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Object characterization with refractometric digital holography

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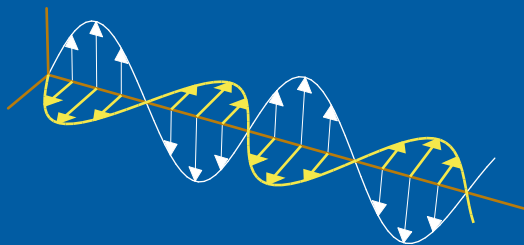
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Abstract

We demonstrate a Digital Holographic method where two different substances in a blend can be discerned. The method requires only one set of exposures and one reconstruction in the plane of focus. The large scale phase variation of the phase image is unwrapped by Flynn's discontinuity algorithm to reveal which regions in the sample the phase has decreased or increased upon transmission of the illuminating wave. Objects with higher or lower index of refraction than the mounting liquid can be detected as regions where the phase has been shifted. We also present a method to calculate the volume distribution of substrates in a sample. Experimental results of both methods are demonstrated with crystals of NaCl and KCl.

1 Introduction

The increasing interest in Digital Holography (DH) in recent years is mainly due to the dramatic development of digital sensors and computers. These two are crucial components for the recording of interference patterns and the reconstruction of images. DH has a number of features that makes it an interesting alternative to conventional optical microscopy. Holography incorporates the registration of the interference pattern between the object field and the reference source. By doing this, all information of an experimental volume is recorded without any mechanical motion of the set up. In one exposure the complete 3D image of an object is compressed into the hologram [5, 8–10].

Several methods have been proposed to use the advantages of DH over conventional microscopy. 3D imaging has been suggested in a number of various solutions [2, 11]. The phase information obtained in holographic measurements have also been used in pattern recognition and depth resolution by the use of low-coherent illumination [1, 6].

We propose a method to discern different types of objects in a sample using digital holographic refractometry [5]. The objects considered here are transparent isotropic crystals of Sodium Chloride (NaCl) and Potassium Chloride (KCl). These objects have one index of refraction irrespective of the direction of the illumination. The method uses digital holography to record the interference pattern between the scattered wave from the objects and the reference point source. The plane of reconstruction is set such that the objects are in focus. A phase contrast image is then calculated which gives a map of the variation of the phase over the objects.

2 Digital holographic refractometry

Consider two isotropic objects, Object1 and Object2, mounted in a refractive index liquid as depicted in Figure 1. The objects have different index of refraction and the index of refraction of the mounting liquid is chosen so that, $n_1 < n_m < n_2$, where n_1, n_2, and n_m are the index of refraction of Object1, Object2, and the mounting liquid respectively. A coherent plane wave illuminates the sample from below. The plane
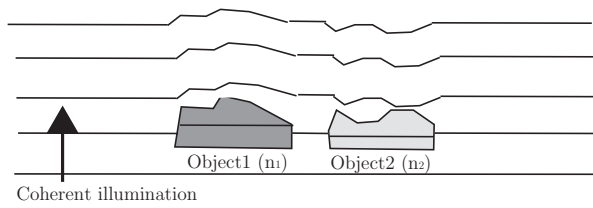
Figure 1: Illustration of the phase shift when a wave impinges on objects with lower and higher index of refraction than the mounting medium. Object1 and Object2 have lower and higher index of refraction than the mounting liquid, respectively. The phase shift is proportional to the variation of the relative index of refraction times the thickness of the object.
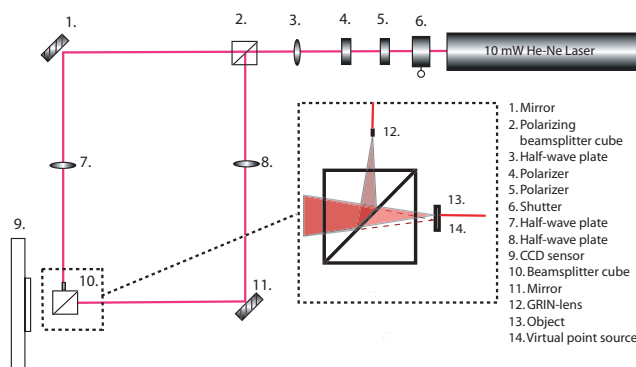

Figure 2: The digital Fourier holographic setup. A beam splitter reflects the reference point source which creates a virtual point source close to the object.

wave enters the sample and the phase shifts upon propagation through the sample. If the phase of the illuminating wave is chosen such that it increases in the direction of propagation, the phase contrast image increases (decreases) in regions with higher (lower) refractive index. This behavior is due to the corresponding increase (decrease) of the optical distance for the illuminating wave in such regions [4].

