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Phase shifting white light interferometry using colour CCD for optical metrology and bio-imaging applications

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ABSTRACT

Phase shifting white light interferometry (PSWLI) has been widely used for optical metrology applications because of their precision, reliability, and versatility. White light interferometry using monochrome CCD makes the measurement process slow for metrology applications. WLI integrated with Red-Green-Blue (RGB) CCD camera is finding imaging applications in the fields optical metrology and bio-imaging. Wavelength dependent refractive index profiles of biological samples were computed from colour white light interferograms. In recent years, whole-filed refractive index profiles of red blood cells (RBCs), onion skin, fish cornea, etc. were measured from RGB interferograms. In this paper, we discuss the bio-imaging applications of colour CCD based white light interferometry. The approach makes the measurement faster, easier, cost-effective, and even dynamic by using single fringe analysis methods, for industrial applications.

Keyword: Phase shifting interferometry, Optical metrology, White light interferometry, Bio-imaging, Red Blood Cells, Onion skin, Fish cornea.

1. INTRODUCTION

Interferometry is a well-established full-filed, non-contact, non-invasive optical tool for both metrology and imaging applications. It has applications in the fields of fiber optics, optical metrology, surface profiling, microfluidics, mechanical stress/strain measurement, velocimetry, biology and medicine etc. It can inspect objects under static, quasi-static or dynamic conditions. Interferometry can handle both smooth and rough samples.[1-5] In interferometry, the desired/phase information about the test object is encoded in the fringe pattern, which in fact represents the phase distribution. By analysing the interferogram produced by a test surface, one can extract the phase information. Various multiple-frame[5, 6] and single-frame[7-11] methods have been used for quantitative analysis of fringe patterns. Single wavelength (1λ) measurements are accurate, but using 1λ greatly limits the wide spread applications of interferometry. The major drawback associated with 1λ –metrology for surface profiling of discontinuous surfaces is the unambiguous step-height measurement range is limited to half-a-wavelength ($\lambda/2$). The approaches adopted to overcome this problems are based on scanning white light interferometry,[12, 13] spectrally resolved white light interferometry,[14, 15] 2λ or 3λ -interferometry,[16-19] and white light interferometry with colour CCD.[20-23].

Scanning white light interferometry (WLI) is a state-of-the-art technique for measuring discontinuities on specular surfaces. WLI makes use of the short coherence length of the white light source. High contrast fringe occurs only when the optical path difference (OPD) is close to zero. The 3-D plot of the axial positions of the zero OPD along the optical axis represents the surface profile of the object under test. Compared to 1λ - (phase shifting) interferometry, the scanning white light interferometry is rather slow, as the number of frames to be recorded and evaluated is large.[12, 13] The spectrally resolved white light interferometry (SRWLI) is a variation of WLI in which the white light interferogram is spectrally resolved into its constituent colour interferograms.[14, 15] All the colour fringes are analysed for respective phases, which are then used to determine the surface profile. The phases at all the wavelengths can be evaluated by phase shifting technique. However this procedure gives only a line profile of the test object, although the requirement on number of frames is similar to the single wavelength phase shifting interferometry. In 2λ or 3λ - interferometric techniques more than one visible (e.g. red (R), green (G), blue (B)) laser wavelengths are used for measurements. Each wavelength will generate its own interference pattern. The acquisition of the multiple-wavelength interferograms can be

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done in two different ways: (i) Sequential illumination mode[16, 24] in which interferograms are recorded with different wavelength one after another sequentially using a monochrome CCD. This is a time consuming process, and (ii) Simultaneous illumination mode[25-28] in which all the interferograms are recorded in one go by using a colour CCD camera. This approach makes the fringe acquisition as simple as in single-wavelength case. Three different sensors have been used for recording colour interferogram: (a) 1-CCD sensor with Bayer filter,[22] (b) 3-CCD sensor,[25] and (c) Foveon X3 sensor.[29] The Bayer filter 1-CCD RGB camera has been demonstrated for many applications including: surface profiling of large discontinuities,[28] simultaneous measurement of shape and deformation,[27, 30] non-destructive testing (NDT) of large defects,[31] simultaneous acquisition of blood flow, blood volume, and oxygenation on human fingers using dual-wavelength laser imaging[32] etc.

