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Real-time phase measurement with a photorefractive novelty filter microscope

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Abstract

A photorefractive two-beam-coupling based novelty filter is sensitive to amplitude and phase changes. However, the phase sensitivity of this device has remained unexploited until now. In this paper we develop and demonstrate a method to overcome the effects of contour formation in novelty filter output and consequently employ the novelty filter as a quantitative real-time transient phase-measuring instrument. A microscope based on a two-beam-coupling novelty filter is implemented and is used for detecting as well as measuring phase changes introduced by moving homogeneous phase objects. The two-dimensional output from the microscope is analysed, and a method based on the intensity dependence of the photorefractive time constant is proposed to overcome the effects of contour formation in the context of real-time transient phase measurement. The phase transfer function of the novelty filter system is experimentally determined and a phase measurement resolution of at least $\lambda/20$ at 532 nm is achieved.

Keywords: Phase measurement, microscope, photorefractive effect, two-wave mixing, optical image processing

1. Introduction

Phase measurement in biological applications has always attracted attention. Phase contrast methods [1, 2], interferometric methods and non-interferometric methods [3, 4] are often used to measure phase information. The drawback of these methods is that the phase information is obtained along with the bright background. Removal of this bright background would considerably improve the contrast and help in visualizing the phase information better. On the other hand, a photorefractive two-beam-coupling novelty filter-based microscope (NFM) [5] converts the phase information of the moving phase objects to the intensity variation and suppresses the stationary background [5–8]. Even though this microscope has been used for phase visualization tasks, not much work has been done to systematically analyse its output and use it for quantitative phase measurements. The aim of this paper is to lay the ground work needed to operate the photorefractive novelty filter as a real-time transient phase-measuring device.

A photorefractive novelty filter can detect changes in the input at the speed of light. Given its speed along with its phase sensitivity, it is surprising to note that it has not yet been successfully integrated into real-time phase-measuring devices. One of the reasons for this may be the unavailability of ideal photorefractive material that is easy to manufacture and easy to integrate in commercial devices. Another reason is the contour formation that is observed in the two-dimensional output of a photorefractive novelty filter [9–11]. This paper addresses the latter issue. As an object moves through the field of view of a novelty filter, the detected output reveals a trail at the front and the back edges of the object due to the finite relaxation time of the photorefractive crystal. This phenomenon is called contour formation [10]. In the case of moving phase objects, the contour contains two pieces of information. The length of the contour gives the velocity of the object: this can be effectively used in particle velocimetry and in object tracking applications. The second piece of information is the phase of the object, which can be determined from the intensity detected at the novelty filter output.

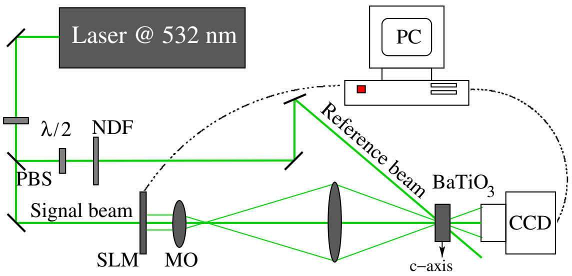


Figure 1. The NFM set-up: PBS, polarizing beam splitter; $\lambda/2$, half-wave plate; SLM, spatial light modulator (LCD or microscope probe); MO, microscope objective; NDF, variable neutral density filter.

(This figure is in colour only in the electronic version)

For the novelty filter to be operated as a real-time phase-measuring instrument contour formation at its output has to be minimized. In this paper we analyse the output of the filter and propose a method to minimize contour formation. To achieve this we utilize the intensity dependence of the photorefractive time constant [12–14]. The second important step which needs to be performed for real-time operation is calibration of the filter. The process of calibration involves determination of the phase transfer function, which is done by translating different homogeneous phase objects of known phase distribution and analysing the novelty filter output. We simulate moving phase objects using a liquid crystal display (LCD) operated in phase-only mode. We also suggest how the NFM with its corresponding phase transfer function can be used to obtain the phase of arbitrary phase objects moving through the field of view of the microscope.

