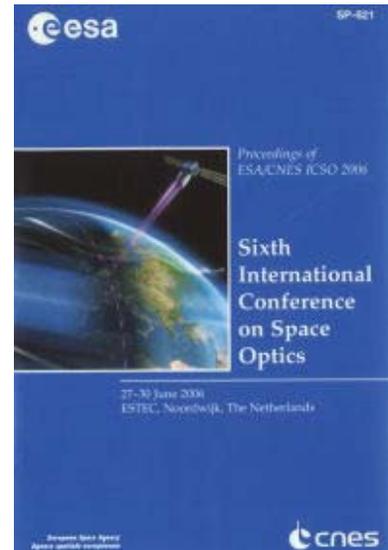# International Conference on Space Optics—ICSO 2006

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HOLOGRAFIC MICROSCOPE ABLE TO GENERATE AN IMAGE WITH EXTENDED FOCUS WITHOUT MECHANICAL MOVEMENT

P. Ferraro[1], S. Grilli[1], P. De Natale[1], D. Alfieri[1], G. Pierattini[2], A. Finizio[2], S. De Nicola[2], G. Coppola[3], V. Striano[3]

[1]Istituto Nazionale di Ottica Applicata, Sez. di Napoli,Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy,
pietro.ferraro@inoa.it, simonetta.grilli@inoa.it, paolo.denatale@inoa.it, domenico.alfieri@inoa.it.
[2]Istituto di Cibernetica del CNR,Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy, g.pierattini@cib.na.cnr.it,
a.finizio@cib.na.cnr.it, s.denicola@cib.na.cnr.it.
[3]Istituto di Microelettronica e Microsistemi del CNR, Via P. Castellini 111, 80100 Napoli, Italy,
giuseppe.coppola@na.imm.cnr.it, vstriano@na.imm.cnr.it.

ABSTRACT

In microscopy high magnifications are achievable for investigating micro-objects but the paradigm is that higher is the required magnification lower the depth of focus is. For an object having a 3D complex shape only a portion of it appears in well focus to the observer that essentially is looking at a single image plane. If an accurate analysis of the whole object has to be performed it is necessary to have a single sharp image in which all details of the object. We propose an holographic microscope to construct an EFI without any mechanical scanning or special optical components. We demonstrate that by this novel approach using DH it is possible to obtain EFI of a 3D object without any mechanical scanning. It will be shown that the unique property in DH, that the phase information of the reconstructed wave front is available numerically allows to construct an EFI image of the object without mechanical movement and by a single image. The proposed microscope could be useful to conduct investigation for experiments in microgravity conditions.

1.   INTRODUCTION

The power of the microscope has always been clear to its discoverers [1]. The microscope allows small objects to be imaged with very large magnifications. At the same time it was clear that there was a trade-off in imaging very small objects in terms of very reduced depth of focus. That means that higher the magnification of the microscope objective, the thinner the corresponding in-focus imaged volume of the object along the optical axis [2]. In fact, the depth-of-field of a microscope, depending on the different conditions of use, is not sufficient in obtaining a single image in which the whole longitudinal volume of the object is in-focus. If an accurate analysis of the whole object has to be performed, it is necessary to have a single sharp image in which all details of the object, even if they are located at different planes along the longitudinal

direction, are still in focus [3]. In this presentation, a new advancement in microscopy based on the use of digital holography (DH) [4-6] leading to a novel concept in optical microscopy is reported. It is demonstrated that by DH it is possible to obtain an extended focused image (EFI) of a 3D object without any mechanical scanning, as occurs in conventional optical microscopy, or by use of a special phase plate used in the wave front coding approach or even can be done by classical optical holography [7]. It will be shown that the unique property of DH, different from classical holography, where the phase information of the reconstructed wave front is available numerically, allows for the reconstruction of an EFI image of the object without mechanical movement and by a single image. For exploring an object having a 3D complex shape with high magnification it is necessary to change the distance between the object and the microscope objective to focus different portions of the object located on different image planes [8,9]. Scientists using microscopes in different areas of research and engineering investigation are very aware of that intrinsic limitation of microscopes. In fact, what is highly desirable in the community of microscopists, is a single image with the necessary magnification but in which the entire object is in focus. That need is very well known by microscope manufacturers. This need has motivated research efforts to find solutions to overcome the aforementioned problems. Essentially, two methods have been found to achieve this. One solution is based on the use of a specially designed phase plate to use in the optical path of the microscope that allows an extension of the depth of focus of the images observable by a microscope [11-13]. An alternate approach consists of a numerical construction of a single EFI image from a collection of images obtained by performing mechanical scanning of the microscope objective on different image planes [8-10]. The latter solution has already found practical application and, in fact, almost all microscopes offered by manufacturers contain a module that is able to create the EFI image [10].

