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APPLIED RESEARCH

Techniques for Constructing Ultra Low Cost Deep Ultraviolet Transmission Microscopes

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ABSTRACT This article presents the design, construction, and results obtained by our new compact, small and inexpensive deep UV microscope. Several methods are described for recording microscopic images of various types in the deep ultraviolet spectral regime (200 nm - 300 nm). The techniques employed utilize CCD cameras, de-bayered webcam sensors, photographic paper and cyanotype coated paper as detectors. In addition, we describe the techniques employed and results obtained by utilizing a cellphone camera to record deep ultraviolet microscopic images. The instrumentation techniques, presented in this paper are expected to be most useful in areas where compact, handheld systems, are needed for in field applications.

INDEX TERMS Microscopy, deep ultraviolet light, deep ultraviolet microscopy.

I. INTRODUCTION

Microscopy in the deep ultraviolet spectral region has several advantages over the visible region, such as higher spatial resolution and higher contrast, especially when observing and recording biological species. This is due to the fact that several important biological molecules such as protein, DNA, RNA and others have a strong absorption in the deep ultraviolet region. To that effect, this instrument provides a means for identifying and quantifying the concentration of these biomolecules at an individual cell level [1], [2]. However, at the present time there are very few optical microscopes available commercially which are capable of imaging such molecules in the deep ultraviolet; and those that are available tend to be of large size and prohibitively expensive.

The reasons behind this lack of deep ultraviolet microscopes are probably due: first, to the fact that traditional glass lenses cannot be used, because of their strong absorption of ultraviolet light [3]. Second, bright enough deep ultraviolet sources were very few, hard to operate and maintain safely, and in addition they require high power to operate. For example, the most common deep ultraviolet light source, mercury vapor lamps, pose health hazard when operating and must be disposed safely especially when they break. In addition to the fact that the traditional camera systems are not very sensitive to ultraviolet light owing to various coatings and protective glasses located on top of the CMOS or CCD sensor, which do not transmit UV light.

There are also concerns about the phototoxicity that deep ultraviolet light exerts on biological systems [4], [5]. However, by controlling, carefully, the exposure time of the samples, to the deep ultraviolet light, the photo-toxicity effects can be minimized [1]. Deep ultraviolet microscopy, on the other hand is widely used in the semiconductor industry, where these wavelengths, from various light sources such as g-line, (365 nm) of mercury lamp, 157 nm and 193 nm lines of excimer lasers and other sources have been used to generate very small features on semiconductor chips, which also help to keep up with the Moore's law [6]. Currently, cutting edge semiconductor manufacturing industry which focuses more on the extreme ultraviolet (EUV) light (13.5 nm wavelength) for fabricating ever smaller size features on semiconductor chips. Unfortunately, the deep ultraviolet lithography revolution in semiconductor manufacturing did not transfer to biological microscopy, probably due to the fact that biological molecules are sensitive to the ultraviolet light and might result in damage to their cellular components. In addition, the 193 nm, and 157 nm excimer laser wavelengths are not very well suited for biological molecules, which absorb at 260 nm (DNA/RNA) and 280 nm (proteins).

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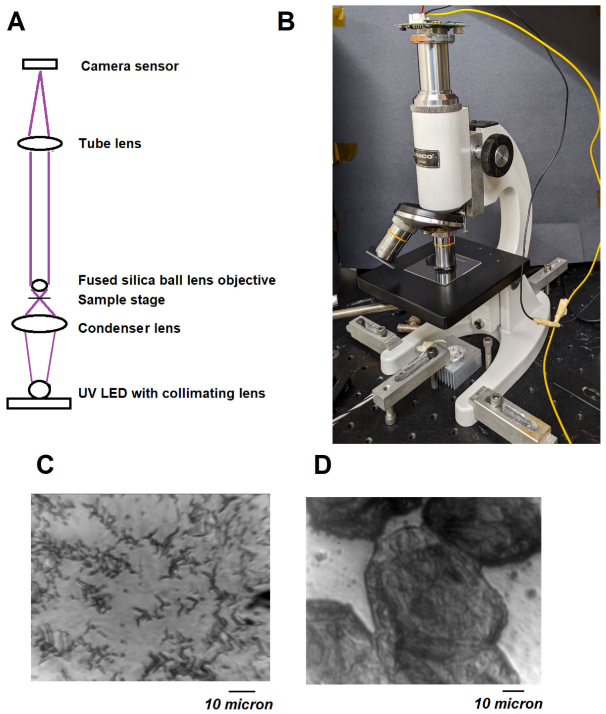

