A digital holographic microscope educational kit for educators and students of holography

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ABSTRACT

Digital holographic microscopy (DHM) has become a powerful diagnostics tool for the sciences, especially biology. In addition, DHM has great potential for use in education because it is so flexible and affordable to schools and students. For many years, the cost and complexity of producing and using holograms limited their use to a narrow range of industry and science. Digital holography has removed these limitations making holography affordable and available to anyone who has a laptop computer. Affordable digital holocameras entering the market can enable teachers and students to produce and view their own holograms, reconstruct and view three dimensional images, and better understand and use holography in research.

This paper describes such a system including exercises and experiments that explain how holograms are made and how they are used to record and analyze dynamic events that take place in three dimensions. MetroLaser's "Holoscope", is a lensless, DHM that produces holographic videos of microscopic objects distributed in a relatively large volume. Each frame in the video is a digital hologram that encodes 3D images that can be focused and viewed, plane by plane in detail. The three-dimensional images encoded in the holograms are electronically reconstructed, scanned, and viewed with sharp images coming in and out of focus on a computer monitor as we scan through the volume. By providing micrometer resolution throughout a cubic centimeter volume, the system effectively freezes time at the moment each frame was recorded and enables precisely tracking the 3D movement of microscopic objects in space and time.

The ability to view each hologram in real-time, shows directly how interference between an object and reference wave produces interference fringes that become the hologram.

Software provided with the system enables the viewer to automatically perform all of the necessary operations from hologram recording to 3D wavefront reconstruction and plane by plane imaging. We also describe more advanced and more expensive DHMs with even higher resolution that are now also available for scientific research.

Keywords; Digital holography, microscopy, education,

1. INTRODUCTION

Present day availability of high-resolution, digital, video cameras have opened the path to producing thousands of holograms (video DHM) of a volume and its contents in a short period of time, where each recorded video frame is a digital hologram. Each hologram contains high resolution 3D images of objects in a relatively large volume at the instant the frame was recorded. When such a volume contain hundreds of bacteria, the holograms provide huge quantities of data in digital form, which is ideal for high-speed image processing and the use of machine learning.

During the past five years, MetroLaser developed advanced digital holographic microscopy (DHM) technology and the Holoscope for applications in biology, specifically to understand complex, dynamic, 3-dimensional interactions and complex movements of bacteria and plant roots in the rhizosphere. Understanding such interactions is critical to the field of biology to optimize growing conditions and soil health for the efficient production of food and biofuels. Figure 1 includes the digital, in-line hologram of a plant root tip and two images of root hairs reconstructed at different depths in the volume, separated by a few hundred microns.

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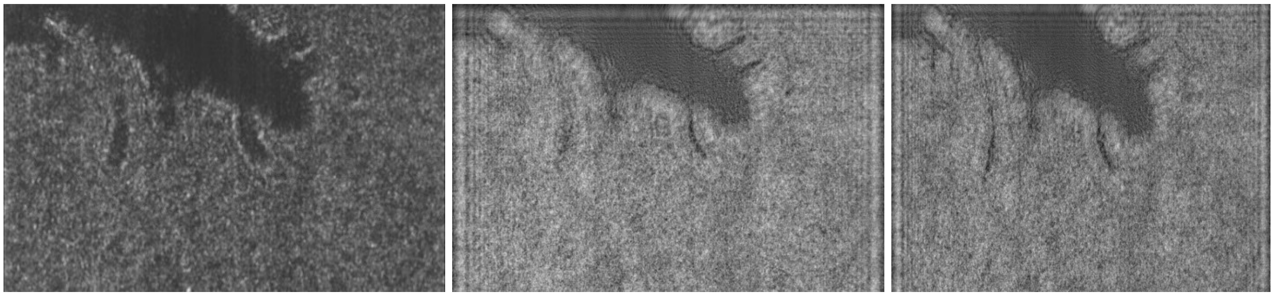


(a) (b) (c)

Figure 1-In-line hologram of a root tip and images reconstructed at different depths: (a) Hologram of a plant root tip; (b) reconstructed image of root hair; (c) reconstructed image of root hair at different depth.

