Generalized Phase Contrast: Perspectives on Information Optics

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1. Introduction

Phase contrast and information optics typically fall into common limiting stereotypes: *Phase contrast* is typically associated phase microscopy for biological imaging while *Information Optics* usually evokes either fiber-optic communication or optical alternatives to electronic computations. It is understandable that Information optics is associated with the enormous bits of information carried by rapidly pulsating light streaming through a worldwide network of optical fibers. Yet, it is arguably also optical communication when a loved one beams an endearing smile, or when we are struck by a powerfully moving painting in a museum, or by a particularly serene scene. The same can be said about a microscope, which can optically communicate information from the microscopic domain. All these examples exploit light's information-carrying ability: fiber-optic light pulses carry temporal information while optical images contain spatial information. Moreover, communication with images underscore light's ability to transfer information in parallel as opposed to the bit-by-bit, serial mode when using light pulses in optical fibers. In a way, the marriage of these two optical communication modes was tacitly enshrined in the Nobel Prize in Physics 2009 that was jointly awarded "for groundbreaking achievements concerning the transmission of light in fibers for optical communication" and "the invention of an imaging semiconductor circuit – the CCD sensor"[1]. In this light, *Information Optics* can be taken to include this expanded view of optical communication, along with its allied optical systems (e.g. information processing, transmission, storage and retrieval) as well as the different applications that it enables. In a similar vein, Generalized Phase Contrast (GPC) [2] is not limited to phase imaging, but is a versatile framework that extends phase contrast to not only enhance phase microscopy but also touch on the diverse areas of Information Optics. In this chapter, we provide perspectives on Information Optics from the point of view of Generalized Phase Contrast and its applications.

2. Optical phase information: from phase microscopy to Generalized Phase Contrast

We can use light to carry information as it propagates by modifying one of its characteristics according to the intended information content. Light as an electromagnetic wave is characterized by amplitude, phase and polarization. However most light detectors, including the human eye, respond to the time-averaged energy flux and, thus, are blind to the optical phase and other phenomena associated with the rapid electromagnetic fluctuations. Thus, it is convenient to work with intensity-based optical information. However, the optical phase information can be vital, such as when observing microbiological systems that tend to have minimal absorption and, hence, are essentially transparent.

Zernike's phase contrast microscope (PCM) was invented to visualize near-transparent biological samples that behave as weak phase objects [3-5]. The PCM is essentially an interferometer that visualizes the weak phase objects by using interference to produce contrast based on the phase perturbations due to the object. In conventional interferometry, light is typically first split into two arms– a reference arm and a signal arm– that are later recombined to reveal the relative phase fluctuations between the signal and the reference. Working as a common path interferometer (CPI), a phase contrast microscope synthesizes its own reference wave. Following Abbe's imaging theory [6], which laid the foundations of Fourier optics, we can treat the phase object as a weighted combination of gratings (i.e., corresponding to different spatial frequencies in the Fourier Optics language). The reference wave can be synthesized by phase-shifting some of these spatial frequency components. A practical way to implement this reference wave synthesis using a generic common-path interferometer is schematically illustrated in Fig. 1.

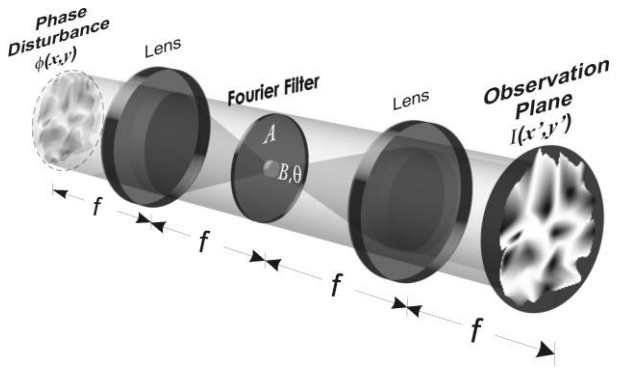


Fig. 1. Generic common path interferometer based on a 4*f* optical system. The diffraction orders representing spatial frequency components of an input phase disturbance, obtained at the back-focal plane of a Fourier transforming lens, are filtered to phase-shift selected components to be used to synthesize a reference wave. Subsequent recombination at the observation plane generates an interference pattern. (adapted from [6], © OSA 2007)