2. EFI WITH DIGITAL HOLOGRAPHY

Since from its discovering optical configuration were settled in holography to combine the 3D imaging property with magnification properties of microscopy [7]. The important need for having an EFI can be satisfied in principle by holography. In fact, holography has the unique attribute that allows to record and reconstruct the amplitude and phase of a coherent wave front that has been reflectively scattered by an object through an interference process. The reconstruction process allows the entire volume to be imaged. In classical film or plate holography the reconstruction process is performed optically by illuminating the recorded hologram by the very same reference beam. An observer in front of the hologram can view the 3D scene. Different image planes can be imaged. For example, by a photographic camera, it is possible to take pictures of different planes at different depths during the reconstruction process by moving the camera along the longitudinal direction. Consequently, by using coherent light, one single hologram obtained using a microscopy set-up is sufficient to reconstruct the whole volume of a microscopic object and by scanning the camera at different depths during the reconstruction process, it is possible to obtain an EFI exactly in the same way as in conventional optical microscopes. It is clear that in the case of holography the scanning process with mechanical movement of the MO must also be performed to image different sections into the imaged volume. However, one very important advantage results using holography: only one image has to be recorded because the mechanical scanning is performed not during the recording process but after the hologram has already been recorded. In this case dynamic events can be studied. That means the EFI of a dynamic process can be obtained by using a number of holograms recorded sequentially. Some advances were achieved in DH, in which the recording process of digital holograms was made directly on a solid state array sensor, such as a CCD (Charged Coupled Device) or CMOS camera. In DH the reconstruction process is performed numerically by processing the digital hologram [17]. In fact, the digital hologram is modelled as the interference process between the diffracted field from the object and its interference with a reference beam at the CCD camera (see Fig. 1). The use of the Rayleigh-Sommerfield diffraction formula allows the whole wave field in amplitude and phase to be reconstructed backward from the CCD array at one single image plane in the interesting volume. Due to the fact that the reconstruction of the digital hologram is fully numeric, reconstructions at different image planes can be performed along the longitudinal axis (z-axis) by changing the distance of back propagation in the modelled diffraction integral. The fig. 1 show the optical set-up for recording digital hologram, were with

1 is indicated the laser source, with 2 the beam splitter, with 3 the beam expander, with 4 the mirror, with 5 the microscope objective, with 6 the object under investigation and with 7 the CCD camera for recording the hologram.
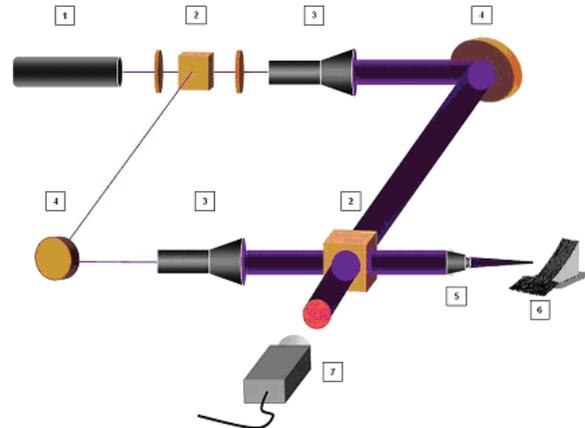