FIGURE 1. Details of the Deep Ultraviolet Microscope (A). Photograph of the newly constructed system (B). (C) Picture of *Halobacteria Salinarum* cells and (D) cheek cells captured at 275 nm with the constructed microscope (using 2 mm fused silica ball lens and 4 mm fused silica half-ball lens, respectively as the objective lens.

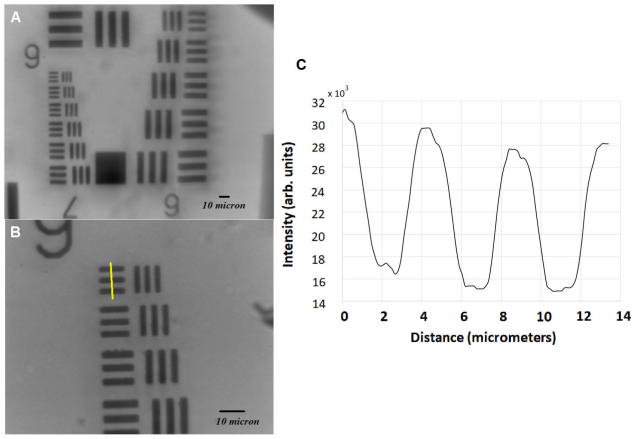

FIGURE 2. Deep ultraviolet microscope images, at 275 nm, obtained with our microscope described in Figure 1. The figure shows the image of a USAF1951 resolution target captured with CCD camera and fused silica half ball lens objective of diameter 4 mm (A) and a fused silica ball lens of 2mm diameter (B). Line profile taken at the 6th element of the 7th group (yellow line) is depicted in (C).

In addition, excimer deep ultraviolet lasers are rather large and not easily portable. However, with the advent of deep ultraviolet LEDs which emit in the desired deep UV wavelengths for biological research, there has been a resurgence of interest in deep ultraviolet microscopy and its applications [2], [7], [8], [9], [10].There have been attempts [9], [10] towards low-cost construction of deep UV microscopes, however they still cost several thousand to a few tens thousands of US dollars to construct. Our designs, presented here, reduces that cost by more than ten times. The research presented here describes the design, construction, and means for recording microscopic images of species, such as bacteria and molecules that absorb in the deep ultraviolet spectral regime (200 nm - 300 nm). In addition, we discuss the use of portable deep ultraviolet wavelength sources, such as deep UV LEDs, to record micron-size objects. The detector(s) utilized in our devices range from CCD cameras to cyanotype coated paper. In addition, we have developed techniques for deep ultraviolet microscopy utilizing a cellphone camera and an intermediate fluorescent screen which have resulted in very compact, portable and high resolution UV microscopes. The instrumentation techniques, described, in this paper, are expected to be most useful in the areas where compact, hand-held systems are desired for in-field applications, particularly, the detection of protein crystals, pathogens such as bacteria, fungi and large molecules.

II. EXPERIMENTAL

A. LIGHT SOURCES

For trans-illumination light sources, the best choice is the use of Deep ultraviolet LEDs. These LED sources are low cost, compact, energy efficient, and emit a band of narrow wavelengths light with FWHM (Full width at half maxima) 10 nm. In addition, these LEDs are available at wavelengths ranging from 250 nm, to 295 nm and even longer thereby providing bright light sources that cover the entire UV to visible region. To record the images and collect the data presented in this paper, we utilized 275 nm UV LED (Model MTSM275UV-F1120S). Utilization of these narrow wavelength band LED sources provide an additional advantage namely: the chromatic aberrations in the recorded image can be greatly reduced, as opposed to the case where the image is recorded by employing a wide band of wavelengths. This reduces the requirements on the achromaticity of the microscope objective lens used; thereby greatly simplifying the system. The remaining non-chromatic aberrations are corrected in the software during the post processing of the images. Another light source choice is the Deuterium or Xenon lamps, coupled to a monochromator which selects the desired wavelength and subsequently scan over a wavelength range from vacuum UV to visible, 190 nm to longer than 500 nm.