A Fourier holography set-up is used to record the holograms, see Figure 2. A JDS Uniphase $10\,\text{mW}$, $633\,\text{nm}$ laser is divided in one reference beam and one object beam by a polarizing beam splitter. A half wave plate is used before the beam splitter to control the intensity ratio of the object beam and the reference beam. To create a reference point source a 0.25 pitch GRIN lens is used to focus the reference beam at the surface of the second beam splitter. The second beam splitter then reflects the point source on to the CCD sensor. The object beam is directed on to the object that is placed at the surface of the second beam splitter. The parallel beam illuminates the object and the scattered light is transmitted through the beam splitter to the CCD sensor. By using the beam splitter to reflect the reference point source, a virtual point source is created and can be placed arbitrary close to the object by alignment [5]. The used sensor is a Kodak KAF-3200E monochromatic CCD with 2184×1472 pixels and a pixel size of $6.8 \times 6.8\,\mu\text{m}$.
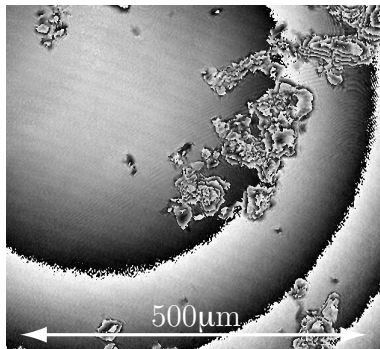
Figure 3: Phase contrast image of KCl crystals mounted in a refractive index liquid. The large scale variation of the phase over the image is due to the change in optical distance the illuminating wave has traveled through the crystals.
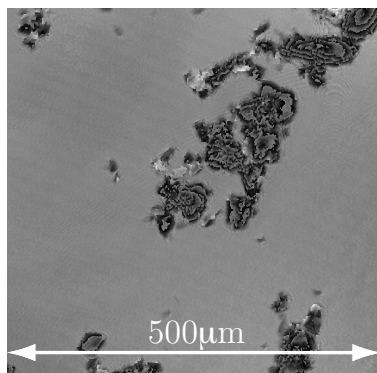

Figure 4: Phase image of KaCl crystals. The KCl crystals appear darker than the surrounding due to their relatively lower index of refraction.

Totally, three images are taken, *i.e.*, the hologram, the reference image, and the object image, respectively. These are then evaluated by the reconstruction algorithm. For the presented setup, a scheme based on 2-dimensional FFTs has been chosen due to its large numerical aperture and the size of the objects. The FFT structure is favorable for a hardware implementation, but still computationally intensive due to the size of the recorded images. Therefore, to improve the reconstruction time, a dedicated hardware solution is being developed [7]. The aim is to develop a dedicated solution that is more powerful than using a standard computer or a modern signal processor.

3   Experimental Results

Figure 3 shows a phase contrast image of KCl crystals mounted in a refractive index liquid. As seen in the image the phase is dramatically changed when going through the crystals. The phase delay can be observed as a color change from white, over gray, to black and back to white. The background also suffers from phase variation due to the slightly divergent beam of the illuminating wave. The
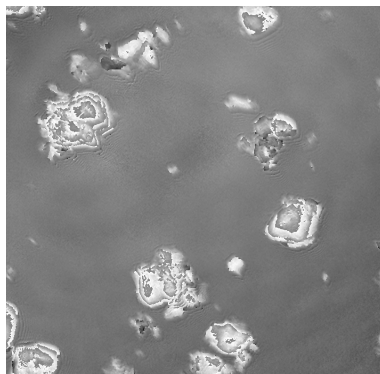
Figure 5: Phase image of NaCl crystals. The NaCl crystals appear brighter than the surrounding due to their relatively higher index of refraction.

phase image is then unwrapped by Flynn's discontinuity algorithm [3] and the large scale background phase variation is removed. The phase variation of the illuminating wave is removed by fitting the unwrapped phase image to a second order polynomial. In the unwrapped phase images the amplitude is proportional to the contrast in refractive index times the propagation length through each crystal, *i.e.*, the optical distance.