In the study mentioned above, we demonstrated that digital holographic microscopy can provide detailed, high resolution tracking of bacteria as they respond to plant attractants (chemotaxis), provide nutrients needed by the plant to grow efficiently, and interact with competing bacteria. The individual bacteria could be identified by their unique swimming patterns. Such microscopic studies have previously been extremely limited by conventional two-dimensional microscopy. While demonstrating the Holoscope as a powerful tool for plant biology, we recognized that it also has exciting potential as an affordable teaching tool for the field of holography, because it can provide real-time, visible, easy-to-understand examples of each of the steps in holography, including the diffraction and interference processes employed in recording holograms and performing wavefront reconstruction. .

Unlike traditional/analog holography, which involves photographic recording and processing, expensive optical components, high stability tables, and complicated setups, digital holography transfers much of the process to relatively inexpensive electronics and software. Not only is such a digital holocamera much less costly, holograms are immediately available for viewing and reconstructing 3D images that can be scanned and viewed with a laptop computer.

Many excellent books on fundamentals of holography are available to provide an academic understanding of holography and its history. One of the mostly highly recommended books is the classic Practical Holography by Saxby[i]. However, a student cannot truly comprehend and “own” holography until he has produced his own hologram, viewed it directly, and used it to reconstruct and view a true 3D image with the hologram. Holoscope makes that possible for any student who truly wants to fully comprehend holography. This paper describes the use of Holoscope to convey a broad and thorough understanding of holography, what it is, how it is done, and what are its uses.

We begin by using Holoscope to demonstrate answers to the following questions.

1. What is a hologram?
2. How are holograms made?
3. How do holograms generate 3D images?
4. How are holograms used?

What is a hologram and how are holograms made?

First, we narrow this definition to “optical hologram”, since holograms can record any type of wave, including acoustical and non-optical waves. An optical hologram is a recording of the information contained in light waves that have passed through or reflected from objects that were illuminated by a beam of light. Figure 2 shows such an illuminated object as it scatters light in the direction of the hologram. This scattered light, the “object wave”, is mixed with a “reference wave”, which interferes with it to form fringes (variations in intensity), known as a diffraction pattern. (Holography is conducted with laser illumination, which is coherent light, since coherent illumination makes interference possible. Mixing light from a standard light bulb does not result in interference fringes.) The recording of the diffraction pattern, produced by mixing an object and reference wave, is a hologram of the object.

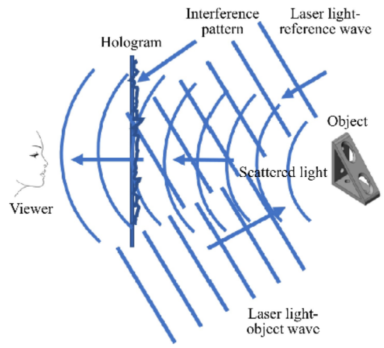


Figure 2. Recording a hologram of an object. [ii]

The diffraction pattern contains enough information about the object wave to enable reconstructing it in full detail, replicating the original object wave. If the hologram is then illuminated again by the reference wave, as in Figure 3, the diffraction pattern in the hologram will, through the process of diffraction, convert some of it into a wave that is identical to the original object wave that was used to produce it, so that what the viewer sees is that same as what was seen when the object was present.

The hologram that was just described is called an "off-axis" hologram, since the object wave and reference wave come from two different directions. If an object field is sufficiently transparent, such as a volume containing particles, holograms can be produced with a single optical wave passing through the volume with a small amount of it being scattered by the particles and the unscattered light serving as a reference wave, which then mixes with the scattered light to form the diffraction pattern that becomes the hologram. This process is called "in-line" holography, since the object and reference waves come from the same direction and overlap. In-line holography is the simplest kind of holography, since it can be done with a single beam of light.

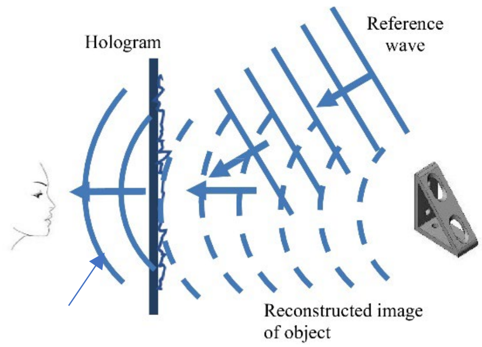


Figure 3.Reconstructing the original object wave. [ii]

Holoscope employs "in-line" holography. Figure 4 illustrates the complete recording and reconstruction process of the Holoscope in recording and reconstructing a three-dimensional image of a volume containing particles. In this figure, a laser beam is passed through a volume that contains three particles. The resulting diffraction pattern is recorded on a digital video camera and is displayed on the computer monitor as shown. Each circular diffraction pattern shown in this image is an in-line hologram of one of the three particles in the volume.