B. MICROSCOPE OBJECTIVE LENS

The systems that we developed, use simple fused silica or quartz lenses as microscope objectives, which are suitable for a narrow wavelength band in the ultraviolet region. These small ball lenses or half ball lenses, composed of fused silica or quartz, transmit in the deep ultraviolet region, without attenuation. The ball lenses have the advantage of a relatively large numerical aperture, thereby providing a high-resolution image. In addition, ball lenses are available rather easily at a very low cost. In our experiments we used 2 mm diameter fused silica ball lens (Edmund optics

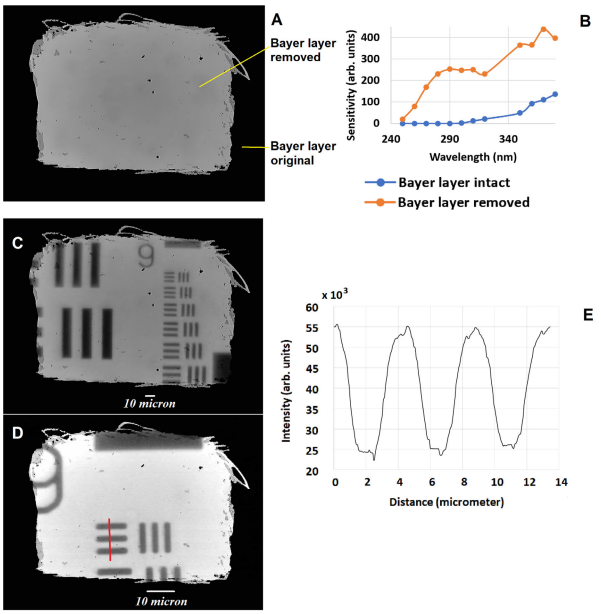

FIGURE 3. Comparison of the sensitivity of a webcam sensor to 275 nm deep UV light in the areas when the bayer filter is intact and after it is removed (A). Comparison of ultraviolet light sensitivity of webcam sensor, with and without the bayer layer. It can be, clearly, seen that the spectral sensitivity increases strongly in the ultraviolet after the bayer filter is removed. The missing data points are due to the filters used to eliminate interference from higher order wavelength outputs from monochromator(B). Image of USAF1951 resolution test target as captured with modified webcam under 275 nm illumination and our deep ultraviolet microscope using 4mm diameter fused silica half ball-lens objective (C) and and 2mm diameter fused silica ball lens objective (D). All the lines up to 6th element of the seventh group of the resolution target were clearly resolved with our system and the line profile taken at the 6th element of the 7th group (red line) is depicted in (E).

67-382) and 4 mm diameter fused silica half-ball lens (Edmund optics # 67-396). Another alternative is to use mirror objectives or reflective microscope objectives which do not suffer from chromatic aberrations and do not attenuate ultraviolet light. These reflective microscope objectives can also be used over a wide range of wavelengths. However, the disadvantage of reflective objectives is their relatively small numerical aperture which limits the resolution; in addition, the cost of these reflective objectives tends to be quite high, roughly several thousand USD for a single lens.

C. DETECTORS

The detectors that we use for the construction of our UV microscopes range from specialized monochromatic ultraviolet sensitive CCDs to paper coated with ultraviolet light sensitive chemicals. In our experiments, monochrome CCDs showed a good response and recorded very accurate microscopic images in the deep ultraviolet, 250 nm wavelength region. In addition, our research has showed that the commonly used CMOS detectors, such as the ones found in webcams, can be made to be sensitive in the deep ultraviolet by removing the IR and UV light blocking filters, and de-bayering the sensor; i.e. removing the red, green and blue layers of filters from the top of the sensor. This can be done by either mechanically removing the bayer layer or by dissolving them using the appropriate chemicals, while leaving the sensor intact. The image exposure times, using CCD and CMOS cameras were in the range of a few milliseconds depending on the sample. Most images presented in the paper are a 'snapshot' of the video frames recorded at $\sim$ 30 frames per second. A video of bacteria swimming in water recorded with this setup at 275 nm wavelength is shown in supplementary section.

