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Optical Fourier techniques for medical image processing and phase contrast imaging

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Abstract

This paper briefly reviews the basics of optical Fourier techniques (OFT) and applications for medical image processing as well as phase contrast imaging of live biological specimens. Enhancement of microcalcifications in a mammogram for early diagnosis of breast cancer is the main focus. Various spatial filtering techniques such as conventional 4f filtering using a spatial mask, photoinduced polarization rotation in photosensitive materials, Fourier holography, and nonlinear transmission characteristics of optical materials are discussed for processing mammograms. We also reviewed how the intensity dependent refractive index can be exploited as a phase filter for phase contrast imaging with a coherent source. This novel approach represents a significant advance in phase contrast microscopy.

1. Introduction

Fourier theorem states that an arbitrary function with spatial period λ can be decomposed into the sum of harmonic functions whose wavelengths are integral submultiples of λ (i.e. $\lambda, \lambda/2, \lambda/3, \dots$). Since λ is considered as a spatial period, $1/\lambda$ is called spatial frequency. It is known that far-field or Fraunhofer diffraction pattern of an aperture function is identical to its Fourier transform. The far-field distribution is nothing but the spatial frequency spectrum of the field distribution across the aperture [1–4]. If a lens is placed after the object, it will shorten the image plane distance and one will have the Fourier spectrum at the back focal plane of the lens. Such an object lens is commonly referred as Fourier transform lens and the process is called optical Fourier transformation (OFT). OFT is a powerful tool widely used for manipulation of optical data. With the availability of coherent sources, OFT is applied in a wide variety of areas with unique features of parallel processing at the speed of light. Applications of OFT in medicine and biology are very broad and an extensive review of OFT for medical imaging is therefore beyond the scope of this paper. In this article we focus on applications of OFT to (1) medical image processing with particular reference to both analog and digital mammograms for early detection of breast cancer, and (2) phase contrast microscopy to observe biological specimens.

The manipulation of spatial frequencies of two-dimensional images using OFT is well studied for applications such as edge enhancement, character recognition, and image correlation [5, 6]. The basic concept of optical Fourier processing is shown in Fig. 1. A well collimated laser beam is incident on the object (amplitude or phase) to be processed and the light bearing the object information is Fourier transformed with a converging lens, mapping different spatial frequencies to different regions in the back focal plane. In the Fourier spectrum, low spatial frequencies occur at the center with high intensity and high spatial frequencies at the edges with low intensity. Various image processing techniques, such as low-pass, band-pass, high-

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pass, and phase-shift, are employed at the Fourier plane to process different spatial frequencies for various applications. An inverse Fourier transform with another Fourier lens is used to image processed spatial frequencies on to the CCD camera, so that the desired components of the image are captured.

Optical Fourier processing is ideal to enhance the features of both amplitude and phase objects. For amplitude objects a spatial (amplitude) filter is employed at the Fourier plane while for phase objects a phase filter is used to alter the phase difference between high and low spatial frequencies. When it comes to application of OFT in medicine, the optical Fourier processing scheme suits well to process medical images like mammograms, hair-line fractures, etc. Similarly optical Fourier processing is widely used in biology – as phase contrast imaging to observe phase objects.

2. Spatial filtering for medical image processing

Processing of mammograms by enhancing the desired features of interest to the radiologist will effectively help in early detection of breast cancer [7–9]. Mammography, the current ‘gold standard’ for breast cancer screening, has been shown to be effective in screening breast cancer [10–12]. Abnormalities detected in mammography can be classified as masses, lesions, and microcalcifications. Features such as microcalcifications are often buried in the background of soft dense breast tissue in a mammogram resulting in low contrast between these essential features and the background. More over, breast density can make it even more difficult to detect these microcalcifications in the mammogram. Therefore, mammogram image analysis is a challenging task both for radiologists and researchers involved in the image processing field [11]. Application of appropriate image processing techniques is required in reading mammograms for better sensitivity and specificity of clinical cancer diagnosis [12–15]. Microcalcifications in the mammogram correspond to high spatial frequencies in the Fourier spectrum, due to their small in size and diffuse in nature, and occur at the edges with low intensity. Thus, they can be conveniently separated at the Fourier plane from low spatial frequencies which are due to the dense soft tissue. When the undesired low spatial frequencies are filtered out, the processed image displays only high spatial frequency components, thus making microcalcifications visible to the naked eye.