2. Photorefractive novelty filter microscope

The photorefractive NFM was first proposed and demonstrated by Cudney *et al* in 1988 [5]. Here, the image information transmitted by a microscope is used as the signal beam in a two-beam-coupling novelty filter. It transmits only those portions of the magnified field of view which have recently undergone change. Thus it can be very well employed for motion detection in biological applications. Our experimental set-up of the NFM is sketched in figure 1. A laser beam of wavelength 532 nm derived from a diode-pumped frequency-doubled Nd:YAG laser is split into two beams. The signal beam carries the magnified image of the probe which is detected by an 8-bit progressive scan CCD camera. The optical magnification of the microscope was 90 and the resolution was better than 4.4 μm (228 line pairs mm^{-1}). The reference beam interferes with the signal beam in a cerium-doped 45° -cut $BaTiO_3$ crystal. Both the beams are extraordinarily polarized and the total power incident on the crystal is less than 1 mW. The crystal is oriented in such a way that the signal beam transfers its energy to the reference beam. Figures 2 and 3 show sequences of images of a twisting nematode without and with novelty filtering respectively. It is clear from the figure 3 that the contrast is improved due to the suppression of the inhomogeneous stationary background.

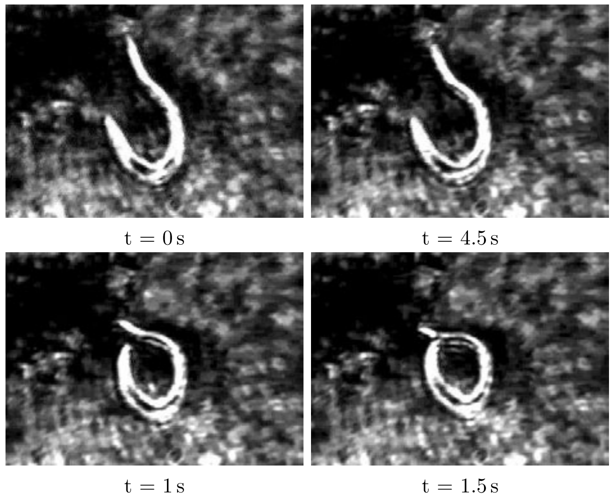


Figure 2. An image sequence without novelty filtering.

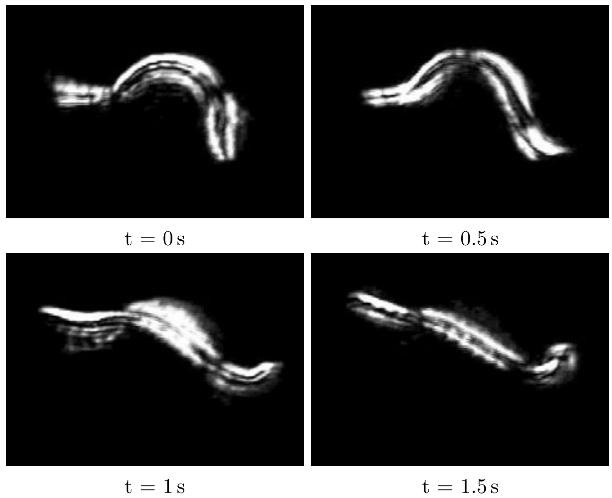


Figure 3. An image sequence with novelty filtering.

3. Contour formation

It is known that photorefractive novelty filters carrying two-dimensional information show contour formation [10]. This depends on the length and the speed of the moving object and also on the relaxation time of the crystal. To operate the NFM as a real-time phase-measuring instrument, contour formation has to be suppressed. To achieve this we propose a method of tuning the filter so that the output is devoid of contour formation.

For this purpose we restrict our investigations to 'simple' rectangular homogeneous phase objects moving from left to right in a constant phase background as depicted in figure 4. The simplification is done for experimental convenience, but the results obtained can easily be extended to complex motions and phase objects of complex shape. The phase object was experimentally implemented using an LCD that was operated in phase-only mode. This LCD has 800 × 600 pixels and a pixel pitch of 32 μm. With this modulator we could achieve a phase modulation of 1.1π rad at 532 nm wavelength. The input to the LCD was controlled by the VGA output from a computer.

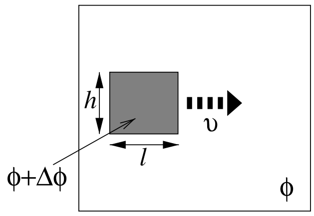


Figure 4. Simplification of the problem: a phase object of length l, height h and of phase information $\phi + \Delta\phi$ moves from left to right with a speed υ in a constant phase ϕ background.