Fig. 1. Digital Holography

In DH the EFI can be composed starting with a stack of amplitude images and using a single phase map obtained numerically in the reconstruction process from a digital hologram. The EFI image can be obtained by reconstructing numerical images at different image planes all from a single digital hologram. For each reconstruction distance d, one single image section is reconstructed. Depending on the optical properties of the employed microscope objective, the depth of focus is limited. If the object under investigation has a 3D shape then at a fixed reconstruction distance d only some portion of the object will be in focus. Of course it is possible to obtain the entire volume by reconstructing a number of image planes in the volume of interest along the z-axis, and with the desired longitudinal resolution. In this way a stack of images of the entire volume can be easily obtained. It is important to note that in DH the reconstruction pixel in the image plane increases as function of the reconstruction distance when the Fourier Transform Method (FTM) is adopted, while it remains constant by using the convolution approach [14,16]. Consequently if FTM is used to obtain a stack of images having each the same size, it is necessary to provide a solution to control the size of the object independently from the reconstruction distance. In addition it is needed to centring the reconstructed image by modelling the reference beam appropriately, as has been demonstrated in recent papers [18-20]. The numerically reconstructed phase map $\varphi(x,y)$ in DH incorporates information about the topographic profile of the object under investigation, where (x,y) are the coordinates of the object point in the object plane. In

fact, the optical path difference (OPD) is related to the phase map by the following equation:

$$OPD(x,y) = \frac{\lambda}{2\pi} \varphi(x,y) \quad (1)$$

If the distance from the lens to the lowest point of the object is p, and q is the corresponding distance of the image of the point from the lens, then any other point of the object at different height Δp(x,y) results in good focus at different imaging planes in front of the CCD, according to the following simple relation:

$$\Delta q(x,y) = -M^2 \Delta p(x,y) \quad (2)$$

where $M=q/p$ is the magnification. In a reflection configuration we have $OPD(x,y)=2\Delta p(x,y)$ and taking into account Eqs.1-2 it results,

$$\Delta q(x,y) = -M^2 \frac{\Delta \varphi(x,y)}{4\pi} \lambda \quad (3)$$

Then the range of distances at which the digital hologram has to be reconstructed to image all the volume in focus is given by Eq. 3.
Fig. 2 show a SEM image of a silicon MEMS structure, a cantilever beam. The silicon cantilever was highly deformed due to the presence of a residual stress induced during the fabrication process.
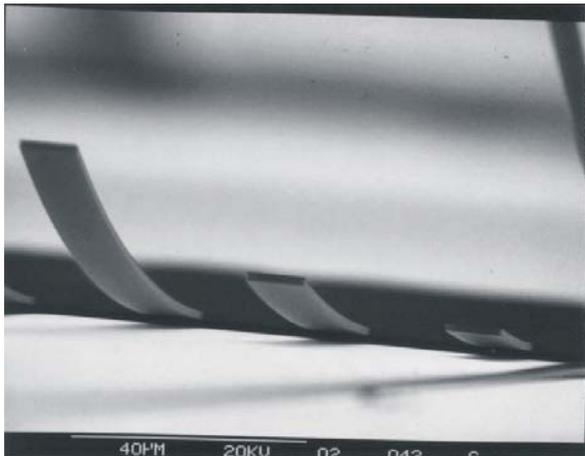

Fig. 2. SEM image of cantilever beam.

From the phase map given in Eq. 1 and by knowledge of the actual magnification M achieved by the DH, it is possible to obtain the extent of the volume occupied by the object along the longitudinal direction (*z-axis*) as given by Eq. 3. The EFI is obtained by "cutting" the stack of reconstructed amplitude images along the entire volume of the object by the surface $\Delta q(x, y)$ given by Eq. 3. Actually, the "cutting" operation means that slices of pixels were taken at the intersections between the surface $\Delta q(x, y)$ and the volume of the stack, from each image of the stack. Those slices of pixels were stitched together to form EFI image. Of course, how

each slice is wide in terms of pixels depends on the resolution required that is related also to the axial resolution. Even if we are considering here for lack of simplicity that surface $\Delta q(x, y)$ has a single curvature requiring the extraction and stitching of slices of pixels the method can be simply extended to more complex surfaces. What should be clear is that the "cutting" operation allow the correct selection of the in focus portions, that have to be extracted from each image stack to obtain the final EFI.