Fig. 2 shows a schematic of the conventional 4f configuration for the image processing with various spatial filters. A diode pumped solid state Nd:YAG laser with 532 nm and output power 10 mW is expanded to a spatially uniform beam. Light transmitted through the object is focused by the Fourier lens. At the Fourier plane, various spatial filters are used for blocking undesirable components. Through an inverse Fourier transformation, the filtered frequency components in the Fourier plane are imaged on to a CCD camera.

For demonstration, we fabricated several spatial filters to selectively display various spatial frequency bands of the binary test object “E”. Fig. 3a shows the spatial filters used in the experiment while Fig. 3b shows the processed images. Using the filters 1 and 2, edge enhancement is obtained by blocking low spatial frequency components. Two very thin filaments are purposely placed in the top left corner of the original image and are indicated by arrows in the Fig. 3b. They are barely visible in the original image, but can be clearly seen in the processed images with filters 1 and 2. On the other hand, filter 3 blocks the high frequency components. Thus, the processed image becomes soft (no sharp edges). In this case, the filaments become more blurry as they correspond to the high spatial frequencies.

The procedure is applied for systematic investigation of mammogram images. Fig. 4 shows the results of a mammogram processed with filter 1 shown in Fig. 3a. The region of abnormal pathological changes is buried in the dense breast tissue background as shown in Fig. 4a. Fig. 4b is the processed image captured by the CCD camera displaying microcalcifications

corresponding to the high spatial frequency band after eliminating the surrounding dense breast tissue which corresponds to the low spatial frequencies.

However, there are some disadvantages with the simple spatial filtering technique. When the object mammogram is changed the spatial frequency spectrum at the Fourier plane will be different due to the changes in the density and region of interest in the mammogram. So each time the size of the spatial filter has to be modified accordingly and has to be precisely placed at the Fourier plane. Thus, it is not a real-time processing as the filter is not all-optically and continually controllable. To overcome these difficulties, researchers have developed several nonlinear filtering techniques for self-adaptive and real-time computing. Organic and biological molecules, photorefractive polymers, and liquid crystals are used as the nonlinear medium.

Kato and Goodman demonstrated logarithmic filtering by placing a halftone contact screen (periodic array of vignettted dots on a flexible support) in the input plane of a coherent optical system [16]. The Fourier spectrum is processed with an appropriate spatial filter and is inverse Fourier transformed. The processed image displays the desired nonlinear function of the original intensity. More recently, Babkina et al. used an acousto-optic modulator at the Fourier plane to perform spatial frequency filtering where the acoustic wave in the paratellurite crystal forms a grating pattern [17]. When the incident optical beam interacts with the grating, the light is diffracted into zero order and first order. By varying the modulator driving frequency, edge enhancement effect is observed in the diffracted beam in real-time. On the other hand hybrid processing techniques are also proposed and demonstrated in the literature where the object information is Fourier transformed with a laser source and spatial filters (generated digitally in the computer) are displayed on the spatial light modulated (SLM) placed at the focal plane for real-time processing. The SLM can be electrically addressed SLM [18], optically addressed SLM [19] or bR film [20]. Such a hybrid processing technique is also used for phase contrast imaging of live biological specimens more recently [21].

3. Photoinduced anisotropy for medical image processing

Photoinduced anisotropy properties of various materials have been used for spatial filtering. Two groups independently exploited photoinduced anisotropy and photoinduced dichroism properties of bacteriorhodopsin (bR) films for real-time image processing. While our group demonstrated a self-adaptive all-optical Fourier image processing system using photoinduced dichroic characteristics [22], Korchemskaya's group exploited photoinduced anisotropy for real-time selective image processing [23]. On the other hand, several other groups exploited similar photoinduced polarization rotation properties of azobenzene dye doped liquid crystal and polymer films for spatial filtering [24–26]. Simply by placing a polarizer sheet at the Fourier plane, Ferrari et al. demonstrated phase visualization and edge enhancement [27]. Most recently, Menke et al. demonstrated optical image processing using photoinduced anisotropy property of pyrrolfulgide [28].