If this image were recorded on a transparent film, we could illuminate it with a reference wave and three images would appear in their original relative positions in space. In digital holography, the information is recorded in digital form and the reconstruction process is completed in computer memory, where the diffraction and propagation equations are used to simulate reconstructing the wavefront and viewing it plane-by-plane to see where the particles come into focus. In the reconstructed image, the top particle focuses first as we scan through the various planes from 9.5 cm to 12.2 cm. After the scan is completed, the particle positions in space are shown in the bottom-left 3D diagram.

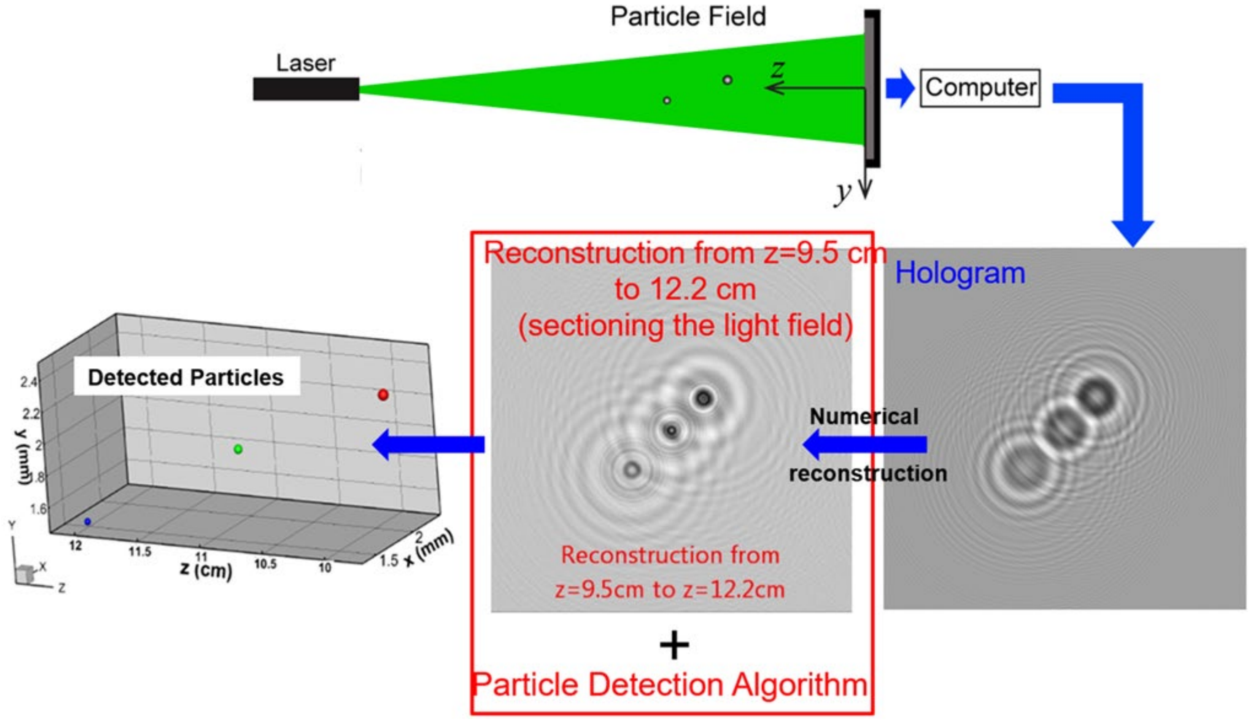


Figure 4. Recording and image reconstruction process in the Holoscope

Figure 5 shows the Holoscope with microscope slides and a cuvette, which are provided to contain the objects of interest that will be recorded in holograms.