3.1. Analysis of contour formation

To systematically analyse contour formation we moved a pixel cluster of 10×5 (10 horizontal $\times$ 5 vertical) pixels through the field of view of the microscope and studied its output by varying the speed of the cluster. The relaxation time of the crystal was 3 s. We identified three different cases as shown in figure 5:

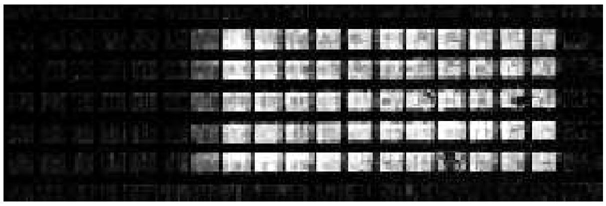
- Case 1: For speeds much larger than the grating formation rate of the crystal, no trail was seen. This case is the simplest to analyse because the input phase object can be directly mapped to the output intensity.
- Case 2: For speeds smaller than the grating formation rate, trails were seen for the front and the back edges. However, these trails were separated and thus with further image processing it is possible to localize the object and to determine its phase.
- Case 3: For speeds about the same as that of the grating formation rate of the crystal, the trail of the front edge overlapped with the trail of the back edge. Determining the phase of the object is much more involved than in the previous two cases and hence such a situation should be avoided.

The phenomenon observed in cases 2 and 3 was reported by Sedlatschek *et al* [10] and called contour generation. It was shown that if the length l of the object is greater than the threshold length $l_t = v\tau$ (where v is the velocity of the object and τ is the relaxation time of the filter), then contour formation occurs. One cannot avoid this due to the finite relaxation time of the novelty filter. However, the finite relaxation characteristic is essential for the functioning of a novelty filter.

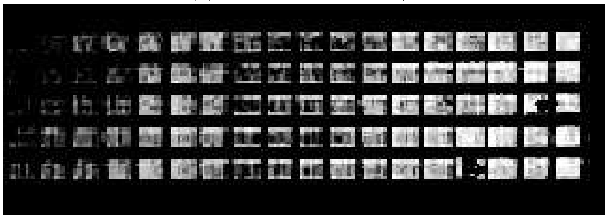
In general, the output of the novelty filter contains phase information and velocity information from the moving phase object. The length of the contour gives velocity information from the object and the intensity of the output corresponds to the phase information. To use the novelty filter as a real-time phase-measuring instrument, it is desirable to suppress the velocity information, or equivalently, contour formation. This implies that the filter has to be manipulated so that its output resembles the one shown in figure 5(a). In this section, we propose a method to achieve this by controlling one of the experimental parameters, namely the relaxation time of the crystal.

3.2. Tuning the novelty filter

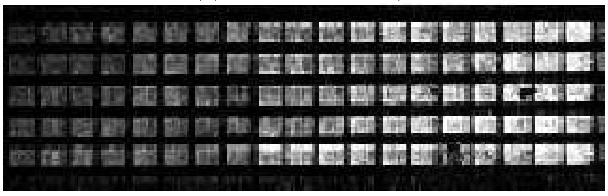
To avoid the situation described in cases 2 and 3, the object should move with very high speeds compared with the grating formation rate. However, in biological or medical applications one does not have much control over the speed or the length of the microorganism. The only other parameter that can be varied is the relaxation time of the filter. It is known from the literature that the photorefractive time constant is dependent on the total intensity of light incident on the crystal [12–14]. We made use of this property to change the relaxation time of the filter. In the experimental set-up, the power of the reference beam was varied with a variable neutral density filter (NDF). We determined the dependence of the relaxation time of the filter on the power of the incident reference beam. Figure 6 depicts the experimental result which shows qualitative similarity to the experimental results obtained by other authors [13, 14]. It is seen that the relaxation time of the filter changes from about 7 to 2 s when the reference beam power is varied from 200 to 800 μW. Thus by changing the reference beam power the relaxation time of the filter can be influenced. The tunability of the relaxation time is a very important feature that can be advantageously used to eliminate contour formation.



(a) $v = 20$ input-pixels/s



(b) $v = 2$ input-pixels/s



(c) $v = 3.3$ input-pixels/s

Figure 5. Novelty filtered images of moving pixel cluster of 10×5 at different speeds. The filter relaxation time is $\approx$3 s.