The basic principle of spatial filtering is same in all these processing schemes – intensity dependent polarization rotation in combination with an analyzer will selectively transmit the desired spatial frequency band. Here we will briefly discuss about such basic filtering operation using bR and its application to processing clinical mammograms and Pap smears [29]. The photocycle of biological photomembrane bR is shown in Fig. 5a. In its stable B state, upon the absorption of a photon within the broad absorption with a maximum at 570 nm, the bR molecule goes through a photochemical cycle of several short lived intermediate states J, K, L and then to the long-lived M state with an absorption peak at 412 nm within 50 μ s. The lifetime of the M state can be altered by several orders of magnitude by altering the reprotonation process. The M state molecules are thermally transformed into the initial B state in milliseconds or they

can go back directly to the initial B state within 200 ns by absorption of a blue photon. The most relevant states in the bR photocycle for our experiments are the B and M states and can be viewed as a two level system as shown in Fig. 5b. The bR can be switched between B $\leftrightarrow$ M states at relatively low powers of milliwatts.

The bR film is initially isotropic as the molecules are randomly oriented. As it is placed between a crossed polarizer (V) and analyzer (H) arrangement, as shown in Fig. 6, no probe beam light gets through. When a linearly polarized light (actinic beam) with polarization at 45° to the vertical is incident on the film, due to the photoinduced dichroism, some light transmits through as a result of actinic induced polarization rotation of the probe beam. The amount of polarization rotation is a function of the actinic beam intensity and is given in Fig. 7.

Fig. 8 shows the experimental data on the degree of rotation of probe beam polarization as a function of probe beam intensity under constant actinic beam intensity of 10 mW/cm². For low probe beam intensities, the degree of rotation is large and it gradually goes to zero as the intensity is increased. This aspect is used for spatial filtering. In the Fourier spectrum low spatial frequencies occur at the center with high intensity and high spatial frequencies occur at the edge of the spectrum with low intensity. Therefore, from Fig. 7, we can say that low spatial frequencies acquire no polarization rotation as they are at high intensity, whereas high spatial frequencies will have large polarization rotation due to their low intensity. Thus, by selectively rotating analyzer, we can image desired features of interest of an image. In bR, switching between B and M states are used to create the photoinduced anisotropy. Similarly in azobenzene molecules, *trans*–*cis* isomerization states are exploited, whereas pyrrolyfulgide operates between its two states – bleached state (E-form) and colored state (C-form) [28].

The concept of spatial filtering using photoinduced anisotropy in bR films is applied for medical image processing. A schematic of the experimental setup for medical image processing is shown in Fig. 9. A linearly polarized (vertical) probe beam carrying the medical image information is Fourier transformed on to the bR film with a lens. As this information passes through the bR film in the presence of actinic illumination, it acquires a range of polarizations of different orientations depending on the intensities in the Fourier spectrum. Thus, there is a correspondence between spatial frequency–intensity–polarization and each spatial frequency band is encoded with a unique polarization. The spatial filtering is accomplished by selectively rotating the analyzer such that undesired spatial frequencies are blocked. When the analyzer is at right angles to the input beam polarization, the low frequency components that experience no polarization rotation are blocked by the analyzer. On the other hand high spatial frequency components corresponding to edges of the object experience polarization rotation due to their low intensity. Thus, the unblocked spatial frequencies are transmitted through the analyzer and imaged on to the CCD camera to yield edge enhancement. By rotating the axis of the analyzer, one can filter out undesirable components enhancing the features of interest.

Clinical mammograms and Pap smears are processed with this experimental system and the results are shown in Fig. 10. Fig. 10a shows the original mammogram. Fig. 10b is the processed image clearly displaying the microcalcification clusters which are not visible in the original mammogram. Low spatial frequencies corresponding to the dense soft breast tissue are filtered out. Fig. 10c shows the original Pap smear where the bright spots represent the cells. Both normal (small spots) and abnormal cells (large cluster) are visible in the original picture. In the processed image, Fig. 10d, the normal cells are filtered out retaining only the abnormal cells. This is achieved by selectively filtering out high spatial frequency components (rotation of the analyzer from the crossed position) in the Fourier spectrum.