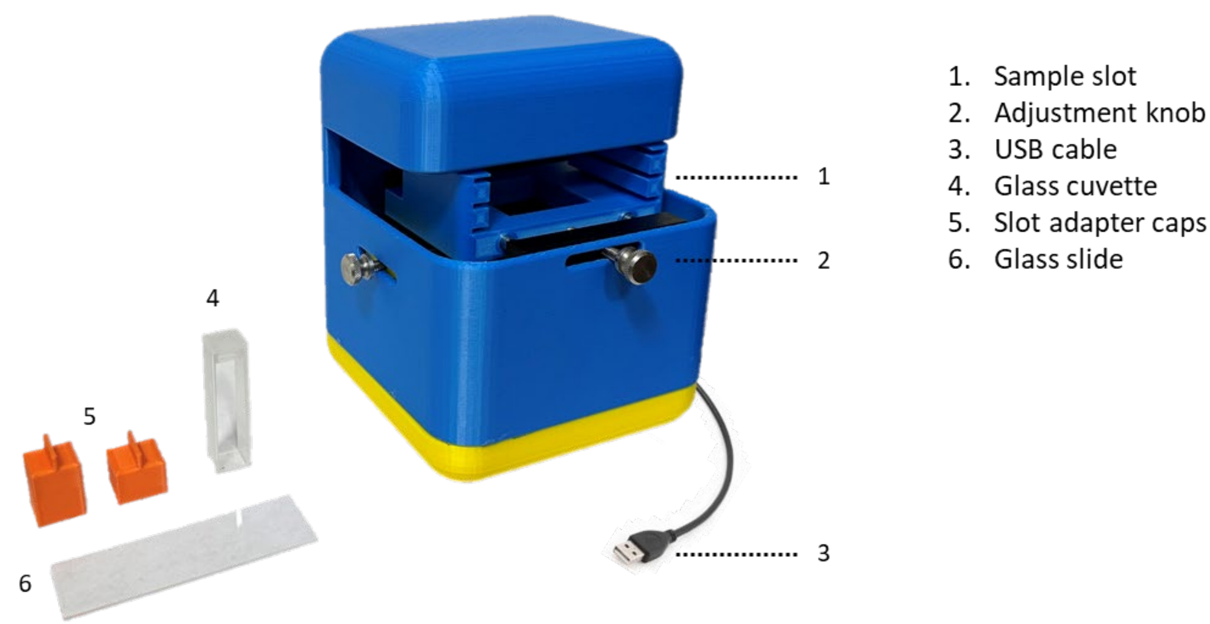


Figure 5-Holoscope with accessories

Holoscope set up

Figure 6 illustrates the Holoscope set up procedure, which begins by attaching to a computer, which provides power to the laser and the camera inside the Holoscope.

The sample under study is inserted into the Holoscope sample slot either on a microscope slide or in a solution in the cuvette and centered in the field of view. For cuvettes, adapter caps are provided to slide the cuvette into one of the slots. The sample position can be viewed on the monitor to assist with alignment.

Adjustment knobs on the front and side are provided to move the sample to the center of the monitor. When the sample is in position, the software provided with the Holoscope enable an operator to record the hologram and reconstruct and focus on images.

