3D profilometry using a dynamically configurable confocal microscope

Sungdo Cha*, Paul C. Lin, Lijun Zhu, Elliot L. Botvinick†, Pang-Chen Sun,

Yeshaiahu Fainman

Univ. of California/San Diego, Dept. of Electrical and Computer Engineering,

La Jolla, CA 92093

ABSTRACT

Confocal microscopy is a powerful tool that has been used in the development of 3D profilometers for depth-section image capture and surface measurements. Previously developed confocal microscopes operate by scanning a single point, or array of points, over the surface of a sample. The 3D profilometer we constructed acquires measurement data using a confocal microscopy technique, where transverse surface (x, y) scanning is performed by a digital micromirror device (DMD). The DMD is imaged onto the object's surface allowing for confocal surface scanning of the field of view at a rate faster than video rate without physical movement of the sample. 3D reconstruction is performed *a posteriori* from stacks of 2D image planes acquired at different depths. A description of the experimental setup with system design issues and solutions are presented. Backscatter noise and diffraction noise due to the periodic micromirror structure is minimized using spatial filtering and polarization coding techniques. Using a 100× objective, the longitudinal point spread function (FWHM) was measured at 2.1 μm, with simultaneous transverse resolution of 228.0 lines/mm. The optical resolution performance of our microscope with real-time scanning provided by the DMD, is shown to be effectively equivalent to those of conventional confocal microscopes. The 3D image capabilities of our scanning system using the DMD were demonstrated on various objects.

Keywords: Profilometer, 3D imaging, confocal, microscope, DMD, spatial modulator

1. INTRODUCTION

Confocal microscopes, which possess such unique properties as superior resolution[1] and depth-section imaging,[2] have become a popular imaging technique for the observation of biological specimens, geological samples, fabricated materials, machined parts, integrated circuits, micro-electromechanical systems (MEMS), and diffractive optical elements.[3] The instrument was invented by Minsky in 1957[4], but most subsequent development occurred in the mid-1980s, when it found widespread use in biological applications due to its ability to accurately localize fluorescence-labels within thick, optically transparent specimens.[5] Even though image resolution can be improved in the transverse direction, the main advantage of the confocal microscope is its unique property of depth-discrimination enabling high-resolution measurements along the longitudinal or depth direction.

The operation of the confocal microscope[6] is based upon the geometrical matching of two imaged conjugate focal (confocal) points, one point corresponding to a point source on the sample surface and the other corresponding to a point detector defined by a pinhole aperture. When the confocal condition is achieved, the two imaged focal points coincide in space, giving maximal signal intensity at the detector. As the sample surface is moved away from the focal plane, both the illuminated spot and the detected spot lose focus, causing light at the detector plane to be broadened. The pinhole serves to filter out the broadened, out of focus scattered light and the detected intensity rapidly decreases. This depth-discrimination feature of the confocal microscope allows relative depth measurements to be performed. Depth-section images are built from the in-focus parts of the sample while scanning over the entire surface of the sample.[7] Thus the confocal microscope can be called a profilometer.

* Dr. Cha is a professor of Physics at Kangwon University in South Korea, currently on sabbatical at UCSD. † Univ. of California/San Diego, Dept. of Bioengineering.

Part of the IS&T/SPIE Conference on Three-Dimensional Image Capture and Applications II • San Jose, California • January 1999
SPIE Vol. 3640 • 0277-786X/99/$10.00

Three-dimensional Confocal profilometry is typically performed in two distinct steps: planar scanning in the transverse direction and displacement in the longitudinal direction. A transverse scan in the (x, y) plane is performed at each depth location (z). Between scanning consecutive depth locations, the objective, or the object is moved axially (in depth) and a new transverse plane confocal image is obtained. 3D reconstruction is performed after all 2D planes have been confocally imaged. Thus scanning can be separated into two categories, transverse (x, y) and depth (z) scanning. Transverse scanning of a focal plane in confocal microscopy is achieved by either moving the specimen with respect to the focal plane, or by moving the focal plane while the specimen is fixed. The specimen is usually moved via mechanical translation stage scanning, providing high accuracy. However mechanical scanning is generally slow and expensive. This method lends itself for use in optical system development, as well as for making large range movements to the specimen in bright field or fluorescence microscopy preceding confocal scans. The raster-scan, is a common alternative to mechanical stage scanning. It uses galvanometric mirrors or acousto-optic deflectors for scanning the illumination spot line by line within the field of view. A photodetector, such as a photo multiplier tube is fixed in space and a pixelated image is recreated by averaging the detector's output over a time corresponding to travel over a small area (or pixel) on the sample. The raster scan increases the rate of scanning as compared to mechanical stage movement, however it is still relatively slow for high resolution applications operating at video-rate. Alternatively, the rotating Nipkow disk consisting of an array of pinholes is commonly used to scan a focal plane.[8] The Nipkow disk has high scan rates as compared to the methods described above. However advantages of the Nipkow disk are achieved at the cost of low illumination efficiency and high backscatter noise cause by reflection from the disk.