4. Fourier holography for medical image processing

In holography, the object and reference plane waves are interfered in the medium to record the hologram. When the recorded hologram is illuminated by the reference wave or another plane wave preserving the angle of incidence, the entire information of the object (both amplitude and phase) is reconstructed. If the recording is done between the Fourier spectrum of an object and a simple plane wave, then the recorded hologram is referred as Fourier hologram. By recording and reconstructing the Fourier hologram one can retrieve both amplitude and phase information of the object. This technique is exploited for spatial filtering and image processing. Feinberg used barium titanate photorefractive material to demonstrate edge enhancement in real-time by recoding and reading gratings of object information [30]. This principle is used by Ochoa et al. to identify defects in a periodic mask using barium silicon oxide as photorefractive crystal [31]. Okamoto et al. presented a real-time optical system to enhance nonlinear diffraction efficiency of holographic gratings written in a bR film [32]. The grating pattern is read using Fourier transform of a periodic pattern to be inspected. In the readout process the diffraction efficiency of the grating depends on the intensity of the reading beam. Therefore, using suitable reading beam intensity, only the defect component is displayed. Similarly, two-beam coupling in photorefractive materials are exploited to spatially amplify the desired frequency band of the Fourier spectrum [33]. Unlike in the conventional 4f spatial filtering system where the undesired spatial frequency components are selectively blocked at the Fourier plane, this technique does not discard any of the incident image information. By selectively overlapping the desired spatial frequency band with the pump beam, only the desired features of the image are amplified in intensity by a factor of 1000 thereby increasing the contrast between the desired and undesired information.

Fourier holography in bacteriorhodopsin (bR) films is applied for processing medical images. Transient Fourier holographic gratings based on photoinduced isomerization properties of bR films are used to perform spatial filtering for detection of microcalcifications in real-time [34]. Fig. 11 shows the experimental arrangement of Fourier holography where the Ar-Kr ion laser beam is expanded and split into two beams. The object beam (mammogram or binary object E), is Fourier transformed by the lens L_1 of 20 cm focal length. At the Fourier plane the bR film is placed for real-time processing of spatial frequency information. The reference beam obtained with the beam splitter BS1 overlaps the Fourier transform of the object beam on the bR film thereby recording a Fourier hologram. When the object beam is blocked, the reference beam performs the reconstruction of the recorded Fourier hologram. The diffraction efficiency is maximum when the object beam intensity matches that of the reference beam intensity. At either low or high intensity region of the object beam, the diffraction efficiency decreases. Thus, the desired spatial frequency components can be selected by matching their intensity to that of the reference beam.

The processing results of clinical screen-film mammogram are shown in Fig. 12. High spatial frequency components of the object are recorded by matching the intensity of reference beam to the intensity of high spatial frequency band. The recording process takes about 5 s. When the object beam is blocked, the reference beam performs the reconstruction of the recorded hologram displaying the spatial frequencies whose intensity in the Fourier spectrum is matched to the reference beam intensity. Thus, the radiologist can easily scan for desired microcalcification clusters by rotating the variable attenuator that controls the reference beam intensity.

A significant feature of this technique is that the enhanced components in the processed image can be separated in time scale, which enables the radiologist to monitor different pathological features in the mammogram at a different time scale. Since different spatial frequency bands correspond to different intensities, all the bands exist simultaneously with different diffraction

efficiencies. However, the optimum diffraction efficiency occurs only for a selected band of frequencies which match with the reference beam intensity. Thus, one can distinguish between different spatial frequencies as they reveal at different times; the band of frequencies with optimum efficiencies last longer. We used a resolution chart (USAF negative target, Edmund Optics) to observe this phenomenon. As shown in Fig. 13 at time $t = 0$ we can observe all the frequency groups (A – low frequency group, B – middle frequency group and C – high frequency group) but at time at $t = 5$ s only the frequency group B which matches the reference beam intensity appears clear while other frequency groups vanish. This could be a potential advantage to the radiologist to view various features in the mammogram at different time scales.