In the weak phase limit, the phase-perturbed input field to this common-path interferometer may be written as

$$p\ x,y\ = \exp\left[i\phi\ x,y\ \right]\Big\|_{\phi\ x,y\ \to 0} \approx 1+i\phi\ x,y\ = 1+\phi\ x,y\ \exp\ i\pi/2\ . \qquad (1)$$

By using a phase-only Fourier filter to introduce a $\pi/2$-phase shift to the unperturbed zero-order component and then subsequently recombining with the phase perturbation, a phase contrast microscope generates an interference pattern,

$$I\ x',y'\ _{\phi\ x,y\ \to 0} = \left| o\ x',y'\ \right|^2 \approx 1+2\phi\ x,y\ , \qquad (2)$$

which exhibits a linear variation with the object phase within its limited range of validity (i.e., the weak phase limit).

Generalized phase contrast extends the phase contrast formulation beyond the weak-phase limit. Assuming a phase-only Fourier filter, the output interference pattern in this case becomes [8]

$$I\ x',y'\ = \left| a\ x',y'\ \exp\left[\mathrm{i}\phi\ x',y'\ \right] + \left[\exp\ \mathrm{i}\theta\ -1 \right] p_l\ x',y'\ \right|^2 , \qquad (3)$$

where the first term is the image of the aperture-truncated input and the second term represents the synthesized reference wave (SRW) whose spatial variation is described as the low-pass filtered image,

$$p_l\ x',y'\ = \Im^{-1}\ \mathrm{circ}\ f_r/\Delta f_r\ \Im\ a\ x,y\ \exp\left[i\phi\ x,y\ \right]\ , \qquad (4)$$

and θ is the phase shift introduced to the low-pass filtered components. This expression reduces to the linear phase contrast of Zernike upon applying similar assumptions of a uniform amplitude assumption, $a\ x',y' = p_l\ x',y'\ = 1$, weak phase approximation, and a filter phase shift of $\theta = \pi/2$.

The GPC framework affords an improved understanding of the phase contrast mechanism and paves the way for deploying GPC-based CPI systems across diverse applications in information optics. For example, the output pattern described in Eq. (3) can be used to analyze the outputs of common-path interferometers to improve the accuracy of quantitative phase microscopy and wavefront sensing in general. Instead of finding unknown phase inputs, we can also use GPC to calculate phase inputs for encoding into spatial light-modulation technologies to generate desired output intensity patterns. Equations derived from the generalized framework can be used for optimizing output metrics such as visibility and light efficiency of arbitrary analogue phase-encoded wavefronts. Moreover, the input-to-output mapping in GPC can be used to create a converse system, referred to as

reverse phase contrast (RPC), which can synthesize desired phase patterns that mimic an input intensity pattern [9].

The diverse applications of GPC can be unified in terms of GPC's role as an information processor under an Information Optics framework (see Fig. 2). In wavefront sensing and measurement, GPC-based optical processing helps optimize the extraction of information from an otherwise invisible phase data. In wavefront synthesis, the GPC prescribes the optimal input field that can synthesize desired outputs. A special case of this synthesis process is the reverse phase contrast, where a phase-modulated output is created from an amplitude-modulated input.

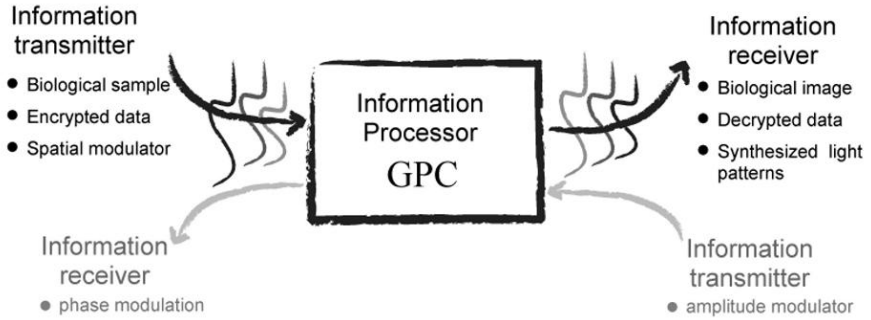


Fig. 2. Generalized phase contrast and Information optics: Depicting the applications of GPC as optical information processing in a generic optical communication channel (adapted from [2], © Springer 2009)

GPC's capacity for processing optical phase information makes it suitable for creating programmable light patterns. Aside from their obvious applications in information display, these controllable light patterns form vital enabling blocks for developing modern optical tools for supporting health and life sciences. One example is interactive microscopy, where light patterns can be used not only to observe the microsystem but also to mechanically manipulate them without using physical contact. These systems are among the many interesting avenues of information optics that GPC is able to access as an optical phase information processor.