Depth scanning is traditionally performed by either mechanical stage scanning in the axial direction, or by moving the objective, and thus the focal plane while the specimen (stage) is fixed. Typically the specimen can be quite large and/or heavy and mechanical movement becomes impractical for high speed operation. Piezoelectric microscope objective micromanipulators are commercially available and allow high-speed movement with high accuracy and repeatability. Ultimately the rate of transverse scanning can affect the rate at which the focal plane can be moved with respect to the specimen, limiting the overall rate of 3D quantitative imaging. The precision of 3D imaging is characterized by the measured system's Point Spread Function (PSF), which can also be affected by the stability of a specific scanning method used. Numerous applications, however, rely on availability of high speed 3D quantitative imaging techniques which in turn depend on our ability to perform high speed transverse and longitudinal scanning.

In the past we have investigated introducing parallelism of optics into confocal microscopy. We have previously shown that chromatic depth scanning in the z direction can be achieved by passing a tunable light source through a diffractive lens.[9] The combination of chromatic depth scanning and slit scanning allow for real time 2D profilometry in the axial and transverse direction.[10] It has been shown that the DMD can be used in multiple-aperture confocal imaging to provide programmable confocal scanning.[11] In this manuscript we introduce a dynamically configurable confocal microscope design that achieves 3D quantitative imaging. The key elements of our design are based on using a DMD, which can generate a 2D array of pinholes operating in parallel for parallel transverse scanning and a diffractive optical element with a tunable source for depth scanning. We are investigating the feasibility of using a DMD to create a dynamically configurable confocal microscope, thereby extending these principles to 3D real time profilometry. We evaluated such critical system characteristics as scanning speed and depth of field, as well as measured experimentally the longitudinal and the transverse resolution and compare it with the results obtained by conventional scanning methods.

In the next section we describe the system design and its experimental implementation. The sources of noise intrinsic to the DMD are discussed and their solutions demonstrated. In section 3, the system's characteristics are evaluated by examining depth and transverse resolution for various objective lenses used in our system. The 3D imaging capabilities of the DMD based confocal microscope is demonstrated by creating 3D quantitative image of a coin, a diffractive optical element with known dimensions and an electric integrated circuit chip. We conclude this paper by discussing the application of our scanning system for real time 3D profilometry.

2. THE CONFIGUABLE CONFOCAL IMAGING SYSTEM WITH DMD ENGINE

Figure 1 shows the setup of the DMD-based configurable confocal imaging system. An Argon-ion laser operating at a wavelength of 514.5nm is used as the principal light source for alignment and measurements. The vertically polarized laser beam is transmitted through a Polarization Rotator to adjust for maximum transmittance through the Polarizing Beam Splitter (PBS). The beam is then spatially filtered, expanded, and collimated. After passing through the PBS, the laser beam is guided towards the DMD surface using mirrors 1 and 2. It is critical that the angles between the two mirrors are set such that, after reflecting from individual micromirrors in the DMD array, the light is parallel to the optical axis of the system(see

Figure 1). The light reflected from the micromirrors in the DMD array (only for micromirrors in the 'on' state) passes through the collimating lens 1 (f = 375 mm), the diffractive lens (f = 180 mm, f/4 at design wavelength of 610 nm), and then through the quarter-wave retardation plate. If a tunable source is used for illumination, the diffractive lens will focus different wavelengths of light onto different focal planes, allowing depth scanning through wavelength tuning.[9,10] The light then passes through an eyepiece (6×) and a microscope objective lens to focus onto the surface of the object. Various microscope objectives were used in the system to evaluate the system sensitivity as well as its performance characteristics.