3. EFI BY DH FOR INSPECTING MEMS

Microscopy is a fundamental diagnostic tool for analyzing, characterizing and testing such structures. Often such structures have complex shapes and are made of different materials. Sometimes during the fabrication process, successive handling operations, or the aging process some damages occur. It can be very helpful for an observer to have an EFI image of the structure to detect, for example, the presence of cracks or defects as they appear in different locations of the structure under observation. The silicon MEMS structures shown in Fig. 2 is a cantilever (50µm x100µm) highly deformed due to the presence of a severe residual stress induced in the fabrication process. A thin layer of aluminium was deposited on the surface of the cantilever. The combination of the initial residual stress and the deposition of the aluminium layer caused a progressive breakage of the structure and in this case it is important to detect and analyze the presence of cracks. By observing the MEMS structure using a microscope with a very high magnification it is evident that in each focused plane only some portion of the object will be in-focus. Fig. 3 show the images as they appear by classical microscope the only tip of the MEMS is in focus. Fig. 4 show the corresponding amplitude reconstructions, as obtained by DH, at the tip of the MEMS structure.
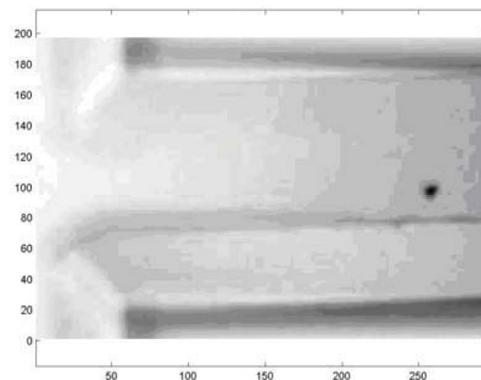

Fig. 3. Classical microscope image of MEMS

The optical configuration as depicted in Fig. 1 was adopted for DH in which the microscope objective was an aspheric lens with focal length f=15.36mm and N.A.= 0.16 equivalent to 10X. The CCD had 1280x1024 square pixel of 6.7µm size. The beam illuminating the object was collimated. A magnification of M=45 was achieved in this case. The source was a laser emitting at wavelength λ=532 nm.
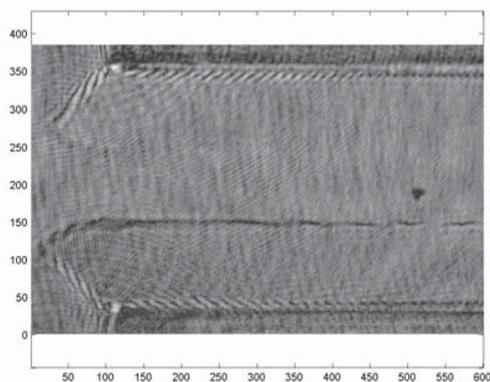

Fig. 4. Amplitude reconstruction

The reconstruction distance to get a good focus at the base of the MEMS was of 156 mm while the entire volume in which the considered MEMS was in focus ranged between 156mm and 190mm (min and max values of the surface $\Delta q(x, y)$). It is clear that in the white light microscope (fig. 3) and the DH picture (Fig. 4) the tip is in well focus while the base is blurred and completely out-of-focus. In the amplitude holographic reconstructions of fig. 4, since a coherent light is used, the out of focus areas at the sharp edges show highly visible diffraction fringes. Finally, figs. 5 and 6 show the EFIs for the optical microscope and the DH respectively.
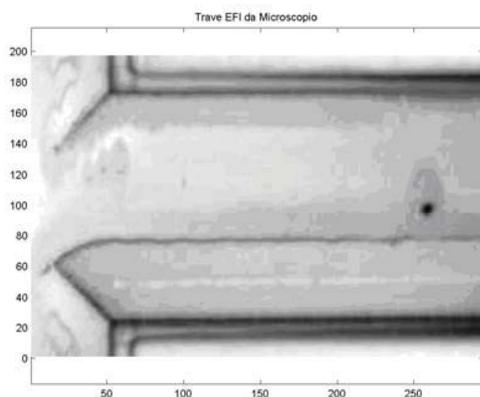

Fig. 5. EFI with classical microscope

The EFI of Fig. 5 was composed by merging a stack of images obtained through a mechanical scanning of the microscope, while the EFI of the DH (Fig. 6) was obtained by numerically reconstructing the digital hologram at different distances, to obtain a stack of amplitude images at different depths.
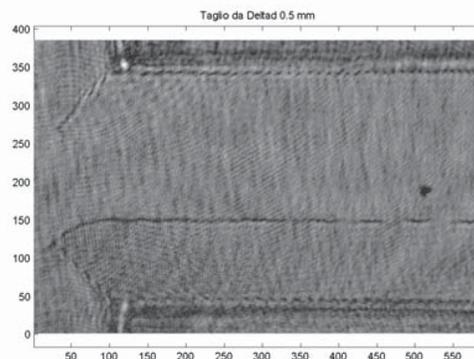