5. Nonlinear transmission for medical image processing

Transmission of nonlinear optical materials is yet another phenomenon which ideally suits for spatial filtering and medical image processing. Recently it is demonstrated that any nonlinear transmission (power limiting) mechanism can potentially be used for image processing [35]. Nonlinear optical principles such as two photon absorption, excited state absorption, nonlinear scattering, self-focusing, etc. exhibit reduced transmittance for high input intensities while offering linear transmittance at low intensities, as shown in Fig. 14 for phthalocyanine sample. Therefore, intensity dependent nonlinear transmission can be used to filter out undesired spatial frequency bands in the Fourier spectrum of the image. The spatial frequency distribution at the Fourier plane can be categorized into different intensity bands and low spatial frequencies at the center with high intensities and high spatial frequencies on the edges with low intensities. At low incident beam intensity all the spatial frequencies are transmitted through the phthalocyanine sample without any filtering. As the incident intensity is increased above the threshold value for power limiting, the low spatial frequencies begin to diminish as they occur at high intensities. Thus, the power limiting curve for a given phthalocyanine sample facilitates the calculation of required input intensities to obtain the desired band of spatial frequencies.

Spatial filtering with nonlinear optical material is illustrated in Fig. 15 using a binary test object “E”. The power measurements recorded by the power meter reveal the optical limiting characteristics as shown in Fig. 14 while the CCD captured the edge enhancement effects of the object at the corresponding incident beam energies. When the intensities are below the limiting threshold, the absorption is in the linear regime and the entire information of the object is transmitted through the sample without any processing as shown in Fig. 15a. The corresponding position on the power limiting curve is marked as (a) in Fig. 14. As the input intensity is increased to the power limiting threshold, the intensity in the low spatial frequency band is sufficient enough to trigger excited state absorption in the sample and begin to diminish as shown in Fig. 15b. When input intensity is increased further, the intensity of low spatial frequency band is beyond the power limiting threshold. So in this region phthalocyanine molecules undergo excited state absorption and thus low spatial frequency band is completely attenuated. But at the same time the intensity of high spatial frequencies striking the sample are well below the power limiting threshold and thus transmitted without being absorbed as shown in Fig. 15c. A near perfect edge enhancement of the object is observed when the input intensities are well above the limiting threshold as depicted in Fig. 15d and e.

This type of spatial filtering technique is relatively user friendly compared to the other image processing techniques that are discussed above – a spatial mask is used at the Fourier plane as in the case of conventional image processing or a reference beam (actinic beam) is needed to perform the image processing or requires cross polarization. While spatial mask is cumbersome and requires precise alignment, the Fourier holography technique that employs reference beam need to be performed on a vibration isolation table as interference is involved. In contrast spatial filtering using nonlinear optical transmission can be performed using two lenses and a neutral density filter. Apart from its simplicity the technique is self-adaptive to the background

intensity (amount of dc) of image. As a fringe benefit, when the same experimental setup is used for both power limiting experiment and optical image processing, as in the case of image bearing intense laser beam, the sensitive detectors are potentially protected by blocking the intense low spatial frequencies, while detecting the essential features of the image by detecting the weak high spatial frequencies.

Depending on the nonlinear material either one or two beams needs to be employed. Xuan et al. exploited two photon absorption and stimulated Raman scattering in nonlinear material like acetone and CS₂ for contrast improvement and contrast reversal [36]. Thoma et al. used photocontrolled transmission properties of bR films for edge enhancement of images [37]. Theoretical simulations show that the transmittance of bR films at 568 nm can be controlled by 413 nm pump beam. Similar results are obtained experimentally and the scheme is applied for medical images [38]. For the case of bR, the transmission of a blue probe beam (442 nm) in a bR film can be controlled with a yellow actinic beam (568 nm). Fig. 16 illustrates the experimental setup and the corresponding results. Intensity dependent transmission characteristics are studied using the experimental setup shown in Fig. 16a. Transmittance of the blue beam through the bR film is measured as a function of the intensity of the control yellow beam. The results displayed in Fig. 16b shows that the local transmittance of the film for blue beam decreases significantly when the intensity of the control yellow beam is increased.