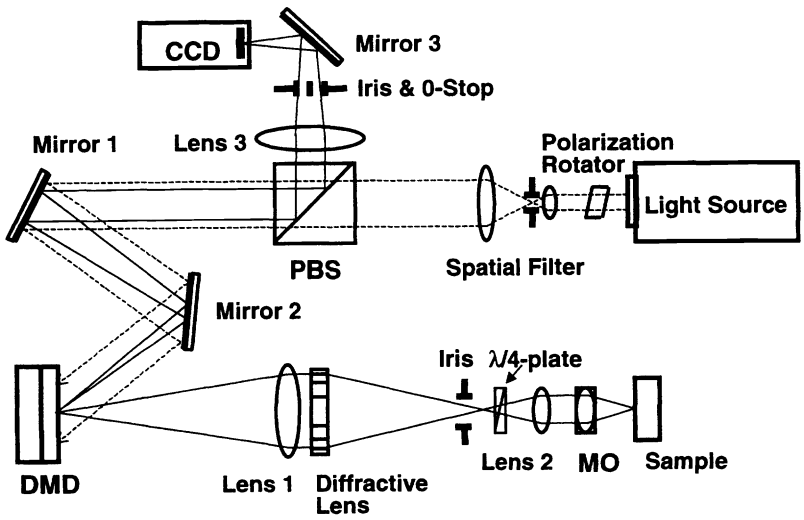

Fig. 1. Schematic diagram of the configurable DMD-based confocal imaging setup.

The light reflected from the object is traced back through the optical components onto the DMD surface. For the reflected light, the DMD serves as a detector pinhole array that filters out the light scattered from the out of focus part of the object and allows only the in-focus back scattered field to be reflected in the direction or the detectors. Because the reflected light passes through the λ/4 retardation plate twice, the returning light will be orthogonally polarized to the incident light. Thus the returning light is reflected by the PBS and mirror 3 to be focused onto the CCD camera (PULNiX TM-7EX, 768 × 494 pixels of 8.4 μm × 9.8 μm) by an imaging lens 3 (f = 200 mm). We use two computers to operate the system, one to control the DMD engine and the other for image acquisition and processing. The DMD engine is driven by the video signal from one of the computers. We can thus design video displays that contain desired pattern that will appear on the DMD display as explained below.

The Texas Instruments Digital Micromirror Device (DMD)[12] is a planar array of 16 μm × 16 μm mirrors that are bistable at ± 10° normal to the chip. Each individual mirror acts as an on/off switch by either reflecting light towards the optical system or by reflecting light away from it. The controller circuits for the DMD converts a SVGA video signal from a PC graphics card into the proper timing signal thereby switching each of the 840 × 600 mirrors in the array to their correct positions. Transit time between the on/off states is 20 microseconds and the entire DMD chip can be refreshed at video rate. By controlling the video signal delivered from the graphics card, individual mirrors can be set to their on/off positions creating any arbitrary pattern of pixels on the chip. The physical design of the DMD allows the projection of an arbitrary pixelated image onto the object. The reflected image will contain noise and losses that are intrinsic to the geometry of the chip. These noise sources include diffraction due to the periodic structure of the mirrors and backscatter from the mirror surfaces. We measured the loss in intensity due to diffraction off the chip to be about 13.5 % during scanning.

The DMD serves dual purposes; scanning the x-y field and configuring the illumination and detection pinhole array necessary for realizing parallel confocal microscopy. The illumination pinhole is created by turning on one micro mirror while surrounding mirrors remain in the off state. Thus only light reflected from this one micromirror will pass through the optical system. The mirror's image in the objective's focal plane serves as the illumination pinhole on the object. Light reflected off the sample at this (x, y) point is then focused back onto the same micromirror of the DMD. Thus the DMD

micromirror in the on state serves as the illumination as well as the detection pinhole. A confocal condition is achieved with the DMD by creating a pinhole array in which each open mirror is surrounded by neighboring closed mirrors. Thus reflection from the object conjugate to the open mirrors is spatially filtered by the closed mirrors and a confocal image is formed. To image the entire field of view, a mosaic is configured by shifting the open and the closed mirrors in a time varying pattern that covers the entire field of view. For example, with weak scattering objects the distance between opened mirrors will be small (e.g., six mirrors in 'off' state between mirrors in 'on' state), whereas strongly scattering objects may requires increasing the distance between the mirrors in the on state. This pattern is then shifted by one mirror several times to complete the horizontal scan. By repeating the horizontal scan for each vertical location, the entire (x, y) field is scanned. The CCD camera is used to acquire one integrated continuous image during the time when a DMD engine performs scanning the entire field of view.

The signal that can reach the CCD plane is weak due to the scattering and attenuation through various optical components, especially the DMD engine itself. It becomes an important issue in our setup to block out the scattering optical noise and to improve the signal-to-noise ratio. The PBS can filter out most of the scattering noise from the DMD surface since the scattered field is mostly horizontally polarized. In addition, the noise that is generated from the periodic structure of the DMD surface is also periodic and can be eliminated using a spatial filter (Iris & 0-Stop) placed in the back focal Fourier transform plane of the imaging lens 3. Finally, the Moiré interference pattern that is created due the spatial periodicity mismatch between the DMD and the CCD arrays can be reduced by using the DMD engine to scan the field of view while taking a time average of the detected CCD image.