Recently, white light illumination combined with single-chip CCD RGB camera was successfully demonstrated for surface profiling of micro-lens array and large-discontinuities,[33] refractive index profiling of biological cells (RBCs),[26] onion skin,[34] fish-eye cornea[35] etc. This approach was further simplified by using single-frame methods instead of multiple-frame methods for phase measurements.[30] Thus the WLI interferometry that uses a RGB 1-CCD camera to acquire R, G, B interferograms simultaneously is a simpler, faster, and dynamic tool for metrology and imaging applications. In this paper, we will discuss the application of WLI with 1-chip CCD for surface profiling of microlens and discontinuous surfaces. Along with our optical metrology applications, we briefly discuss the applications of WLI with colour CCD for bio-imaging.

2. WHITE LIGHT INTERFEROMETRY WITH 1-CHIP CCD CAMERA

A typical white light interferometer with a RGB 1-CCD colour camera is depicted in Fig.1. The interference device is a micro- Mirau interference system equipped with PZT for phase shifting. A convex lens is used to collimate the white light. The collimated beam illuminates the micro-specimen via the beam splitter (CBS) and the Mirau objective (MO). The test specimen is mounted on a 3-axis stage for alignment. The measurement system consists in microscopic objectives with different magnifications. The white light interference pattern is acquired using a colour camera (1-CCD JAI BB-500GE 2/300 GigE vision camera). The colour CCD is interfaced to the computer with a frame grabber card. A piezo-electric transducer (PZT) is attached to the Mirau objective to generate phase shifted frames. The PZT is driven by the PC with a Data acquisition card (DAQ) card. Real-time fringe visualization, storing phase shifting, and quantitative fringe analysis were carried out in LABVIEW and MATLAB software.

3. SURFACE PROFILING OF MICROLENS

Measurement of the shape, surface quality, and optical performance of micro-lenses, as well as, the uniformity of the parameters across the wafer is an important issue. Laser interferometry is not ideal for surface profiling of smooth surfaces, because the long coherence length results in speckle noise, which could introduce error in the surface profile. Hence white light is ideal source for testing microlens array. 3-D surface profiling of a fused silica microlens is demonstrated using the system shown in Fig.1. The microlens and the reference mirror were illuminated with a white light source and interferograms were collected using a 1-CCD RGB camera. The three wavelengths corresponding to the three interferograms were determined by the spectral bands of the colour CCD used. The CCD has three separate spectral bands, R, G, B centered at Red (620 nm), Green (540 nm), and Blue (460 nm) wavelengths respectively. The PZT was calibrated at 540 nm for phase shift 90° , so the maximum phase shift error involved is $\sim 16^\circ$, which can be compensated by using 8-step algorithm. Eight phase shifted frames were acquired and decomposed each frame in to its individual components and the corresponding phases at R, G, B were calculated using 8-step algorithm. Any one of them can be used to reconstruct the surface profile of microlens array. The white light colour interferogram obtained on a micro-lens is shown in Fig. 2(a). Figs. 2(b-d) shows the individual interferograms decomposed from interferogram in Fig. 2(a). The wrapped phase map at 620 nm and unwrapped 3-D surface profile of the array are shown in Figs. 2(e) and 2(f), respectively.

Fig. 1. Schematic of a phase shifting white light interferometer with a colour CCD: CBS- Cubic beam splitter, MO- Microscopic objective, RM - Reference mirror, PBS - Partial reflecting beam splitter, PZT - Piezoelectric transducer; DAQ - Data acquisition card.

Fig. 2. 3-D Surface profiling of a fused silica micro-lens: (a) white light colour interferogram, interferogram at (b) 620 nm, (c) 540 nm, (d) 460 nm, and (e) wrapped phase map at 620 nm, (f) 3-D view of the micro-lens.