Fig. 17 shows the experimental setup for medical image processing based on this mechanism. The information bearing blue beam (442 nm) is Fourier-transformed to the bR at the focal plane and the desired spatial frequencies are selectively blocked by illuminating the film with a 568 nm control beam. Since different spatial frequency components are spatially separated in the Fourier plane, the physical location of the yellow control beam on the bR film determines the components blocked. If high spatial frequency information is desired, then the low frequency components are blocked by focusing the control beam at the center of the Fourier spectrum.

Fig. 18 shows the image processing of clinical screen-film mammogram and the corresponding processed image which display only microcalcifications, not visible to the naked eye in the original mammogram.

As digital mammography is becoming popular, we also performed image processing of digital mammograms. An electrically addressed spatial light modulator (SLM) is used to facilitate the interface between digitally stored mammogram in the computer and the optics used in the experiment. The collimated He–Cd 442 nm laser beam illuminates the SLM and the output of the SLM is a coherent optical signal bearing the image being displayed on the SLM. Fig. 19 shows the original and the corresponding processed images of a digital phantom containing simulated microcalcifications buried in the gray background and a clinical digital mammogram. In presence of yellow control light beam, low spatial frequencies (gray background) are blocked and the reconstructed image shows only high spatial frequencies.

6. Phase contrast imaging of biological specimens

Spatial filtering techniques are used for enhancing the features of amplitude objects where part of the Fourier spectrum is either blocked or transmitted depending on the filtering scheme. Mostly these filters can be described as amplitude or binary filters. In the conventional 4f spatial filtering scheme, however, if the amplitude object is replaced with a phase object and the spatial filter with a phase filter, the phase filter only alters the phase of part of the Fourier spectrum. Such a processing scheme is nothing but phase contrast imaging which is widely used to observe phase objects.

Phase objects are transparent – they provide no contrast with their environment and alter only the phase of the wave. The optical thickness of such objects generally varies from point to point due to changes in either the refractive index or physical thickness or both. Since eye cannot detect the changes in phase variations, phase objects are invisible to the naked eye. However, if an additional phase difference is created between the undeviated (low spatial frequencies) and deviated (high spatial frequencies) light, then they interfere either constructively or destructively (depending on the amount of phase added) thereby converting the phase variation into amplitude contrast. Phase contrast imaging was developed in 1933 by Zernike [39] to observe phase objects. He used a phase plate to create a $\pi/2$ phase difference between the undeviated light and the light diffracted by the object thereby transforming minute variations in phase of the object into corresponding changes in the image contrast. This principle is exploited in the phase contrast microscope which is widely used in teaching and research labs to view high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles).

Several alternative concepts for phase contrast imaging are demonstrated to avoid the complications with the usage of phase plate as a phase filter. It is difficult to place the phase plate at the exact location so that the required phase shift is induced between low and high spatial frequencies, and manufacturing the phase plate is also not trivial. Liu et al. used photorefractive crystals at the Fourier plane to introduce uniform phase shift to low spatial frequency components [40]. A C-cut $\text{LiNbO}_3\text{:Fe}$ crystal sheet served as the phase filter and good phase contrast images are obtained. Glückstad worked out both theoretical and experimental ways to improve imaging of phase objects [41,42]. As an extension to Zernike phase contrast configuration, they showed that phase-only encoding utilizing full-range ($0-2\pi$) yields phase-only imaging with high energy efficiency.

The optically addressed spatial light modulator (OASLM) also serves as a nonlinear filter when placed at the Fourier plane and edge enhancement and phase contrast imaging are demonstrated [43]. The required phase change is obtained through the dependence of the extraordinary index of refraction of OASLM on voltage. Similarly, Komorowska et al. used an OASLM made of a planar nematic liquid crystal layer sandwiched between a photoconducting polymer and a polyimide orienting layer and performed phase contrast imaging [44]. The induced phase modulation is proportional to the intensity of the incident light on the OASLM.

Popescu et al. developed Fourier phase microscopy by combining phase contrast microscopy and phase shifting interferometry (PSI) to quantify the phase shifts induced by the phase objects [21]. Fourier transform of the object is projected onto the surface of a reflective programmable phase modulator (PPM) and the phase of diffracted light is shifted into four increments of $\pi/2$ with respect to the average field (undeviated or dc), as in typical PSI measurements. After recording four interferograms, the phase shift associated with the object is evaluated qualitatively at each and every point of the field of view.