Another means that we utilize these sensors and cameras without modifying them is to use a fluorescent screen, such as ZnS:Ag; which fluoresces brightly, when illuminated with deep ultraviolet light. The Images recorded by our microscope can be projected on to these fluorescent screens, which in turn are imaged with an unmodified cellphone camera. This type of arrangement makes our ultraviolet light microscope system extremely accessible, inexpensive and compact. It has already been shown that a high-quality instruments such as spectrometer and microscopes for the visible light region can be build using a cell-phone camera [11], [12], [13], [14], [15], and now we have extended it to deep ultraviolet wavelengths. In addition, we have utilized photographic paper as an ultraviolet light sensitive detector because these papers, inherently, exhibit higher sensitivity in the blue and ultraviolet wavelength region than the visible. The use of photographic papers may also be one of the most cost effective and straight forward means for recording high quality deep ultraviolet microscopic images. Another related means for recording microscopic images is the use of cyanotype printing chemicals, which although do not show as high sensitivity as photographic paper in the ultraviolet region, but are very easy to work with and can serve as a great educational tool in ultraviolet imaging and microscopy. There have been some previous studies on the utilization of cyanotype paper for studying the effectiveness of the sunscreens against the UV radiation of the sun [16], [17].

D. SOFTWARE

The software system which we utilized to capture the deep ultraviolet microscopic images from monochrome CCD (Videology 21D379H.4) and webcam (Logitech C270) was Sharpcap. The bayer layer from the webcam sensor was removed.

III. CONSTRUCTION AND RESULTS

A. DEEP ULTRAVIOLET MICROSCOPE USING A SIMPLE MICROSCOPE AND A MONOCHROME CCD CAMERA

To construct our deep ultraviolet microscope, we used the body of very simple straight tube microscope (Tasco LM400). Such microscopes are usually supplied with a mirror to redirect ambient light to the sample. After removing this mirror, there was ample space to insert our custom made deep ultraviolet light source for transillumination of the sample. The deep UV LED (275 nm) was mounted on a heat sink and the ultraviolet light from this deep ultraviolet LED, is first collimated, roughly, with a fused silica half-ball lens. The collimated light then passes through a quartz condenser

lens which focuses it onto the sample. The sample substrate slide consists of a UV transmissive quartz plate. The fused silica ball lens microscope objectives were constructed by embedding the ball lens in a plate and attaching the plate to a standard microscope objective lens shell (with all the internal optics removed). This allowed us to easily insert our fused silica ball lens objective in to the microscope turret. Various diameter ball lenses provide different amounts of magnification. We used a 4 mm diameter half-ball lens and a 2 mm diameter ball lens for the objective lenses. Because the CCD and CMOS sensors are small in size, the field of view of an image formed directly on them by the objective lens is very small. To widen the field of view we used a quartz tube lens with a focal length of 50 mm. With this CCD arrangement, the image field of view was about 288 μm X 230 μm using a 4 mm half ball objective lens, and 110 μm X 88 μm using a 2 mm ball lens objective.

Figure 1 shows a schematic and picture of the constructed microscope system, and an image of *Halobacteria* cells captured with our constructed microscope while Figure 2 shows the picture of fused silica USAF1951 target taken with this microscope. We were able to resolve, clearly, all the lines upto 6th element of the seventh group, which is the smallest separation in this target that has a line width of 2.19 microns. Based on this data we estimate that the resolving power of this microscope is better than 0.5 microns, which gives a numerical aperture of the system better than 0.35. The numerical aperture of this system can be further improved by utilizing ball lenses of smaller diameter. In addition to a good resolution, the improved contrast in the unlabeled bacteria images due to strong absorption, of deep ultraviolet light, by protein and nucleic acids is clearly seen. In addition to good resolution, the improved contrast in the unlabeled bacteria images due to strong absorption, of deep ultraviolet light, by protein and nucleic acids, is clearly seen. In addition, with the visible light transmission microscope, it is very hard to see bacteria without labelling them or using rather expensive phase contrast systems because bacteria are almost transparent to the visible light. The illuminated non-uniformities in the sample plane could also be improved in the software by recording a flat field image. Our UV microscope system is very easy to build and affordable, in fact any simple microscope body with a straight through tube can be utilized to build this system. The microscope with tilted tubes which use glass prism, can-not be used due to their strong absorption of deep ultraviolet light by their glass optics. We estimate that our entire UV microscope system can be built for less than 300 USD, including the cost of the microscope body.