3. Biophotonics: Information Optics in support of health and life sciences

One of the Generalized Phase Contrast applications is in Biophotonics– a rapidly developing field driven by the interplay between biology and photonics. Optical phase processing with GPC can support the development of enabling tools for biology and medicine. This area of GPC application is a fertile ground for Information Optics.

How can Information Optics support the health and life sciences? Let's start by considering laser surgery where lasers are used as optical scalpels. At first glance, it appears that this does not concern Information Optics since it deals with the delivery of optical energy, rather than optical information. However, the role of Information Optics becomes apparent when we consider the required optical transformation to mitigate the mismatch between the inherent beam shape from the laser source and the optimal beam shape for optimal light-tissue interaction. We will revisit this point when we discuss optical forces and optical addressing in succeeding sections.

The previous example illustrates the indirect, yet key role of Information Optics in the health and life sciences. The microscope, for example, which is a ubiquitous instrument in biological laboratories, played a key role in medicine by displaying optical information on what would otherwise be invisible microscopic organisms. Understanding the role played by microscopic constituents in diseases was a defining point in the history of scientific medicine and continues to be a vital element in our continuing effort to understand the underlying mechanisms in health and diseases. Emerging optics and photonics technologies are poised to develop the "new microscopes"– the next-generation optical technologies for exchanging information with the micro-world– that can become crucial enabling tools in biological and medical research centers, point-of-care facilities and, eventually, even as part of consumer devices.

Major attention is being focused on unraveling the underlying microscopic and nanoscopic mechanisms that regulate different biological processes. Information Optics can play a key role in developing the necessary micro- and nanotechnologies for investigating these mechanisms and the development of future solutions: From goading stem cells to grow into replacements for damaged human organs, to assisting the innate human mechanisms for self-repair, or coaxing cancerous growths to commit cellular suicide and, maybe, even arresting the cellular and human aging process. This challenge involves gathering information at multiple scales: Even something as innocuous as mechanical material behavior, e.g. deformation and fracture of collagenous tissue (see Fig. 3), may be controlled by an interplay of mechanisms from the different scales.

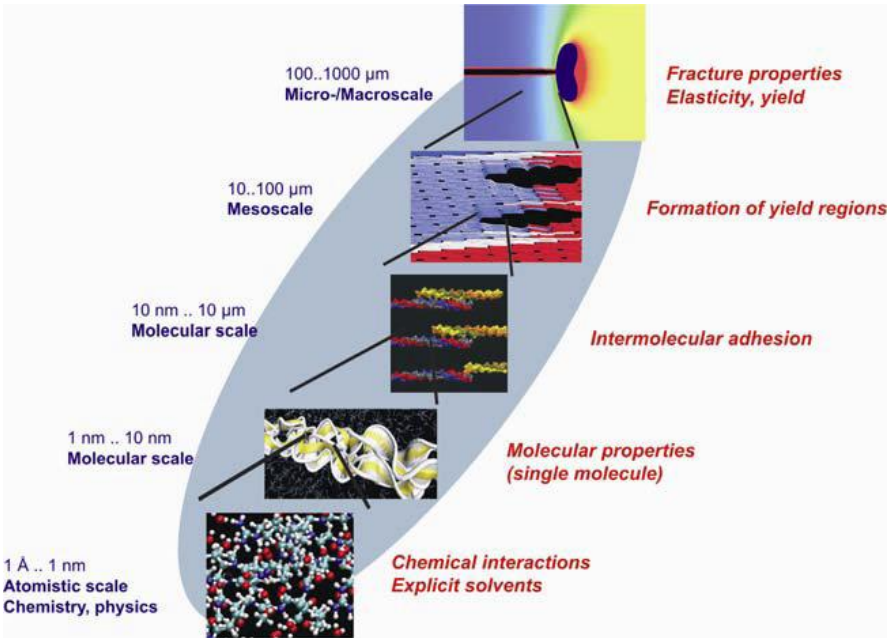


Fig. 3. Overview of the different material scales for that factor into the mechanical material behavior of collagenous tissue (adapted from [10], © Springer, 2007)

