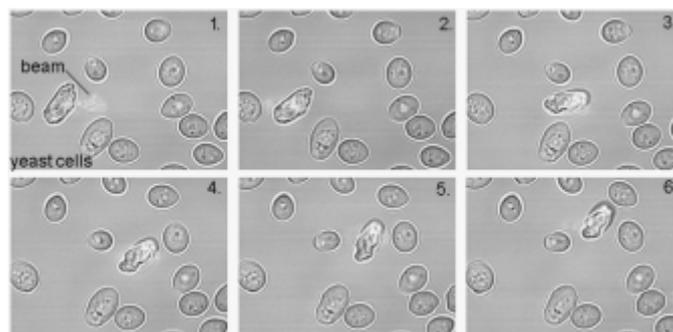


Fig. 7. A set of images showing the trapping and manipulation of an individual yeast cell. (1) shows the introduction of the laser beam into the sample - initially moved to the left where it traps an irregularly shaped yeast cell (2), this is then manipulated (3)-(6). As the trapped cell is manipulated, it twists and moves in the trap changing the cross section of the cell that is in focus. The typical size variation of the cells of approximately 5-10 μm . A short Real Player movie sequence of the optical trapping can be seen at the web-site: <http://www.risoe.dk/aktuelt/video/aktuelt.htm>

The GPC method for programmable optical tweezers

The recent development of optical tweezers represents an extremely interesting and useful application of optics to the field of cell biology and micromanipulation in general.⁵ Optical tweezers use the radiation pressure effect from a highly focussed laser beam to trap and manipulate micron-sized cells and particles with pN sized forces. It is often desirable to simultaneously operate a number of optical tweezers to independently control the relative movement or placement of molecules and a number of different techniques for achieving this have been suggested.

We wish to apply the generalised phase contrast technique in conjunction with a phase-only spatial light modulator to generate a dynamic, reconfigurable and computer controlled multiple beam tweezer system.⁶⁻⁷ In such a system, the number, shape and position of tweezer beams can be modified to best suit the trapping task at hand. Using our approach, a phase-only liquid-crystal spatial light modulator (SLM) encodes an image directly in the phase component of the collimated monochromatic wavefront of an expanded laser beam. This phase-encoded information serves as the input for a phase-contrast system, in which the phase-contrast filter (PCF) generates a high-contrast amplitude pattern as described in the preceding section. This amplitude pattern can then be focussed down using a microscope objective in order to produce a suitable wavefront for the optical trapping of microscopic particles. The layout for the optical system is shown in Fig. 5.

The first step of this project is the construction of a conventional single beam tweezer system a photograph of which is seen in Fig. 6. This system uses a 200mW, 830nm diode laser, which is coupled into a high-resolution microscope and through the objective lens onto the sample to produce a trapping beam in the focal plane of the microscope. In this basic tweezer system, beam steering is achieved by displacing a lens in the optical path to manipulate a single trapped particle.

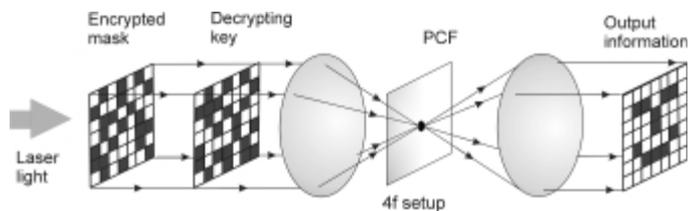


Fig. 8. Schematic diagram showing the generic system architecture for the phase-based decryption operation and the visualisation of the decrypted information with a phase contrast filter (PCF).

The image sequence in Fig. 7 shows optical trapping and manipulation in a biological specimen. In this case, a single yeast cell is selected, trapped and subsequently moved around in the field of view. Trapping is possible with laser powers in the 5-20 mW range. Referring to the image sequence, the position of the laser beam can be followed by the light reflected and scattered from the cover slip and the yeast cell during the trapping and manipulation process.

The GPC method for optical encryption

There is widespread interest in the development of encryption systems, which operate in the optical domain. The advantages inherent in an optical approach to encryption, such as a high space-bandwidth product, the difficulty of accessing, copying or falsification and the possibility of including biometrics are widely recognized.⁸ In an encryption system, we wish to encode information in such a fashion that even if it is viewed or copied only the application of the correct key will reveal the original information. Our encryption approach is based on the direct mapping of an encrypted phase-mask and a decrypting phase key, resulting in the decryption of information completely within a phase-only domain.^{9,10} A schematic diagram of the generic phase-only encryption system is shown in Fig. 8.

In this system, an encrypted binary phase mask is decrypted with a binary phase-only key and the decoded information is subsequently visualised using the GPC method. A plane polarised monochromatic wavefront illuminates the encrypted phase mask, which consists of a random array of binary phase-shifting pixels. This phase-mask is produced by electronically scrambling the original binary-format information we wish to encrypt with a random binary pattern and using this to generate an encrypted phase mask. The decrypting key effectively reverses the scrambling operation in the optical domain and results in the production of a wavefront in which the information of interest is encoded as a relative phase shift between different sections of the wavefront, in this case corresponding to the pixels.