B. MODIFIED WEBCAM FOR DEEP ULTRAVIOLET MICROSCOPY IMAGING

Simple web-cameras are widely accessible and to that effect we have utilized them in conjunction with our deep ultraviolet microscope, because use of a webcam can make the deep ultraviolet microscope extremely affordable and simple.

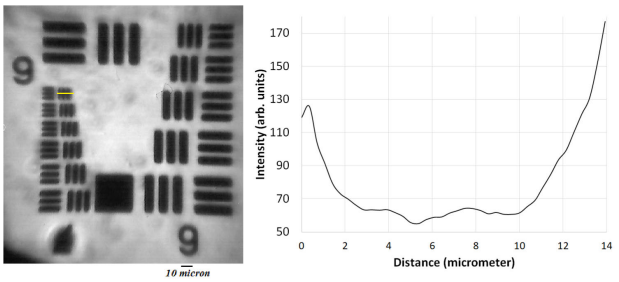

FIGURE 4. Image of a fused silica USAF1951 test target captured on a B&W photographic paper with our microscope under 275 nm illumination (Left) (made with 4mm fused silica half ball-lens objective and the exposure time was about 25 milliseconds). The corresponding line plot of the 6th element of the 7th group (right). The low contrast and resolution as compared to the CCD and webcam images is mostly attributed to the scanning artifacts, paper properties, and to a smaller extent to the camera shake during the shutter operation.

An unmodified webcam has several elements which make it insensitive to deep ultraviolet light, such as Infrared and Ultraviolet blocking glass filter/s; bayer filter on top of the sensor consisting of red, green and blue filters. We show that it is possible to remove this infrared blocking glass, and the bayer filter array from the sensor, thereby greatly enhancing its sensitivity in the ultraviolet wavelength region. Figure 3(A) shows a webcam under 275 nm illumination, with the bayer filter layer partly removed. It can be, clearly, seen that the areas where the bayer layer is intact, no ultraviolet light is reaching the detector thereby the region is completely dark. However, the areas where the bayer layer was removed are very bright, showing highly increased sensitivity to the 275 nm deep ultraviolet light. Figure 3(B) show the spectral sensitivity of the webcam sensor to ultraviolet light with the bayer array removed compared to the same system when the bayer array is present, intact. To compare the spectral sensitivity, we first, removed partly the bayer layer from the top of the sensor. We then illuminated the sensor with several different wavelengths of ultraviolet light using a monochromator and a xenon lamp for light source. We made certain, that light is illuminating the sample uniformly, while the band pass filters were placed at the monochromator output to eliminate interference from higher spectral orders. Then, we compared the output of the pixels where the bayer layer was removed to the pixels where the bayer layer was intact. The output power at every wavelength was measured using a photodetector whose sensitivity curve was known (Hamamatsu S1336-8BQ).

Deep ultraviolet microscopy images of the USAF 1951 target captured at 275 nm with our modified webcam are shown in Figure 3(C,D) and again, we can see that all the lines are clearly resolved by our deep UV microscope. We also note that owing to the fact that the webcam sensor is smaller (about 140 μm X 180 μm for 4 mm half ball objective lens, and 60 μm X 78 μm for 2 mm ball lens objective) in size than the CCD sensor, the field of view is smaller when we utilize webcam sensor as a detector in our deep ultraviolet microscope.