4. Microscopy and nanoscopy: Information Optics for optimal information collection

Generalized phase contrast is, at its core, a phase imaging tool and so it naturally lends itself to solutions geared towards accurate phase microscopy. Similarly, the goal of creating tools for extracting accurate information in the micro- and nanoscopic regimes naturally falls within the realm of Information Optics.

How can we extract more information from micro- and nano-scale phenomena? One major hurdle in conventional optical microscopy is the so-called diffraction limit, which was first expounded by Abbe [6]. In short, we cannot just keep optically zooming in and expect progressively better clarity with the higher magnification lenses. Diffraction prevents light from being brought to a point-like focus but rather spreads it out in a blur about the size of the illuminating wavelength. In optical processing terms, the limiting apertures in the microscope apertures set a finite cut-off frequency that blurs the image. This limits our power to observe and probe microscopic phenomena down to a certain length scale, below which the overlapping images of adjacent structures makes it difficult to discriminate the different features.

A way to improve optical resolution is to impart information into light and sculpt it into more functional shapes. This active information gathering can be achieved, for example, using structured illumination like fringes. Combined with post-processing, structured illumination microscopy can double the resolution compared to uniform illumination in conventional passive microscopy [11-13] (e.g. see Fig. 4). Replacing flood illumination with a light sheet, a technique called selective plane illumination microscopy (SPIM) [14], can achieve optical sectioning to image selected slices that is not corrupted by other regions in the volume. A shaped spot [15], or a sequence of shaped spots [16], can ensure that light only comes from a very small region in the sample. A raster scan can then produce an image with much higher resolution. Information optics can contribute to versatile light-shaping, in space and in time, and help tear down the diffraction barrier in future microscopes.

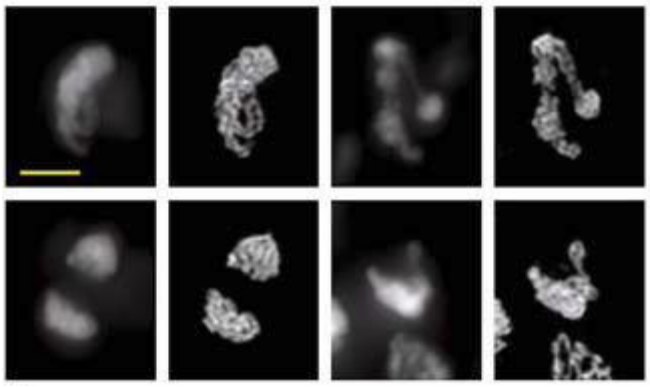


Fig. 4. Conventional microscopy (blurred images) of chromosomes of the transition zone of meiotic prophase in *C. Elegans* and resolution improvement (corresponding sharp images) using structured illumination microscopy (adapted from [13], © Springer 2008)

An upcoming approach in microscopy is to combine the strengths and functionalities of different microscopic modalities by generating a composite image that merges results from the different techniques. By replacing lamps with lasers in modern microscopes, light serves to not only illuminate but also to supply energy for triggering a variety of atomic and molecular processes. The reemission of excess energy as modified light enables nonlinear microscopy, which can reveal structures and biochemical signatures that are otherwise too small or too fast to be visible under normal illumination. Enhancing this microscopic modality requires ultrashort laser pulses to deliver large amounts of energy in a very short time interval. This can, among others, cause a sufficient number of otherwise rare events to occur, such as the simultaneous absorption of two photons and subsequent reemission as a single more energetic photon which can be used to form the image.

Quantitative phase imaging remains a relevant approach in the face of modern super-resolving microscopes. An attractive feature of phase microscopy is its innate non-destructive nature. Using the inherent phase perturbations from the sam-

ple for contrast avoids external contrast agents and can work even at low light levels. This can be highly useful when using highly transparent samples, or when energy from laser absorption can present unwanted side-effects such as heating and photodamage. Hence, quantitative phase microscopy can find a comfortable niche for enriching the information derived from multimodal microscopy.