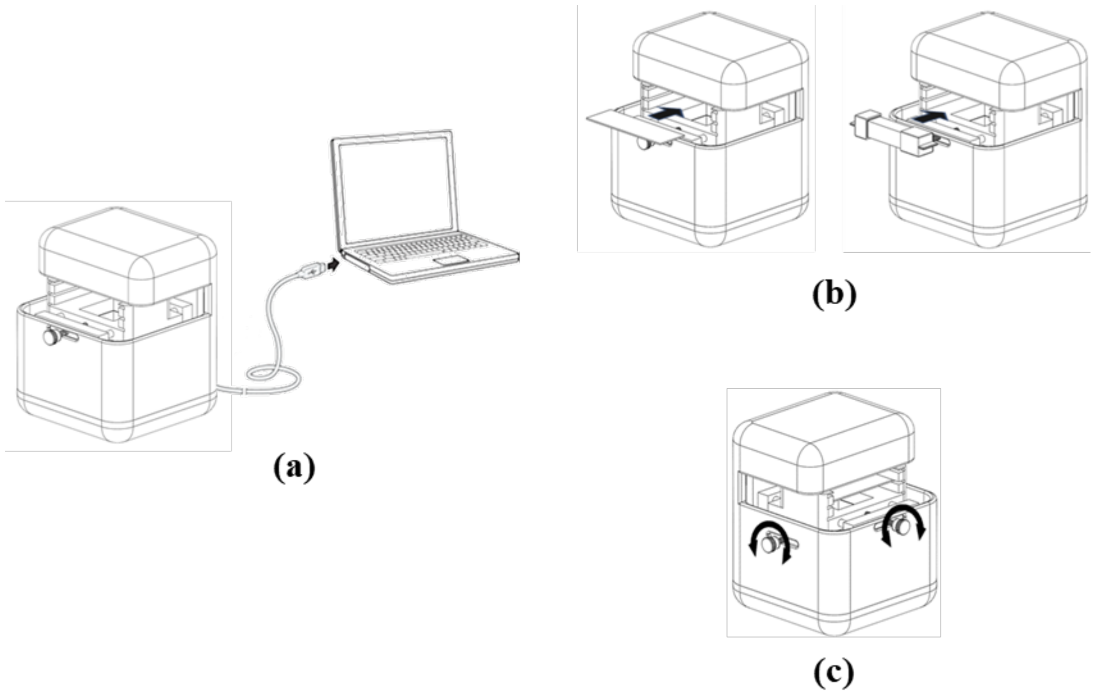


Figure 6. Holoscope set up with a sample inserted: (a) Connecting Holoscope to a computer; (b) inserting sample into the Holoscope slots on a microscope slide or in a cuvette; (c) rotating adjustment knobs to move the sample.

The Holoscope features three slots for sample mounting shown in Figure 7. Slot 1 (top) is the closest to the image sensor, providing the smallest magnification, while Slot 3 (bottom), located furthest from the sensor yet closest to the light source, offers the greatest magnification in the resulting image.

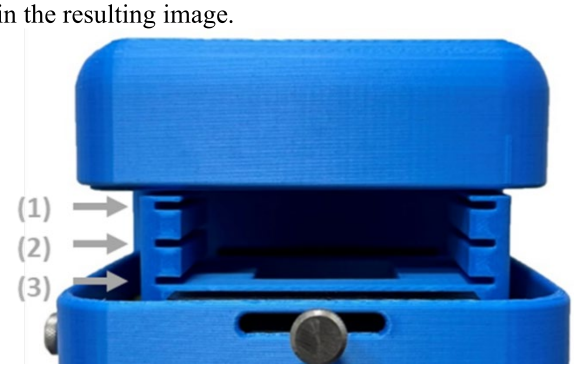
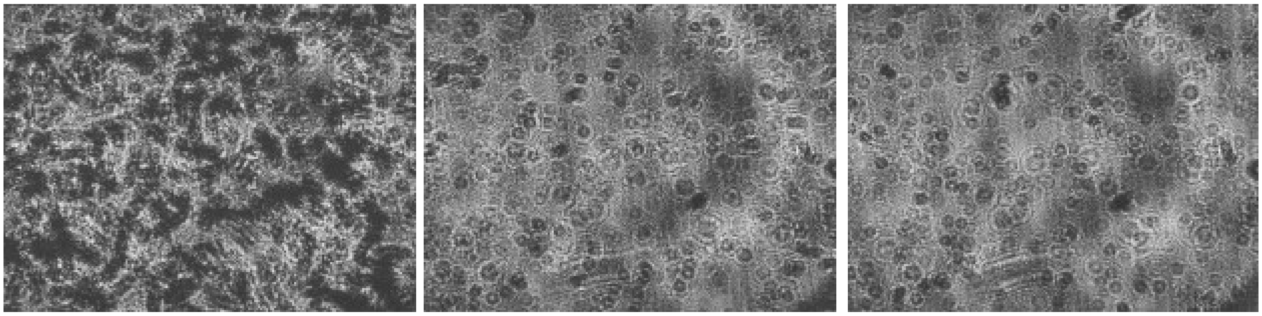


Figure 7. Three available slots for inserting a microscope slide or a cuvette.

In the following discussion, we illustrate Holoscope examining a 3D volume of particles. For this example, we created a 3D scene, a volume of water in the cuvette, dropped some chalk dust into the water, and recorded video holograms of the dust slowly settling to the bottom. The holograms can be used to view the dynamics of these particles and their three-dimensional position over time by reconstructing their focused images at different propagation depths and times.
Holograms of the water and dust particle mixture were captured at three different times, shown in Figure 8, immediately after shaking the sample, 5 minutes later, and 10 minutes later, to illustrate how particle density affects the appearance and quality of the hologram and focused images.