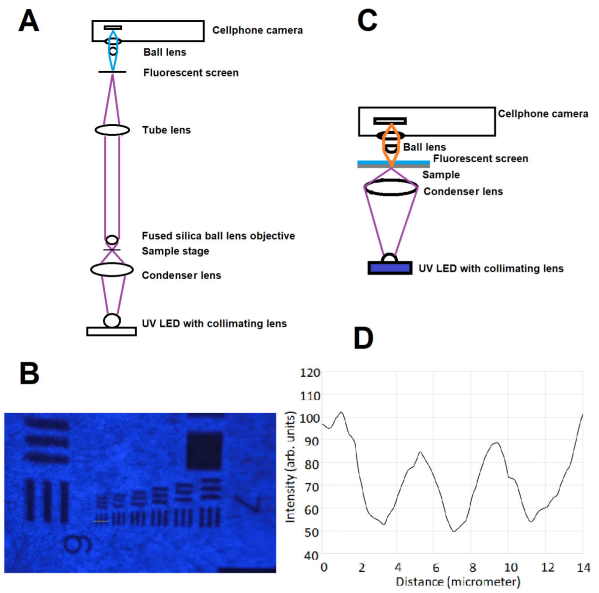

FIGURE 5. (A) Utilizing a visible light camera to record deep ultraviolet microscopic images by means of a fluorescent screen, (B) image of USAF 1951 resolution target obtained with this Microscope system at 275 nm. The fluorescent screen used was a piece of printer paper which fluoresce in blue with 275 nm excitation. Despite the paper texture blurring the image, we could still resolve all the lines in the group 7 of the resolution target. (C) a compact deep ultraviolet microscope system utilizing a cellphone camera and fluorescent screen. (D) shows the line profile of 6th element of the 7th group from (B).

C. CAPTURING DEEP UV MICROSCOPIC IMAGES ON PHOTOGRAPHIC PAPER

Conventional black and white photographic papers/film possesses high sensitivity in the deep UV region of the spectrum. Use of these photographic papers provide an affordable and convenient means for capturing deep UV microscopic images. In order to enable our system to capture deep UV microscopic images on a photographic paper or film, we modified a kodak brownie box camera by removing its lens and adding an attachment which slides into the microscope tube thereby attaching the camera firmly onto the body of the microscope. Subsequently, we focused the image by placing a fluorescent screen on the film plane. Before the image is captured, the fluorescent screen is replaced by photographic paper (Arista Edu Ultra VC RC) or film, under red light illumination, to prevent accidental exposure of the photographic paper. After loading the paper, we recorded the image by operating the shutter in conventional manner and exposing the paper for about 25 milliseconds. The exposed paper was developed in conventional black and white developer (Kodak dektol) and fixed in a hypo (sodium thiosulphate) solution. The image of the fused silica USAF1951 resolution target captured on a photographic paper is shown in Figure 4. The negative picture was scanned at 1200 dpi resolution using a hp 4630 scanner and inverted in GIMP software. Despite some reduction in the contrast, it is possible to clearly resolve all the elements of the seventh group of the test target. The reduction in contrast is attributed to the scanning artifacts and small amount of camera shaking during manual operation of the camera shutter.