5. Optical trapping and manipulation: Information Optics for controlled momentum transfer

Processing optical phase information with GPC can contribute to microscopy through quantitative phase imaging. It can also be used to synthesize patterned light that can help improve the optical resolution when combined with *a priori* knowledge about how light interacts with the sample. In general, light patterning can serve as a versatile tool for controlling light-matter interaction. Information Optics can contribute much to this light patterning process and, consequently, to the light-matter interaction that it controls.

Light carries momentum and can exert forces by transferring some of this momentum as it interacts with matter during its propagation. Hence, light shaping can be used, among others, to achieve controlled optical forces. The programmable of light patterns controlled through Generalized Phase Contrast, when relayed into a microscope (e.g. see Fig. 5), has been successfully used to exert controlled optical forces that can independently trap and manipulate multiple microparticles simultaneously. Information optics is a rich resource for creating these dynamic light patterns and can contribute in realizing novel modes of optical trapping and manipulation.

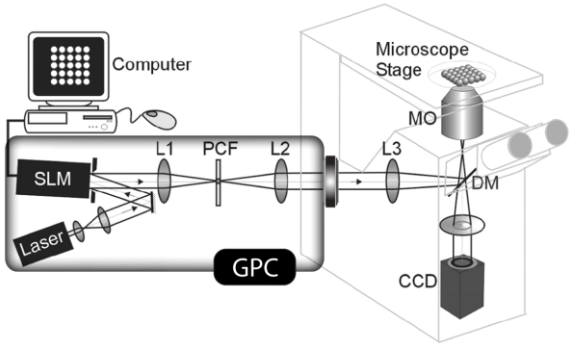
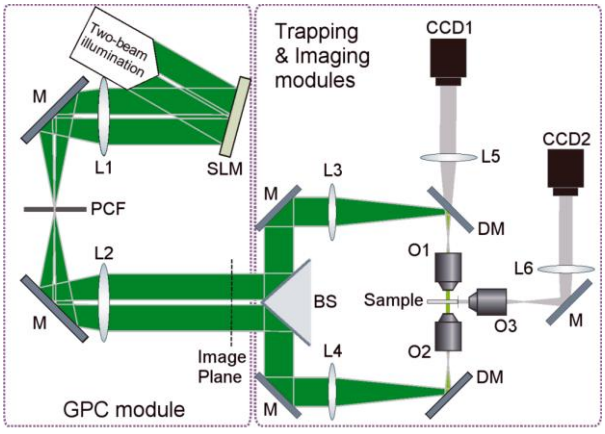


Fig. 5. Optical trapping and manipulation. Light patterns synthesized by a GPC illumination module are relayed to a microscope as optical traps for manipulating microparticles. (adapted from [17], © OSA 2002)

Harnessing optical forces enables interactive microscopy where the user not only observes but is also capable of dictating where, how and when microscopic

processes would occur, either through direct manipulation or programmed and computer-optimized sequences. Interactive microscopy helps advance systematic hypothesis testing and requires not only the ability to observe, but also to manipulate the different constituents in a microsystem. Conventional microscopes only imbibe us with superhuman vision to peek into microscopic worlds. On the other hand, an interactive microscope will not only magnify the miniscule components for accurate observation but also demagnify our physical attempts to manipulate the system. Shaped laser beams can exert forces that are so gentle, yet precise, that they are suitable for safely grabbing and manipulating microscopic constituents with highly calibrated precision.

Information Optics can provide a framework for improving the design and exploring the limits of the next generations of interactive microscopes. These tools can then optimally pack information into the controlling beams and maintain optimal light efficiency in the process. They will also incorporate clever ways of extracting information from the system under control. These elements are incorporated in a GPC-based interactive microscope (see Fig. 6). GPC efficiently creates multiple pairs of diverging beams that are introduced from opposite faces of a sample to optically trap and manipulate microparticles without introducing intensity hotspots that might adversely affect the system. Moreover, this counter-propagating geometry liberates sufficient working distance for auxiliary observations along an orthogonal axis.