At this point, we claim that there exists a critical response time τ_c which depends on the length and the speed of the object:

$$\tau_c = \frac{l}{v},$$

such that if $\tau > \tau_c$ no trail will be seen and if $\tau < \tau_c$ a trail will be seen: τ is the relaxation time of the filter.

3.3. Suppressing contour formation

To substantiate our claim, we performed an experiment with a moving pixel cluster of $l = 10$ pixels, $h = 5$ pixels, $\Delta\phi = \pi$ rad and $v = 3.3$ input pixels/s which corresponds to

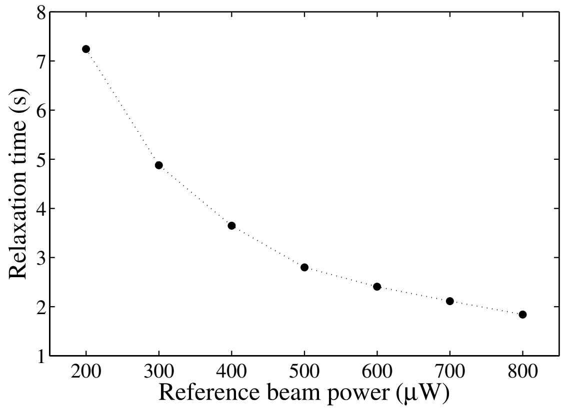
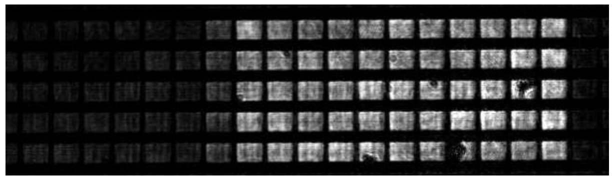
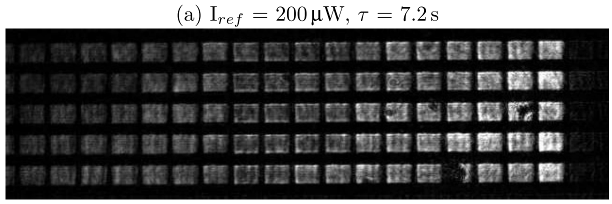


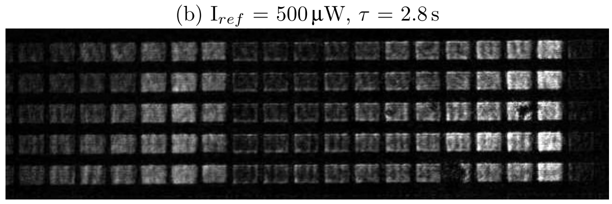
Figure 6. Intensity dependence of the relaxation time of the novelty filter. The dotted curve between the data points serves as a guide to the eye. The error bars are so small that they are within the data points.



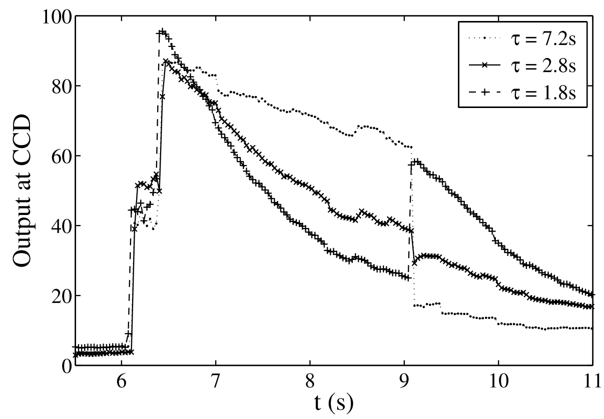
(a) $I_{ref} = 200\,\mu W$, $\tau = 7.2\,s$



(b) $I_{ref} = 500\,\mu W$, $\tau = 2.8\,s$



(c) $I_{ref} = 800\,\mu W$, $\tau = 1.8\,s$



(d) Intensity at a point as the phase object moves across it

Figure 7. Novelty filtered images of a moving pixel cluster under different relaxation times. The speed of the cluster is 3.3 input pixels/s.