The mask and key can be placed, either directly in contact with one another so that the decryption takes place in the same image plane, or alternatively they can be imaged onto one another with an optical system. By using a spatial light modulator, the phase key can be scrolled electronically until it overlies the phase pattern of the encrypted mask removing the necessity for precise mechanical positioning in the optical system. An experiment utilising this electronic alignment feature is shown in Fig. 9 with the dynamic phase key displayed in Fig. 9(c) and the fixed scrambled phase mask in Fig. 9(b). Superposition of these two phase

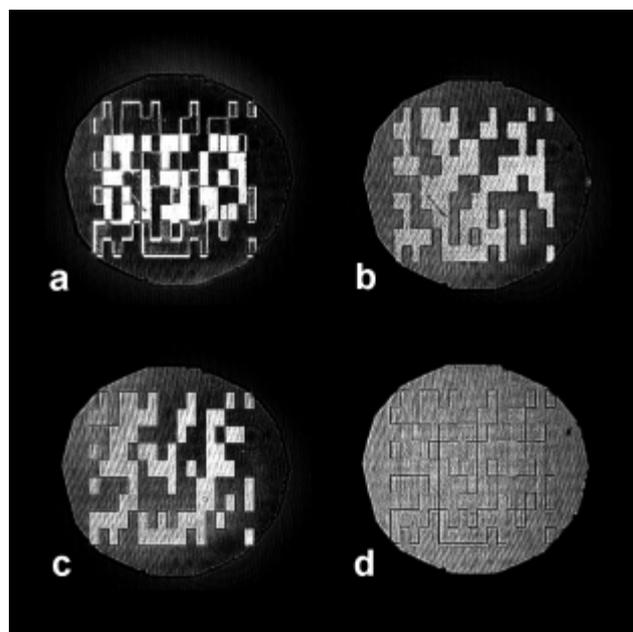


Fig. 9. Decryption of a 17×9 pixel fixed mask with a 17×9 pixel dynamic key. (a) shows a successful decryption which reveals the text RISØ, (b) shows an image of the fixed phase mask viewed with the PCF and (c) shows the corresponding image for the decrypting key. If the PCF is misplaced then the decrypted information is not visualised (d). The fixed mask size is 3mm square.

patterns results in the decrypted phase pattern shown in Fig. 9(d) and visualised by use of the GPC method in Fig. 9(a).

It should be noted that although we use binary phase masks for the key and the encrypted information, we are in fact not limited to this case. The encryption technique we present could equally well be applied to systems in which multiple phase levels are used for the masks and keys. However, the fabrication issues involved in the production of a multiple phase level fixed phase mask are more complicated than for the production of a binary mask, so for the purposes of the experimental demonstration of decryption binary $0/\pi$ phase masks and phase keys have been used.

Acknowledgements

The phase masks and phase contrast filters have been produced by; E. Eilertsen, E. Rasmussen and J. Stubager using the holoplotter facility at Risø National Laboratory. We thank A. Nordskov, T. Petersen and B. Sass for the construction and design of a number of the opto-mechanical components used in these experiments.

We acknowledge the help of the 4th research group at the Hamamatsu Photonics Central Research Laboratories, particularly H. Toyoda, Y. Igasaki and T. Hara, for providing phase-only spatial light modulators and expertise as part of a joint research agreement.

The Programmable Phase Optics program is funded as part of an award from the Danish Technical Scientific Research Council (STVF). Our work on optical tweezers has been made possible by an equipment grant from the centre for biomedical optics and new laser systems (BIOP).

References

1. F. Zernike, "How I discovered phase contrast", *Science* **121**, 345-349 (1955).
2. J. Glückstad, US patent no. 6,011,874, "Phase contrast imaging", issued Jan. 2000.
3. H. Toyoda and J. Glückstad, US patent no. 6,021,223, "Image forming apparatus", issued Jan. 2000.
4. J. Glückstad and P. C. Mogensen, "Optimal phase contrast in common path interferometry", *Appl. Opt.* **40**, 268-282 (2001).
5. A. Ashkin, J. M. Dziedzic, and T. Yamane, "Optical trapping and manipulation of single cells using infrared laser beams", *Nature* **330**, 769-771 (1987).
6. J. Glückstad and P. C. Mogensen, "Reconfigurable ternary-phase array illuminator based on the generalised phase contrast method", *Opt. Comm.* **173**, 169-175 (2000).
7. P. C. Mogensen and J. Glückstad, "Dynamic array generation and pattern formation for optical tweezers", *Opt. Comm.* **175**, 75-81 (2000).
8. B. Javidi, "Securing information with optical technologies", *Physics Today* **50**, 27-32 (1997).
9. J. Glückstad, "An optical encryption and decryption method and system", PCT patent application PCT/DK99/00331 (published under 'world-patent' classific. no. WO 002339A1, Feb. 2000).
10. P. C. Mogensen and J. Glückstad, "Phase-only optical decryption of a fixed mask", *Appl. Opt.* **40**, 1226-1235 (2001).

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Jesper Glückstad obtained an MSc in photonics from Aalborg University in 1990 and a PhD in optical neural computation from the Niels Bohr Institute in 1994. In 1998 he was recipient of a Talent Project Grant (refunding obtained in 2001) "Programmable Phase Optics" from the Danish Technical Scientific Research Council. Prior to that he was a visiting scientist at Hamamatsu Photonics K.K. Central Research Laboratories, and "Kawata Lab" at Osaka University, in Japan. He received the DOPS Annual Prize in 2000.

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René Lyng Eriksen received his BSc in Electrical Eng. from the Engineering College of Odense followed by a few years work as Project Engineer at ABB Industri Odense. Subsequently, he obtained an MSc in applied physics at the University of Odense in 1999. Following this, he was employed in the R&D department of Lucent Technologies, Denmark. In 2000, he joined the Programmable Phase Optics program at Risø as a PhD student.