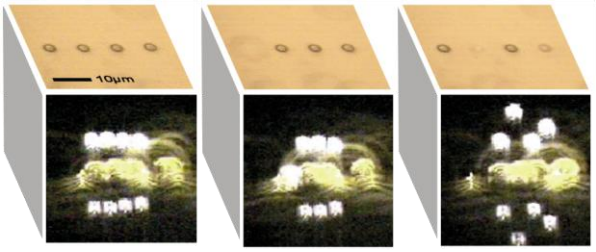


Fig. 6. Interactive microscopy using GPC-generated beams for controlled manipulation of multiple particles. Top: Optical setup with GPC-generated light patterns creating multiple counter-propagating beam traps. Another microsope is fitted orthogonally for enhanced observation. Bottom: Composite of top- and side-view microscope images obtained from the system. (adapted from [18], © OSA 2005)

Contemporary work on optical trapping and manipulation explore the boundaries of possibility with this versatile tool: From demonstrations with miniaturized plastic balls to directed experiments in living cells, bacteria and viruses. Optical forces have already been used to find the stiffness and other physical properties of vital biological components such as DNA [19] and molecular motors [20]. Future optical manipulation technologies, supported by Information Optics, can become invaluable tools in carrying out fundamental biological studies and generating biomedical solutions.

6. Optical addressing and microfabrication: Information Optics for controlled energy transfer

Dynamic light patterns created with GPC and other approaches based on Information Optics provide a means for realizing controlled distribution of optical energy. The calibrated optical forces from light distributions can be used for measuring the physical characteristics of key cellular components, which can contribute in advancing fundamental knowledge. Furthermore, these light distributions provide a means for controlling energy transfer that can support studies of biochemical processes. As expressed in [21], shaped light pulses can 'effectively act as a new class of "reagents" to alter the course of molecular dissociation and rearrangement'. Information Optics can help realize the spatio-temporal shaping for carrying this potential to its fullest.

The high level of precision in optical manipulation can perform microchemistry with minute amounts of reagents. This tremendously shrinks the test tube for in vitro experiments and can pave the way for setting up microlaboratories that perform experiments within the living organism. For example, optical forces can be used to deliver microscopic doses of inactive biological compounds to target sites where another illumination can then activate these compounds; initially, to understand the process and, subsequently, to achieve a desired well-controlled response. The second illumination must likewise be shaped accordingly to achieve the desired specificity and localized response. Patterned light may also be used to achieve a synchronized or programmed initiation or inhibition of light-sensitive processes at multiple target sites.

Light activation is achieved through photo-chemical processes that use light's energy to modify the target. Going a step further, light's energy can be used to create new structures out of existing precursors, as for instance by two-photon photopolymerization [22]. Photopolymerization is routinely used for hardening

dental fillings by exposure to UV light. In two-photon photopolymerization, the concurrent absorption of two low-energy photons mimics the high-energy effects of ultraviolet light for polymerization. This rare absorption only occurs in sufficient amounts within the highest intensity regions of a pattern and, hence, capable of highly selective hardening. This provides a means for creating structures with finer features than the light spot used. Creating such fine features is important as human cells are known to respond to nanoscopic features of its environment [23].

7. The Next generation BioPhotonics Workstation: Information Optics approaches integrated under one platform

Research on the different applications of information processing with Generalized Phase Contrast, particularly those relating to light sensing and synthesis, together with microscopy and micromanipulation, accumulates instrumental knowledge for the development of a BioPhotonics Workstation [24]– a device that builds upon previous results to serve as enabling tool in biophotonics research (e.g., see Fig. 7). Information Optics can supply a wealth of optical tools that can be integrated into a Next Generation BioPhotonics Workstation that can serve as a scientific workhorse for carrying photonics technologies towards biomedical disciplines.