3. SYSTEM CHARACTERIZATION AND MEASUREMENT

The system shown in Figure 1 was constructed and then characterized in terms of such performance characteristics as longitudinal and transverse resolutions. We have also performed 3D quantitative measurement of various objects such as a coin, a diffractive optical element and an electronic integrated circuit chip.

3.1. Longitudinal and Transverse Resolution

The longitudinal Point Spread Function (PSF) is a measurement characterizing the confocal imaging depth resolution.[2] It was obtained by turning on one micromirror in the DMD array and measuring the intensity of light at the corresponding pixel at the CCD camera while moving a planar mirror throughout the confocal imaging object plane. The planar mirror was mounted on a mechanical stage with submicron position resolution and accuracy. Figure 2 shows the measured longitudinal PSF for a 40× (Oriel, f/0.65), 60× (Olympus, f/0.80) and 100× (Leitz Wetzlar, f/0.90) microscope objective lens. The Full Width at Half Maximum (FWHM) measurements were estimated by interpolation of the measured data. The depths of field were calculated to be 6.0 μm for the 40×, 4.1 μm for the 60×, and 2.1 μm for the 100× objectives. The system's transverse resolving power was characterized from imaging the USAF Resolution Target. Figure 3 shows the image of the USAF resolution target taken with the 100× objective. The line patterns with highest spatial frequency (228.0 lines/mm) was clearly resolved (see Figure 3).

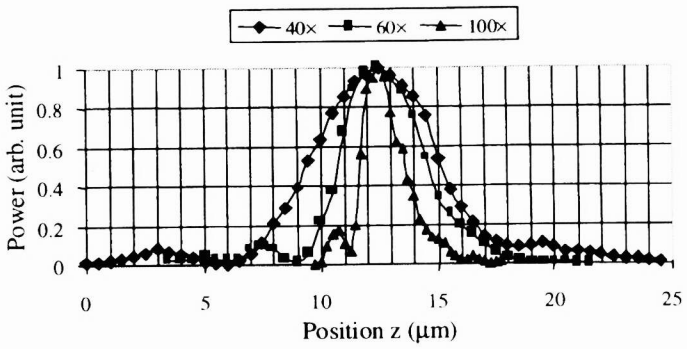

Fig. 2. Experimental results of the measured longitudinal PSF: confocal microscope with 40× objective(◆), 60× objective(■), and 100× objective(▲).

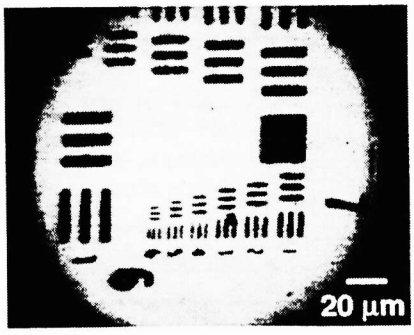

Fig. 3. Images of the USAF 1951 Resolution Target taken with the 100× objective.

3.2. 3D Quantitative Measurements

In order to demonstrate the capability of our confocal imaging system we have performed quantitative measurements of various samples. The measurements were done by first acquiring a sequence of confocal images from different depths of the object, and then using the center of mass method to find the depth position for each pixel. Figure 4 shows images of the letter 'R' on a US dime coin using a 20× objective. Figure 4-a is taken when all DMD mirrors are in "on" positions, which is essentially an image obtained from a conventional microscope (i.e. confocal conditions do not exist). Figures 4-b and 4-c show confocal images when we switched the system into the confocal mode, and depth focusing was performed on the top (Figure 4-b) and the bottom (Figure 4-c) of the "R" respectively. Notice that confocal images shown in Figures 4-b and 4-c show high rejection of out of focus scattered light as compared to the conventional microscope image shown in Figure 4-a, demonstrating the depth sectioning capabilities of our confocal system. Figure 4-d shows a 3D surface contour plot of the letter "R" measured by the confocal imaging system. The plot is generated from 10 consecutive confocal images, each separated by 5 μm through the depth of the letter "R". The total depth from the bottom to the top is estimated to be 45 μm.