$\tau_c = 3$ s. This configuration of the pixel cluster corresponds to that described in case 3 in section 3.1. The pixel cluster was moved through the field of view and the novelty filter output was recorded for different relaxation time (or reference beam power) settings. Figures 7(a)–(c) show snapshots of the output. It is clearly seen that for a large relaxation time $\tau = 7.2$ s, there is minimal contour formation. Thus by adjusting the reference beam power incident on the filter, it is possible to obtain an output that is devoid of contour formation. Such a novelty filter output is a necessity for real-time phase measurement as it contains only the phase information.

Figure 7(d) shows the profiles of output detected at a single CCD camera pixel as the phase object moves across it ($6\text{ s} < t < 9\text{ s}$). For large relaxation times, the profile tends to get flatter, which in turn means that contour formation becomes less obvious. Though contour formation cannot be completely eliminated, it can be minimized. The condition for choosing the optimal time constant for a given length l and velocity v of the object can be calculated as follows. Requiring that the variation in the output for a moving homogeneous phase object should be less than a fraction p (where $p < 1$) of the output as soon as the change took place and considering the exponential decay of the novelty filter output after a change in the input, one can derive

$$\tau > \left(\frac{1}{\ln(\frac{1}{1-p})}\right)\tau_c = \left(\frac{1}{\ln(\frac{1}{1-p})}\right)\frac{l}{v}.$$

If we require that $p < 0.1$ (10%), then $\tau > 10l/v$.

4. Phase transfer function

In [8] we showed that the two-beam-coupling novelty filter can be used to quantitatively measure phase changes. We established the existence of a characteristic response curve for the novelty filter. This response curve, the *phase transfer function*, relates the input phase change with the detected output intensity. It is unique for phase changes from 0 to π rad. However, the phase measurement reported in [8] was done with signal beams carrying no two-dimensional spatial information. In this paper, we extend that work for phase measurement of two-dimensional moving objects.

The novelty filter output which does not exhibit contour formation contains phase information from the input moving phase object. To use the novelty filter as the phase-measuring instrument, its phase transfer function has to be determined. Once the phase transfer function is established, it is simple to create a look-up table and map the detected intensity to the corresponding phase.

To determine the phase transfer of our NFM, we translated a pixel cluster of 5×5 pixels with a speed of 20 input pixels/s ($\tau_c = 0.25$ s) through the field of view of the microscope and observed the output. The relaxation time of the crystal was set to 2.4 s which is much larger than τ_c, thus avoiding contour formation. Each time a different phase value ($\Delta\phi$) was set to the pixel cluster and the output of the filter was captured by the CCD camera as the cluster moved through the field of view. The average output intensity detected at the camera is plotted against $\Delta\phi$ in figure 8. This plot is the phase transfer function of the NFM. From the error bars in the graph, it can be deduced that phase changes of 0.1π rad or a $\lambda/20$ phase difference can be distinctly detected. Using a camera with a larger dynamic range, still better phase-change resolution can

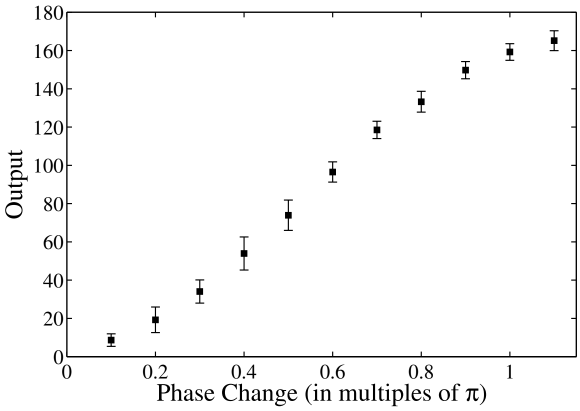


Figure 8. Experimentally determined phase transfer function.

be obtained. Currently, we are working on extending the phase measurement range up to 2π rad.

5. Conclusion

We exploited the phase sensitivity of the two-beam-coupling NFM and used it for implementing a real-time two-dimensional phase-measuring instrument. We investigated homogeneous phase objects moving in a constant phase background. We demonstrated that contour formation in the novelty filter output can be minimized by changing the relaxation time of the filter. We measured the phase transfer function of our NFM in the range of 0–π rad and found that our system can measure phase changes with a resolution of at least $\lambda/20$ at 532 nm.

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