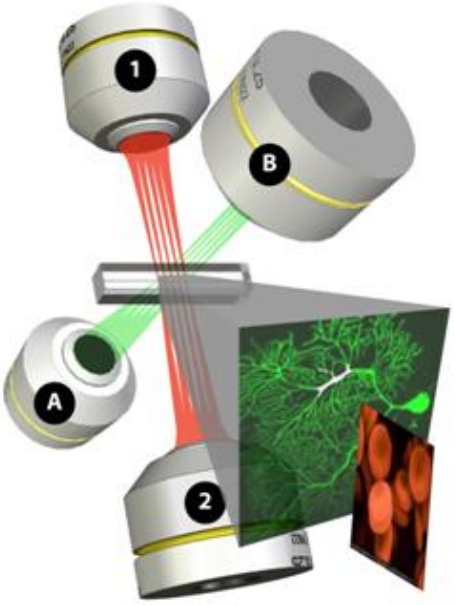


Fig. 7. BioPhotonics Workstation: Information Optics drives the creation of purposely patterned light can perform assorted functions, such as enabling higher microscope resolutions, initiating spatially controlled light-driven biochemical processes, and exerting finely calibrated forces, while leaving enough room for integrating other auxiliary systems. (adapted from [24])

At present, the power tolerance of commercially available spatial light modulators presents a hurdle for achieving higher output powers that could potentially exert higher optical forces, or reveal more information by exciting higher-order nonlinear processes. Similarly, compact and portable lasers, which are essential for moving the technology from the optics laboratories to the end-users in the biomedical field, are still not available with acceptable power and beam quality at biologically safe wavelengths. The capacity for sensing and manipulating microbiological systems can be integrated into a Next Generation BioPhotonics Workstation that packs a variety of photonics tools from Information Optics for the systematic investigations of biological questions. Future technological developments can only feed more solutions into such biophotonics workstations, which will then incorporate modern developments in lasers, spatial light modulation and detection and supported by the solid theoretical framework of Information Optics.

7. Beyond biology: Information Optics for microfabrication, micro-assembly and information security

7.1 Micro- and nanotechnology

Microscopes magnify optical phenomena and extend our vision into microscopic worlds. We can use these same microscopes, in reverse, to send miniature versions of meaningfully patterned optical fields into these microsystems. Experimental demonstrations using interactive microscopy with GPC [17,18,25,26], together with results from the BioPhotonics Workstation [27,28], show its potential functionality extends beyond biophotonics into the broader area of micro- and nanotechnology. Beyond the biological applications mentioned earlier, a convergence of techniques from Information Optics can generate useful allied techniques for micro- and nanotechnology. As discussed earlier, micropatterned light can be used to fabricate microstructures with nanoscopic features through photopolymerization and be used to extend the capacity of microscopes for resolving finer features. Moreover, these light patterns can redistribute energy to designated sites and can create “optical landscapes” that can trap and guide the microcomponents along user-defined trajectories. In general, they can serve to extend our eyes and fingers for observation and controlled manipulation in the microscopic and nanoscopic regimes. A vision for such a system is depicted in Fig. 8, together with a snapshot from a demonstration of optical microassembly of microstructure fabricated by two-photon photopolymerization reported in [27].

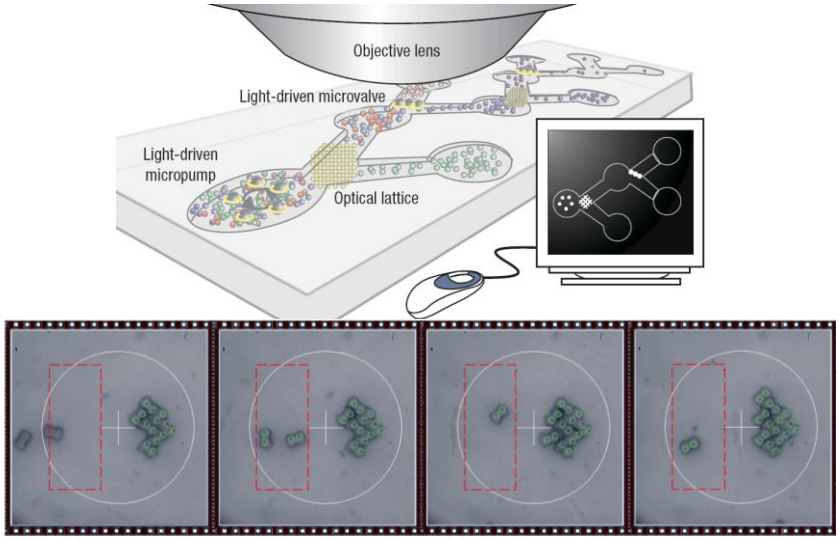


Fig. 8. Top: Vision for an all-optical lab-on-a-chip [29] (© Nature 2004); Bottom: Optical microassembly of microstructure fabricated by two-photon photopolymerization [27]. (© OSA 2009).