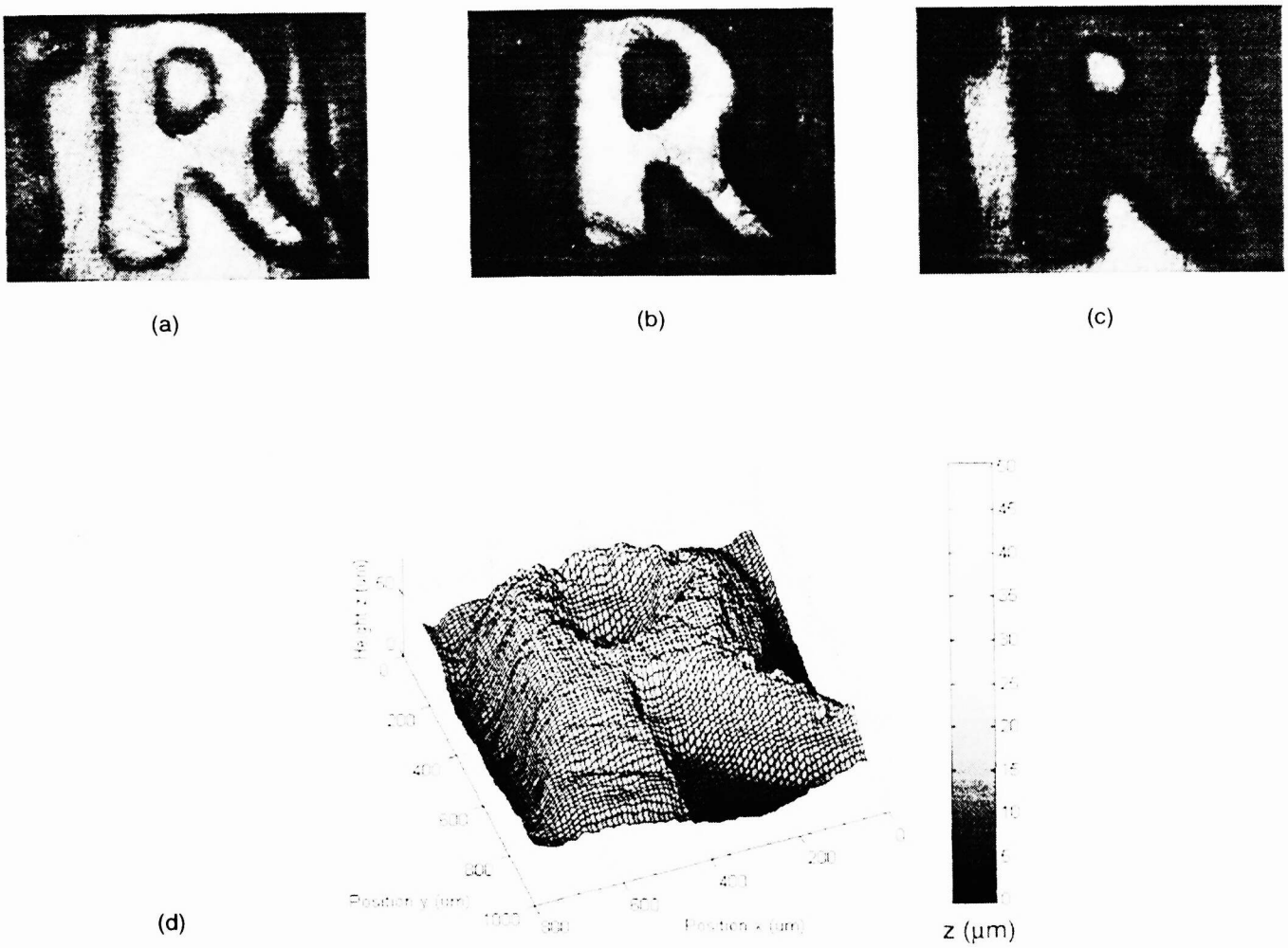

Fig. 4. Images of a dime coin (the letter '**R**' from 'IN GOD WE TRUST'): (a) a conventional microscope image of the coin, (b) a confocal microscope image of the top of the coin, (c) a confocal image of the bottom of the coin, (d) 3D mesh reconstruction of 10 consecutive image planes (5 μm depth spacing).

In the second experiment, we measured (using 100× objective) the surface profile of a four-phase-level diffractive optical element. Figures 5-a and 5-b show images of the diffractive element taken when the confocal imaging plane is adjusted to the top and to the bottom level of the elements, respectively. Figure 5-d shows the 3D mesh plot that is reconstructed from 8 consecutive confocal images through out the depth of the diffractive element with 0.2 μm depth spacing. For comparison, we used a Dektak profilometer to measure the same optical element and the resulting 2D surface profile is shown in Figure 5-c. The two measured values are consistent indicating that the performance of our method is comparable with other existing surface profiling techniques.