Nonlinear optical materials are also used as phase filters. Castillo et al. utilized the Kerr-type nonlinear property of bacteriorhodopsin film for self-induced Zernike-type filter and obtained phase contrast images [45]. Sendhil et al. exploited the intensity dependent refractive index of zinc tetraphenyl porphyrin for phase contrast imaging [46]. In these methods, the zero order of the Fourier spectrum induces intensity dependent refractive index changes thereby modifying its phase. Since only the zero order induces a phase shift and not the higher orders, a phase filter is created and phase contrast imaging is performed.

Photothermal induced birefringence property of dye doped twisted nematic liquid crystals are exploited for self-adaptive all-optical Fourier phase contrast imaging of biological species

[47]. When the dye doped twisted nematic liquid crystal cell is placed at the back focal plane of a converging lens, high intensity low spatial frequencies induce local liquid crystal molecules into isotropic phase, whereas low intensity high spatial frequencies are not intense enough and molecules in this region remain in an anisotropic phase. Liquid crystal molecules in the anisotropic phase add certain amount of phase to the incident polarized light, whereas the molecules in the isotropic phase do not add any additional phase. Therefore, the high spatial frequencies acquire an additional $\pi/2$ phase as they transmit through the self-induced anisotropic phase of local liquid crystal molecules. The low spatial frequencies, however, transmit through the self-induced isotropic phase of liquid crystal molecules without acquiring any phase difference. This leads to a relative phase difference of $\pi/2$ between high and low spatial frequencies, primary criteria for phase contrast imaging, at the exit plane of liquid crystal cell.

Fig. 20 illustrates images of paramecium. Paramecia are unicellular microorganisms belonging to the protoctist phylum Ciliophora. Members of this phylum (ciliates) are characterized by their cigar or slipper shape and external covering of continuously beating, hair-like cilia, and these fine structures in particular are not always easy to visualize with bright field microscopy unless the rest of the specimen is out of focus. The bright field image obtained with our system (Fig. 20a) shows the distinguishing specimen outline and oral groove of the paramecium but not much more. Fig. 20b displays the Fourier phase contrast image where the outline of the Paramecium is identifiable, and the external fine hair-like structures called cilia can be seen at the posterior end (top of the image). The feeding structure, the oral groove and other internal structures are clearly visible.

We also applied the Fourier phase contrast technique to view onion peel. Onion cells from the skin of an onion bulb are commonly used for early training comparisons between plants and animals. The thin layer of cells is so translucent that phase contrast or staining is needed for viewing. Fig. 21a shows a bright field image of such a preparation of onion cells, the walls and nuclei are visible but that is greatly enhanced with the phase contrast image seen in Fig. 21b.

7. Conclusion

We reviewed the applications of various optical spatial filtering techniques with specific applications for processing of mammograms and phase contrast imaging of biological specimens. For medical image processing each filtering technique has its own merits and demerits. Employing a spatial mask at the Fourier plane is the simplest of all, but the same filter size may not work for all mammograms. However, if a spatial light modulator is used at the Fourier plane, then the filtering using a digital spatial mask is more convenient. Therefore, all-optical self-adaptive spatial filtering is an ideal choice for real-time image processing. One disadvantage with most of these filtering techniques is the need for a second beam: (1) Photoinduced anisotropy – the amount of polarization rotation that can be induced is very small ($\sim 5^\circ$ in bR) and hence the rotation of analyzer is very critical. Therefore, this technique can be of practical importance only for materials with large polarization rotation. (2) Fourier holography – the major drawback is the requirement of vibration isolation as it involves interference. However, this type of image processing scheme has the additional ability of displaying different pathological features of interest in time scale. During the readout process, when the spatial frequency information is unfolding, a movie can be recorded using a fast CCD camera. The radiologist can view the movie at his leisure – look at the features corresponding to different spatial frequencies one by one in sequence or freeze the frame to concentrate on a particular feature. (3) Nonlinear transmission – the focal spot size of the control beam on the bR film has to be adjusted for different mammograms. However, such a drawback can be avoided when a single beam induced nonlinear transmission is exploited in commercially available reverse saturable absorption materials. As digital mammography is becoming more

prominent, any of these optical processing techniques can be used for digital mammograms by employing spatial light modulator at the object plane.