4. SURFACE PROFILING OF DISCONTINUOUS SURFACES

Fig. 3 shows the surface profiling of a silica sample with small (Figs. 3(a-c)) and large (Figs. 3(d-f)) discontinuities using the system shown in Fig. 1. Figs. 3(a) and 3(d) show the colour white light fringes generated on a small and large step, respectively. The colour white light interference pattern, the interferogram at 620 nm, and corresponding 3-D phase map calculated using 8-step algorithm are shown in Figs. 3 (a-c), respectively. The step-height is about 130 nm, which is less than 310 nm if 620 nm data is used. Figs. 3(d,e) show the colour white light interference pattern, and the interferogram at 620 nm. The 3-D profile obtained using fringe order method is shown in Fig. 3(f). The step-height value measured from fringe order method is about 640 nm. The fringe order method could resolve the large discontinuity, and also retain the single-wavelength resolution. The maximum step-height that can be measured using this approach is determined by the spectral band widths of the R, G, B channels. For example, the bandwidth of R channel of the 1-CCD JAI BB-500GE 2/300 GigE vision camera used for this experiment is ~ 80 nm, and hence the coherence length of this channel become ~ 3600 nm ($L = \lambda^2 / \Delta\lambda$). So, the maximum step-height measurement is limited by the coherence length of the channel. To increase the measurement range the channel spectral width needs to be reduced. To make this measurement further simpler, and a single-shot approach using Hilbert transformation single frame analysis was demonstrated for profiling of large discontinuities.[33]

Fig.3. 3-D Surface profile analysis of an etched silicon sample with small (a-c) and large (d-f) step: (a) white light interference pattern generated on a small step, (b) the extracted interferogram at 620 nm, (c) 3-D profile showing 130 nm step height, (d) white light interference pattern generated on a large step, (e) the extracted interferogram at 620 nm, (f) 2π ambiguity corrected 3-D surface profile using fringe order method.

4. APPLICATIONS FOR BIO-IMAGING

Quantitative measurement of the refractive index of biological cells is important for extracting vital information, such as, concentration of hemoglobin, thickness, average mass of cell, etc.[36] The refractive index $[n(\lambda)]$ of a biological cell is wavelength dependent, hence requires several wavelengths for measuring the refractive index. Recently, quantitative measurement of phase and wavelength-dependent refractive index of RBCs was reported using a white light phase shifting interferometry and 1-CCD colour camera.[26] The measurement system used is similar to the system shown in Fig. 1. To make the measurements simpler and single-shot, a Hilbert transformation single-frame method was used for quantitative phase analysis of polystyrene spheres, biological (RBC) cells, onion skin etc.[37] The quantitative phase map of onion skin obtained using single-shot Hilbert transformation method. The refractive indices of the onion cell for three wavelengths red, green and blue and was measured to be 1.59, 1.55 and 1.51, respectively.[37] Cornea is a smooth membrane that covers the front of the eye and plays an important role in focusing images on the retina. Any minor changes in the surface shape of the cornea will affect clarity of the image. The cornea is a major refractive surface of the eye which contributes $\sim 70\%$ to the total dioptric power. Corneal topography information is helpful for finding the health of cornea, and for treatment or surgery. White light source which has short coherence length can provide high axial

resolution for precise cross-sectional imaging of Cornea. White light was already used for biological tissue imaging applications.[35] Using the same WLPSI with 1-CCD RGB camera system, topography and tomography of fish cornea were obtained.[35] Thus the white light interferometry with colour 1- CCD camera is a promising tool for biological imaging applications.

5. CONCLUSIONS

In this article, we presented a white light interferometry with single-chip colour CCD that can be used for optical metrology and imaging applications. The system was demonstrated for surface profiling of micro-lens and measurement of small / large discontinuities on micro-samples. Along with our optical metrology results, we briefly discussed the bio-imaging applications of the system reported recently. The system has several advantages over single wavelength techniques: (i) along with small step-height measurements, large step-height measurements are possible, (ii) spectroscopic imaging of biological cells, (iii) the use of colour CCD allows the image acquisition as simple as single wavelength case. Thus the described interferometric system could be a promising tool that can provide simpler, cheaper, and faster quantitative measurements for optical inspection and imaging applications.

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