7.2 Phase-only cryptography and matched-filtering GPC: completing the circle of Information Optics

Generalized Phase Contrast can be used not only for visualizing information about invisible optical phase perturbations, but also utilizing phase processing to secure information through phase-only cryptography [30] (see Fig. 9). The absence of direct methods for measuring phase offers a natural first layer of security. Another security layer is applied by using a second invisible phase pattern to encrypt the original pattern. This creates a phase-encrypted phase pattern that is both invisible and unintelligible. The original pattern can be retrieved by shining the encrypted phase onto a phase mask with the correct key at the input plane of a GPC system.

Nonetheless, optical cryptography is already traditionally associated and remains an active research field within Information Optics. Thus, this GPC application does not seem to illustrate an atypical application of Information Optics. However, it does highlight the broad scope of Information Optics when a technique associated with qualitative biological microscopy creates a solution to a quantitative and computational problem like cryptography. This example also illustrates the wide area of Information Optics, which has been the recurring theme in this chapter: GPC applications illustrate that Information Optics can have multiple, wide-ranging applications beyond its stereotypical disciplinary boundaries.

As GPC boosts phase contrast to make a leap from qualitative biological imaging to quantitative cryptography, it suggests that a leap in the opposite is also possible. We have attempted to make this reverse leap more explicit ourselves in our recent work on mGPC, where we apply matched filtering and GPC to propose a new technique for creating reconfigurable light fields [31, 32], which can have possible applications in interactive microscopy [33].

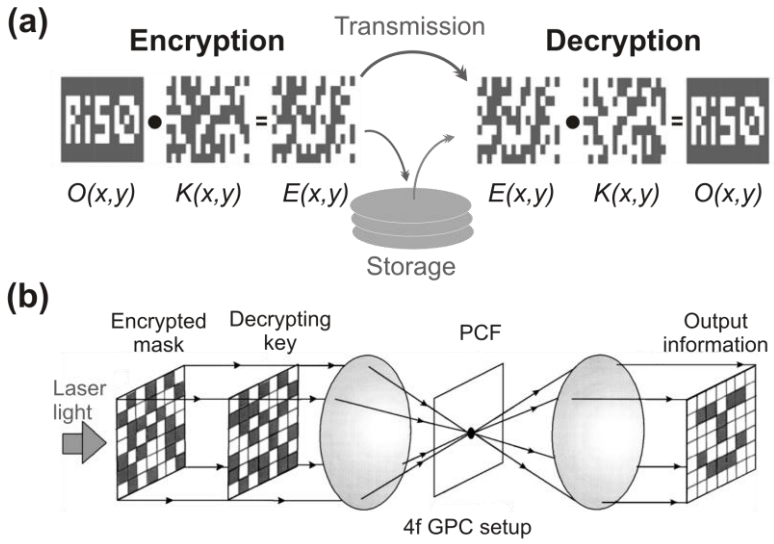


Fig. 9.. GPC for information security (a) Phase only optical cryptography (b) Decrypting an encrypted phase mask using a GPC-based system. [30](© OSA 2000);

8. Summing up

Light's innate ability to carry information, combined with human attempts at harnessing this ability, has placed optics at the forefront of scientific revolutions, from Galileo's telescopic observations of heavenly bodies to Leeuwenhoek's investigations of the micro-cosmos. In this chapter, we have presented perspectives on Information Optics as seen from the framework of Generalized Phase Contrast and its applications. Information Optics provides a useful concept for unifying diverse optical technologies that essentially exploit optical information: From optical imaging for sensing and measurement to synthesizing optical fields for not only display information but also achieving interactive control of light-matter interactions and associated phenomena. The capacity to extract useful information from light, together with the ability to mould light's physical properties according to specified information, serves to use light as our eyes and hands into physical

phenomena. Carrying this biological “hands-and-eyes” analogy for optical sensing and control one step further leads us to an important component– the brain– which functions as an information processor. In conclusion, the diverse applications of GPC are unified with GPC’s role as an information processor under the framework of Information Optics.

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