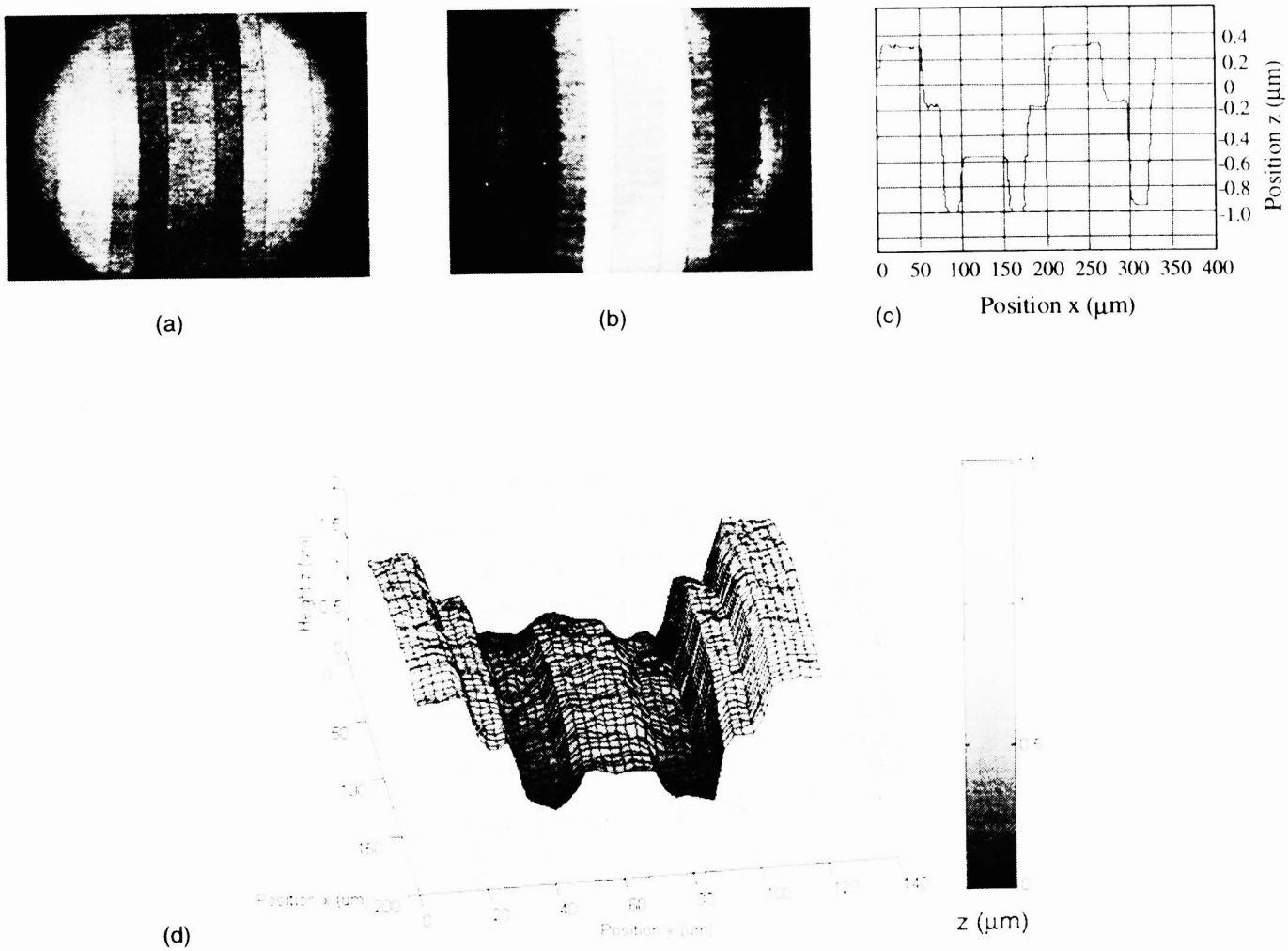

Fig. 5. Images of a four phase level diffractive element with levels at 0.45, 0.85 and 1.35 μm relative to the bottom (using 100× objective): (a) image of the diffractive element when the confocal plane is adjusted to the top, (b) to the bottom levels, (c) the depth profile measured by a Dektak profilometer, (d) 3D mesh reconstruction of 8 consecutive image planes (0.2 μm depth spacing).