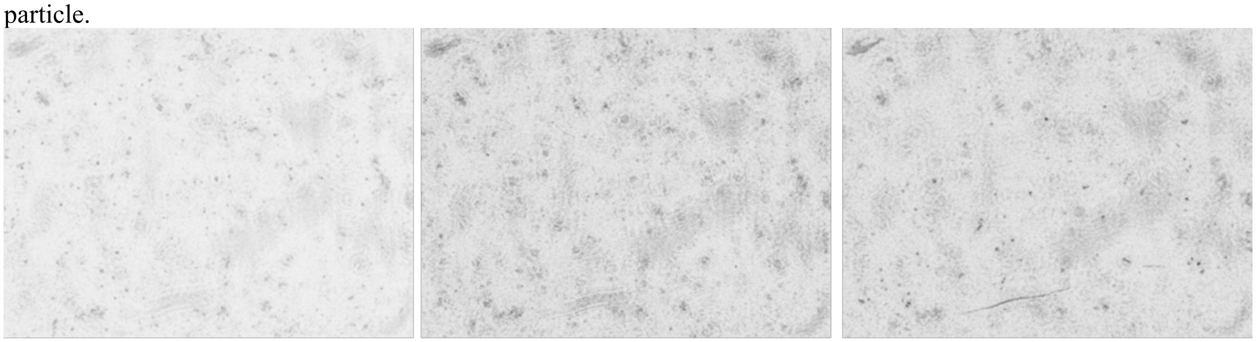
The initial hologram displays densely packed overlapping diffraction patterns. As time passes and the particles settle, holograms from later intervals show clearer and more distinct diffraction patternsdue to a lower particle density.



(a) (b) (c)

Figure 8. *In-line h*olograms of dust particles suspended in water; (a) t = 0 mins; (b) t = 5 mins; (c) t = 10 mins.

Figure 9 shows the reconstructed and focused images produced, using the Holoscope's software, from one of the holograms, at three different depths, and at one instant in time in the cuvette. A particle image that is in focus in one of the planes will be out of focus in other planes and can be observed as a diffraction pattern in the out-of-focus plane for that particle.



(a) (b) (c)

Figure 9. Reconstructed images of the dust particles in water with different propagation depth; (a) 12.0 mm, (b) 16.5 mm, (c) 21.0 mm.

Holograms of Insects

We show here examples of holograms of insects to examine their intriguing shapes and movements in 3D. We placed a small caterpillar on a slide, inserted the slide in the middle sample slot of the Holoscope and recorded the hologram shown in Figure 10a. The hologram was then used to reconstruct and scan the 3D image recorded in the hologram. Figure 10b shows the image focused on the caterpillar legs. In this image plane, we can also see that particles that were on the slide coming into focus also, since they are in a plane that is close to that where the legs focus.

The adjustment knobs are used to center the caterpillar within the image frame, ensuring optimal viewing. The caterpillar's movements can be observed in real-time on the preview monitor in the software interface.
Insects have many interesting microscopic features that require 3D scanning, which are more difficult to analyze with conventional microscopy. DHM enables recording and tracking of the relative movements with high resolution of such parts over time, which is difficult to achieve with conventional microscopy. Figure 11 shows reconstructed images of an aphid and a mosquito.