Fig. 6. EFI with digtal holography

The stack was composed of 35 amplitude images each obtained by reconstructing the hologram at step of 1mm from 156mm to 190mm. Through the reconstruction of the phase-map, the profile of the cantilever was recovered. In both the EFIs, by microscope and by DH, the crack is clearly visible and in good focus all along its length. Fig. 7 show another image of a cantilever obtained with optical microscope.
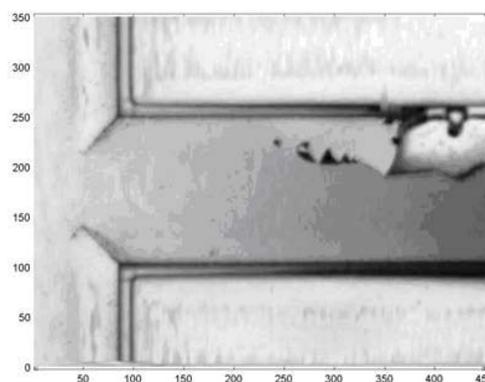

Fig.7. EFI image of cantilever

The silicon MEMS shows abrupt breaks and cracks in different locations on its surface, the breaks and cracks are all in in-focus in the EFI images of Figs 7 and fig 8 which show the EFI image obtained with DH, the EFI images fig. 7 and 8 shows very clear that some details are perfectly visible in the figures.
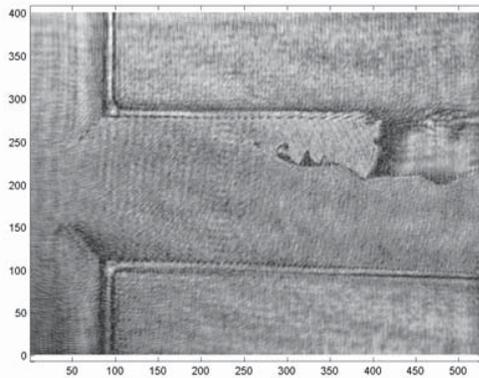
Fig. 8. DH EFI image of cantilever

Fig. 9 show another result obtainable by combining the phase imaging and EFI imaging capabilities of DH. In fact in fig. 9 the real accurate topography of the MEMS cantilever with all cracks well in focus is shown. Hence it is possible to construct an EFI image by a single image recorded by means of DH showing a cracks along the entire length of the MEMS, avoiding any mechanical translation.
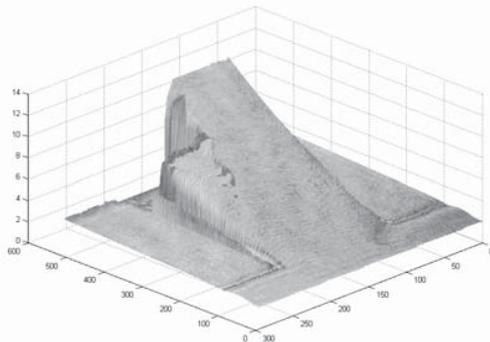

Fig. 9. 3D topography image of cantilever

4. CONCLUSION

A new approach has been developed and demonstrated for constructing EFI by using DH. The EFI image is obtained by reconstructing numerical images at different image planes from a single digital hologram. The very important advantage of the proposed method is the possibility of obtaining an EFI of a microscopic object without a mechanical scanning operation. The whole 3D information intrinsically contained in the digital hologram is usefully used to construct a single image with all portions of a 3D object in focus. In addition it is important to note that it is not possible to obtain an EFI image without mechanical

scanning by classical film or plate holography. The concept is demonstrated here for investigating material properties of MEMS structures made of silicon. Results show that the EFI image obtained by a digital holographic microscope (DH) is comparable to that obtained by an incoherent light microscope. The method could be, in principle, extended to all holographic techniques applied with X-rays, coherent sources, or by holography made with electrons, opening the way to obtaining EFI images for investigation in nanotechnology sciences. By DH it is possible to construct an EFI image of an object or systems experiencing dynamic evolution since the recording of only one image is required avoiding mechanical scanning to record several images at different focus planes. However application of the technique to objects with diffuse surface or complicated structure would be difficult because of the phase-unwrapping and for conduct investigation for experiments in microgravity conditions.

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