In the last experiment we measured (using 100× objective) a Rozier 10/95 Photonic SRAM chip. Figures 6 shows confocal images when the confocal plane is adjusted to the bottom (6-a), to the middle (6-b, 3.4 μm from bottom) and to the top (6-c, 7.9 μm from bottom) of the chip. The fine structure inherent to each level of the chip can be resolved in its corresponding image. Notice how the posts shown in Figure 6-c show up as 'shadows' in figures 6-a, b even though the reflecting surfaces of the posts are proximal to the objective relative to the image planes in figures 6-a, b. We have also shown in Figure 6-d the top view of the measured chip where the depths are coded by different gray scales. Figure 6-e shows a 3D mesh reconstruction from 17 consecutive confocal images through out the depth of a chip (0.5 μm depth spacing).

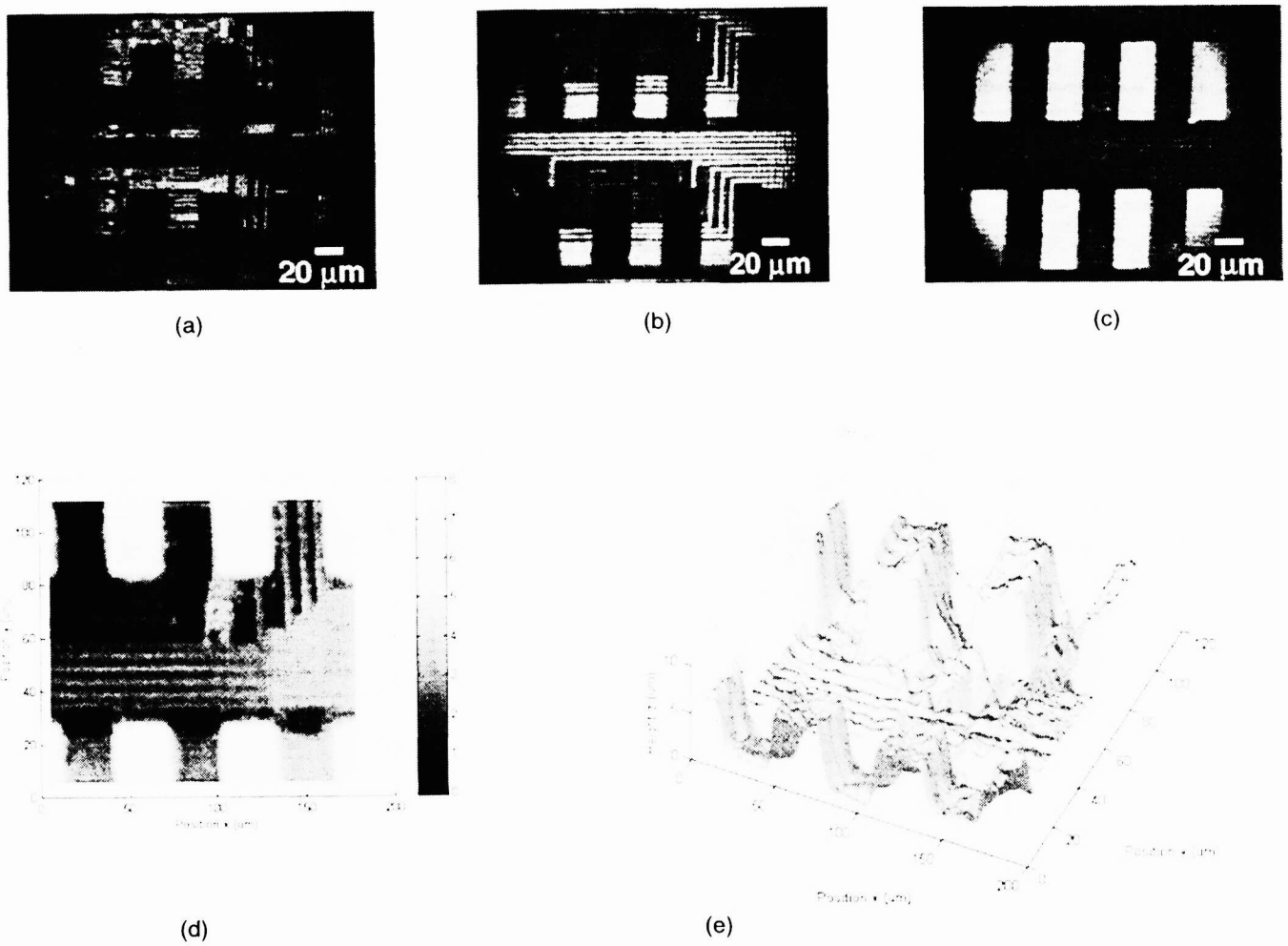

Fig. 6. Images of the chip (Rozier 10/95 Photonic SRAM) using 100X objective: (a) image of the chip when the confocal plane is adjusted to the bottom, (b) to the middle (3.4 µm from bottom), (c) to the top (7.9 µm from bottom), (d) the top view of a 3D rendering of the chip with depth coded by grayscale, (e) 3D reconstruction of 17 consecutive image planes (e, 0.5 µm depth spacing).