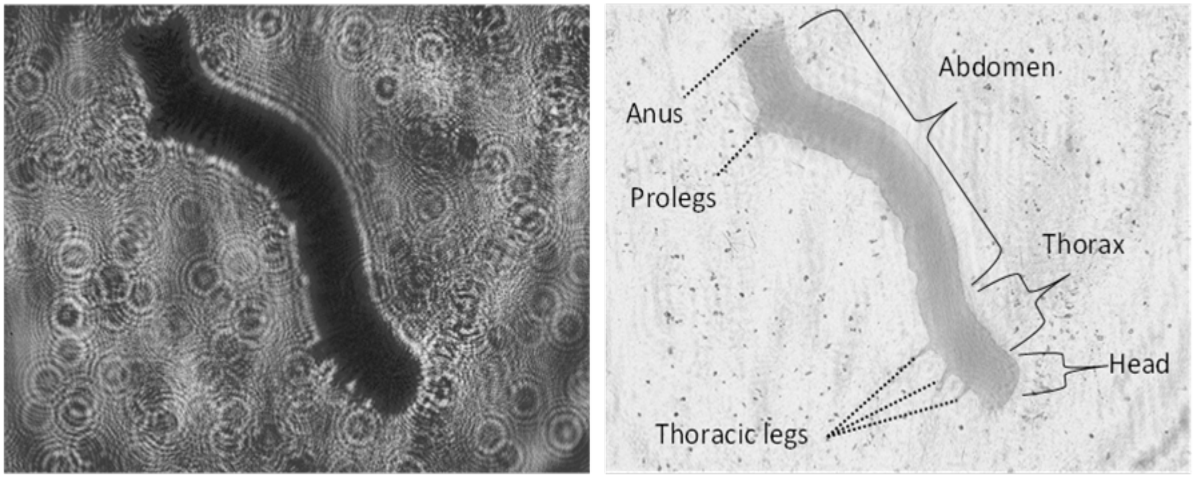


Figure 10. Hologram and a reconstructed image of a caterpillar: (a) Hologram of caterpillar and (b) reconstructed image focused on the legs.

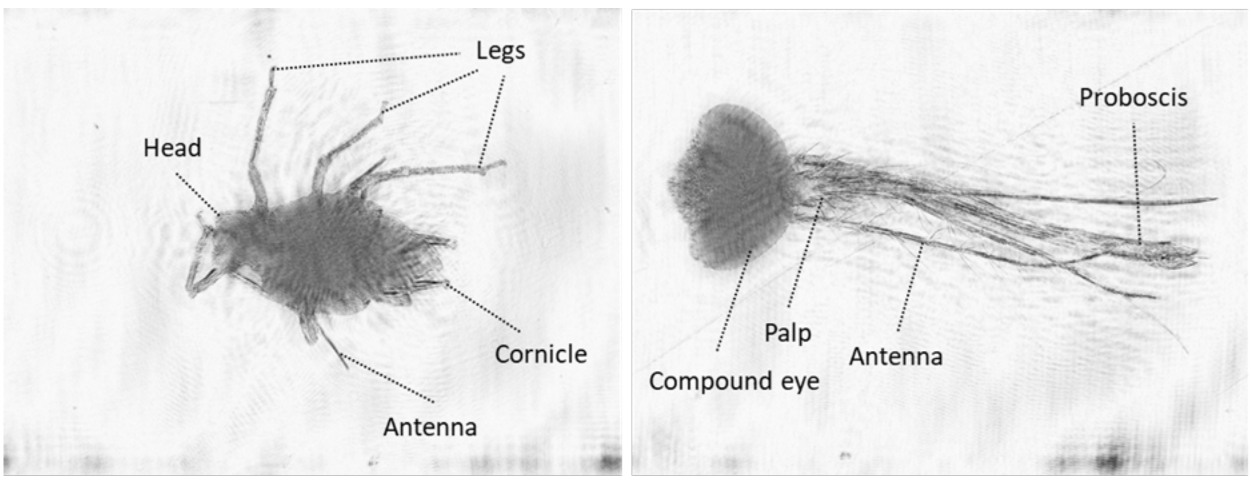


Figure 11. Reconstructed images of an aphid and a mosquito: (a) Reconstructed image of an aphid and (b) reconstructed image of a mosquito's head.

The legs of the aphid are clearly visible, and closer focusing reveals the small tube-like cornicles near the posterior end of the aphid's body. When aphids are threatened by predators, they release a defensive fluid through these cornicles that contains chemicals that deter or repel attackers. Looking at mosquito's head under the microscope, its eyes, palps, and a needle-like fiber called a proboscis are clearly displayed. Mosquitoes use their long antennae to sense things around them. The proboscis is like a needle they use for feeding. They also have palps near the needle that guide it to the blood vessels. These palps help the mosquito find a good spot to feed.

2. CONCLUSION

The foregoing examples of microscopic 3D viewing using the Holoscope were presented to demonstrate its effectiveness as a valuable tool for exploring and understanding the anatomy of insects and the movement of bacteria in 3D over time. The ability to access and see the holograms as they are recorded, the diffraction patterns that contain the 3D information, and reconstructed images as they come in and out of focus in a reconstruction process can be valuable in helping students understand how holography works and how it is used. We conclude that the Holoscope can have a real value as an affordable teaching tool for the field of holography.

REFERENCES

[i] Practical Holography, Graham Saxby and Stanislovas Zacharovas, textbook 4th Edition, CRC Press, ISBN 9781482251579 (2016)

[ii] James D. Trolinger. The language of holography[J]. *Light: Advanced Manufacturing* **2**, 34(2021). doi: 10.37188/lam.2021.034