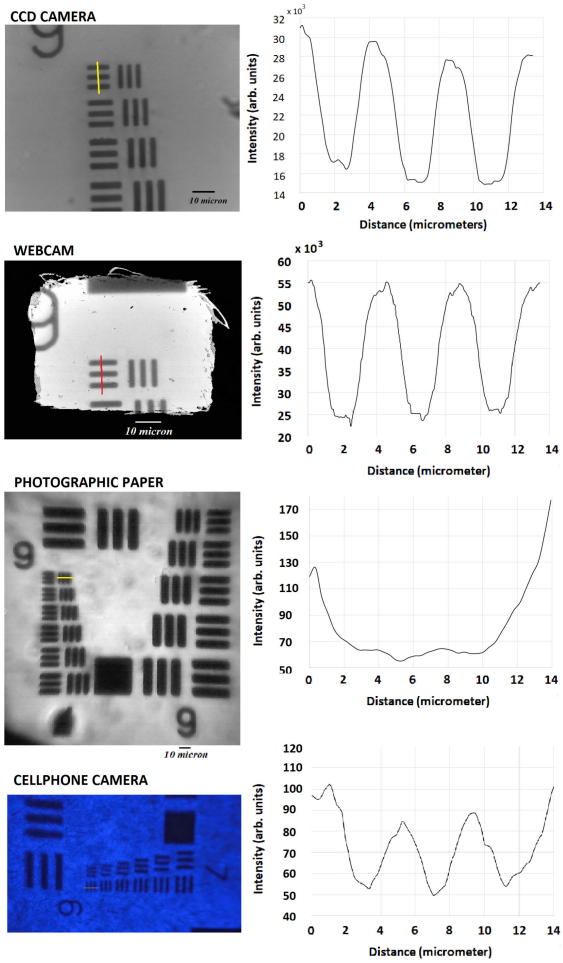

FIGURE 6. A comparison of the images and line profiles of the USAF 1951 target (6th element of the 7th group) captured using different imaging techniques. The reasons for reduction in the reduction of the resolution for the images captured with cellphone camera and photographic paper are explained in the text.

D. DEEP ULTRAVIOLET MICROSCOPE USING A CELLPHONE CAMERA

It is also possible to capture deep ultraviolet microscopic images using an unmodified cellphone camera. In order to achieve this, a UV fluorescent screen was used to convert the deep ultraviolet image to visible image at the image plane, which subsequently, is imaged, either with a normal camera & lens arrangement, or with a cellphone camera (Figure 5 A). A deep ultraviolet microscopic image of USAF 1951 resolution test target recorded with this arrangement is shown in Figure 5 B. The fluorescent screen used for capturing this image was a piece of white printer paper which fluoresces under UV light. Despite blurring of the image due to paper texture, we resolved all the lines in the 7th group of the test target. For this capture, the tube lens was removed to obtain higher magnification, at the image plane, in order to reduce the effect of the paper texture. Another alternative, for fluorescent screen could be a ZnS:Ag screen, which fluoresces blue under UV light illumination. Such screens are typically used to image scintillations from alpha particles. Even though texture of the fluorescent screen affects the resolution of deep

UV microscopic images, there are several fluorescent screens available (such as those used for X-ray imaging, or CRT screens) which have much less texture because they are coated on smooth surfaces. By using them we can practically eliminate the resolution limits due to screen texture. This system can be made compact by attaching a half-ball lens directly in front of the cellphone camera and sandwiching the sample plane with the fluorescent screen (Figure 5 C).

E. DEEP ULTRAVIOLET MICROSCOPE IMAGING WITH CYANOTYPE COATED PAPER

Another interesting and useful means for recording deep ultraviolet microscopic images with our microscope can be achieved by using a paper coated with cyanotype chemicals (Potassium Ferricyanide and Ferrous Ammonium Citrate). Cyanotype chemicals have high sensitivity to deep ultraviolet light. Use of such paper media, to record deep ultraviolet microscopic images may be easily utilized for teaching due to the very low cost of these chemicals and ease of processing, which, simply, involves washing the paper under water after exposure to the UV light. Cyanotype deep ultraviolet images can be made by using contact printing techniques, where the sample is placed on a UV transmissive glass plate. The sample side is placed in contact with the cyanotype paper while the sample is illuminated by ultraviolet light from the other side.

IV. CONCLUSION

In this article, we describe the design, construction and operation of compact inexpensive microscope instruments that has enable us to record deep ultraviolet sub micrometer images utilizing relatively widely available and inexpensive components. Such compact ultraviolet microscopes may find multiple applications, ranging from biomolecular spectra to identification & quantitation of biological species and molecules at ultra-low volumes, concentrations, and of micron sizes for the study of protein crystals, lignins to high contrast biological cells, molecules and semiconductor devices observations. Our designed microscope performs microscopic imaging in the transmitted light, however, it could be modified to operate under reflected or angled illumination [18], [19]. Reflected and angled illuminations are usually utilized for recording ultraviolet light induced fluorescence emissions from biological samples.

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CONFLICT OF INTERESTS

Authors declare no conflicts of interests.

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