Finally, optical Fourier processing techniques using coherent source offer several distinct advantages over conventional screening of mammograms using white light; the same facts also hold for phase contrast microscopy. As the deviation angle depends on the wavelength, the monochromaticity in the Fourier techniques facilitates clear and well resolved spatial frequency bands for the Fourier processing. Intensity of the laser source makes object features bright and clearly visible. As such high spatial frequencies are enhanced and can be observed with good contrast.

Further in the case of phase contrast imaging, the high degree of phase coherence preserves phase retardation introduced by the phase filter. Thus, the phase information can be converted to amplitude with good contrast. Phase quantization studies using Fourier phase microscopy already demonstrated potential applications for basic research. Thus, optical Fourier transform in medicine and biology is a promising area of research leading to significant advances in both basic science as well as technology.

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Fig. 1. Optical Fourier processing scheme for amplitude as well as phase objects. Spatial filter for amplitude objects, whereas phase filter for phase objects.

Fig. 2. Image processing with spatial filters. CL: collimating lens; L: Fourier lens; NDF: neutral density filter.

Fig. 3.

(a) Spatial filters for image processing. 1 and 2 are high-pass filters and 3 is low-pass filter.
 (b) Spatial filtering of binary image “E” using these spatial filters.

Fig. 4. Processing of mammogram using a spatial filter. (a) Original mammogram where the desired features of interest are buried in the background of soft breast tissue. (b) Filtered image displaying only the features of interest.

Fig. 5.
 (a) Photocycle of bacteriorhodopsin molecule. (b) Equivalent two level model.

Fig. 6. Schematic of the experimental setup for the measurement of photoinduced polarization rotation.

Fig. 7. Dependence of photoinduced polarization rotation on actinic beam intensity with constant probe beam intensity [29].

Fig. 8. Polarization rotation induced in the probe beam as its intensity is increased under constant actinic beam [29].

Fig. 9.

Medical image processing with bR films. 1: Beam from 532 nm diode pump laser; 2: microlens and pin hole filter; 3: collimating lens; 4: vertical polarizer; 5: input image; 6: Fourier lens; 7: nonlinear optical bR film; 8: inverse Fourier lens; 9: analyzer with horizontal axis; 10: processed image; 11: actinic 532 nm beam; 12: 45° polarizer.

Fig. 10. The experimental results of medical image processing: mammogram (top) and Pap smear (bottom) [29].

Fig. 11.

Experimental arrangement for study of edge enhancement using transient Fourier hologram recorded in the bR film. M: Mirrors; BS: beam splitters; L: lenses.

Fig. 12. Image processing of clinical mammogram using Fourier holography. (a) Mammogram with region of interest marked. (b) Processed image where the clusters of microcalcifications are clearly identified [34].

Fig. 13. Transient display of spatial frequency information of grating resolution chart captured at times: (a) $t = 0$ s and (b) $t = 5$ s [34].

Fig. 14.
Power limiting characteristic display of sample [35].

Fig. 15. Processed images showing the edge enhancement of the object E. (i)–(iv) The points where the processed images are captured using the CCD camera [35].

Fig. 16. Experimental setup of nonlinear transmission through bR film with control of actinic beam. The results are displayed in the plot.

Fig. 17.

Schematic of image processing using nonlinear absorption in bR films. L: converging lens; NBF: a narrow band filter to block 568 nm at CCD plane; CL: collimation lenses; NDF: neutral density filters. Inset: Fourier spectrum and the spatial overlap of two beams at the bR film plane [38].

Fig. 18.
(a) Film mammogram with magnified ROI and (b) its processed image of ROI [38].

Fig. 19.
Region of interest of a digital mammogram and its processed image showing only microcalcifications [38].

Fig. 20. Fourier phase contrast imaging of Paramecium. (a) Bright field image of live Paramecium. (b) Corresponding Fourier phase contrast image.

Fig. 21.
(a) Bright field image and (b) Fourier phase contrast image of onion cells.