4. SUMMARY AND DISCUSSION

In summary, we have designed and constructed a DMD based confocal imaging system for 3D profilometry. By using the DMD as a spatial light modulator, confocal surface scanning can be performed at speeds faster than video rate without physical movement of the sample. In addition, it allows us to programmably configure the source and detection pinhole array to achieve the best signal and to reduce the crosstalk noise. Longitudinal and transverse resolution of the system was estimated by measuring the longitudinal point spread function and the USAF resolution target, respectively. Finally, we have performed

performed 3D quantitative measurements of several samples and compared the results to those obtained from using an existing surface profilometer. The measured values are found to be within good agreement. We have also resolved some of the technical issues to improve the confocal imaging system performance by using polarized light in conjunction with a PBS and a quarter wave plate to filter out scattered noise, as well as by using a spatial filter to remove the diffraction noise created by the periodic structure of the DMD.

The next step in our development of a DMD based 3D confocal imaging system is to integrate the lateral spatial scanning capabilities of the DMD with a suitable method of axial scanning to retain the high scan rate. This step will require replacing the light source with a tunable Ti-Saphire laser. Depth scanning can be performed by changing the wavelength of light introduced into the diffractive lens thereby changing the axial position of its focal plane. The DMD based confocal system can also be modified to perform a different function: producing images with very large depth field-of-views. This is done at the cost of a decrease in lateral resolution. This method replaces the monochromatic light source with a white light source. The diffractive optics will map each constituent wavelength of the white light source onto a continuum of focal planes. Since each chromatic focal plane is focused onto the DMD, when used in a confocal mode, the DMD will perform filtering of the out of focus information at each wavelength of light. Therefore, we can acquire images of different colors in parallel, where each color is scattered from a distinct depth of the object. The integration of all color images would create an image which is in focus through a very large depth of field. Finally, we are in the preliminary stage of exploring applications of our system in confocal fluorescence imaging of biological samples.

ACKNOWLEGEMENTS

This research is supported by DARPA and the National Science Foundation, and in part by Kangwon National University.

REFERENCES

1. M. Bertero, P. Boccacci, R. E. Davies, and E. R. Pike, "Super-resolution in confocal scanning microscope: III. The case of circular pupils," Inv. Prob. 7, 655-674 (1991).
2. D. K. Hamilton, T. Wilson, and C. J. R. Sheppard, "Experimental observations of depth-discrimination properties of scanning microscopes," Opt. Lett. 6 (12), 625-6 (1981).
3. S. G. Anderson, "Confocal laser microscopes see a wider field of application," Laser Focus World 30 (2), 83-6 (Feb. 1994).
4. M. Minsky, "Microscopy apparatus," U.S. patent 3,013,467 (19 December 1961).
5. J. B. Pawley, *Handbook of Biological Confocal Microscopy*, Plenum Press, New York (1989).
6. T. Wilson and C. Sheppard, *Theory and Practice of Scanning Optical Microscopy*, Academic Press, London (1984).
7. D. K. Hamilton and T. Wilson, "Surface profile measurement using the confocal microscope," J. Appl. Phys. 53 (7), 5320-2 (1982).
8. G. Q. Xiao, T. R. Corle, and G. S. Kino, "Real-time confocal scanning optical microscope," Appl. Phys. Lett. 53 (8), 716-8 (1988).
9. S. Dobson, P. C. Sun, and Y. Fainman, "Diffractive lenses for chromatic confocal imaging," Appl. Opt. 36 (20), 1749 (1997).
10. Paul C. Lin, P. C. Sun, Lijun Zhu, and Y. Fainman, "Single-shot depth-section imaging through chromatic slit-scan confocal microscopy," Appl. Opt. 37 (28), 6764 (1998).
11. Minhua Liang, Robert L. Stehr, and andrew W. Krause, "Confocal pattern period multiple-aperture confocalimaging system with coherent illumination," Opt. Lett. 22 (11), 751 (1997).
12. J. B. Sampsell, "An Overview of Texas Instruments Digital Micromirror Device (DMD) and Its Application to Projection Displays," *Society for Information Display Internatl. Symposium Digest of Tech. Paper*, 24, 1012 (1993).