In Figure 4, KCl with $n = 1.490$ has been mounted in a refractive index liquid with $n = 1.520$ and the unwrapped phase image has been re-wrapped to the range -2π to 2π to increase the contrast. The size of the larger crystals are about $40\,\mu$m and considering the relative small difference in index of refraction between the crystals and the surrounding liquid the method gives a substantial contrast in the phase. It is noticed that all crystals in the image appear darker than the surrounding and hence confirms the crystals relatively lower index of refraction.

In Figure 5, crystals of NaCl mounted in the same liquid as was used in the images of KCl are depicted. NaCl has an index of refraction of 1.540 which means that the phase should decrease on propagation through the crystals. The unwrapped phase image shows that all crystals appear brighter than the background. Here, the difference in refractive index between the crystals and the mounting liquid is approximately the same, but positive, and again the phase contrast is large.

In Figure 6, KCl and NaCl have been mixed and mounted in a mounting liquid with $n = 1.520$. In this image the dark crystals of KCl are easily distinguished from the brighter crystals of NaCl. This image shows that digital holography can be used to separate two different isotropic substrates in a blend. The unwrapped phase image clearly separates the two substrates and an analysis of the distribution of each crystal can easily be made.

As the phase contrast image gives a map of the change in optical distance through the crystals the index of refraction can be determined if the thickness of the crystals are known. However, this is difficult to accomplish as the crystals are highly asymmetric and there is no reliable method to determine the thickness. However, in this case the index of refraction is known of both the crystals and the mounting liquid. The unwrapped phase image can then be used to calculate the thickness
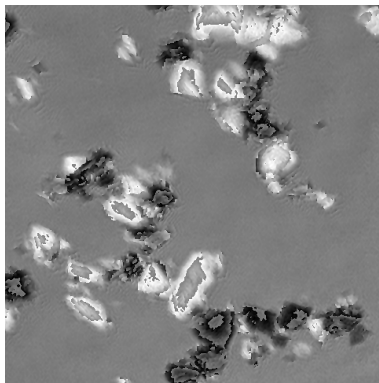
Figure 6: In a mixture, the KCl and NaCl crystals are identified as dark and bright objects, respectively.
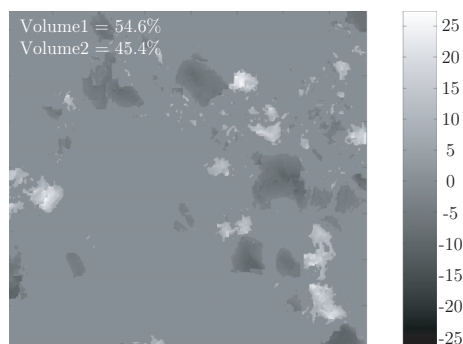

Figure 7: Volume image of a mixture of NaCl and KCl.

of each individual crystal. In Figure 7, the volume distribution of NaCl and KCl in the sample has been calculated. This is done by using the relative index of refraction as $\Delta OP = d\,\Delta n$, where ΔOP is the change in optical path length. For KCl $\Delta n_{\mathrm{k}} = -0.03$ and for NaCl $\Delta n_{\mathrm{n}} = 0.02$. The background is set as zero level and a noise level is used to reduce the errors in the volume calculation. The image amplitude is now proportional to the distance the illuminating wave have traveled through each crystal. Adding the values of each pixel of positive and negative sign respectively in the image gives the volume distribution of each substance in the sample. Dispersion effects have not been considered in these calculations. At the wavelength $633\,\mathrm{nm}$ the index of refractions of the liquid and the crystals are lower than the tabulated values at $589.3\,\mathrm{nm}$ used here, and, hence, the results presented here should be considered approximative.

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