MetroLaser, Inc.

Application Note: Lensless Digital Holographic Microscope for Measuring various samples on a Glass Slide and Cuvette

MetroLaser's Holoscope is a Lensless Digital Holographic Microscope (LDHM) that uses cutting-edge imaging technology to let us see things in 3D without needing special labels or dyes. It's the go-to tool for scientists studying microscopic stuff. In this note, we're going to explore how the Holoscope helps measure samples on a glass slide or in cuvettes and how it creates holograms and 3D images.

For this investigation, you'll need clean glass slides or cuvettes to hold your samples and the Holoscope, which comes with a laser, image sensor, and software package. To get started, prepare your samples by putting them on the glass slide or in a cuvette. Then, place them in one of the Holoscope's slots for accurate optical alignment.

The Holoscope features three slots for sample mounting. 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 1. The face of the Holoscope, showing the three slots for sample mounting.

Exploring Hair with the Holoscope

Introduction:

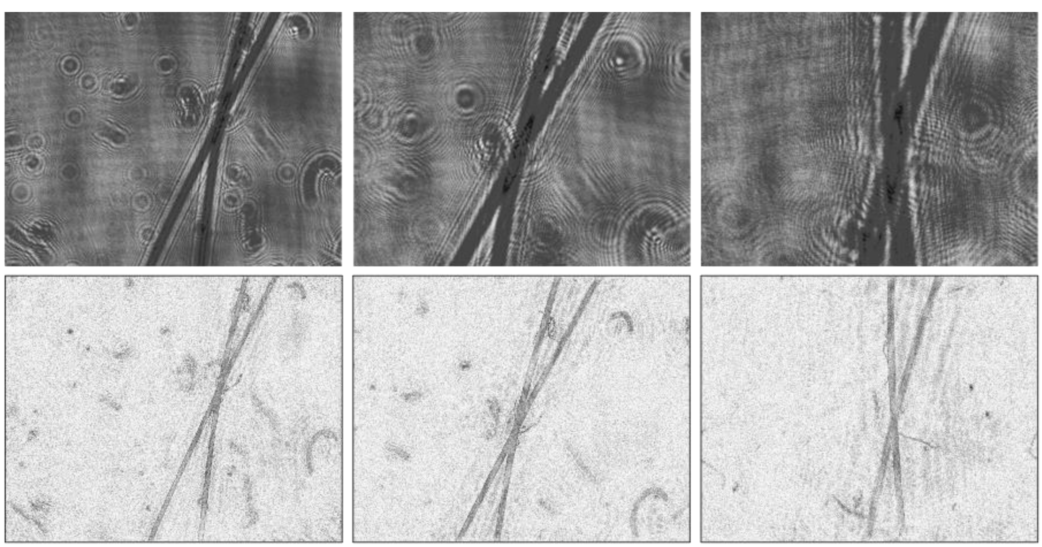
Our goal in this experiment is to investigate the capabilities of the Holoscope, a Lensless Digital Holographic Microscope (DHM), in capturing and reconstructing detailed images of hair strands on a glass slide. We are about to explore how magnification levels vary with the positioning of the hair sample.

Materials and Methods:

The experiment begins by carefully placing hair strands on a glass slide. This slide can be inserted into one of the Holoscope's slots. Using the knobs on the side of the Holoscope, the lateral position of the hair sample is adjusted. The adjustment process is captivating, observable through the live hologram displayed on the preview monitor. Holograms of the hair are recorded and electronically focused on a PC using the provided software package. The software allows us to play around with the depth by choosing top, middle, or bottom for the slot, and the coarse and fine sliders are used to facilitate further fine-tuning until we get sharp and clear images on the reconstructed image panel. The depths we figured out are 5.5 mm, 18.2 mm, and 30.0 mm for holograms captured from the sample in the top, middle, and bottom slots, respectively.

Result:

Microscope images of the hair varies in magnification due to the different heights of the slots. The hologram is formed through the interference between the hair and the laser light. So, the farther the hair is from the image sensor, the more magnified it looks. That's why the bottom slot shows the highest magnifications, while the top slot gives us the least magnifications.



(a) (b) (c)

Figure 2. Holograms (top row) and reconstructed images (bottom row) of hair at different sample slots; (a) top slot; (b) middle slot; (c) bottom slot

Conclusion:

Our experiment with the Holoscope provides valuable insights into the holographic recording process and magnification variations. Microscope images at different magnifications unveils a direct correlation between distance from the image sensor and magnification, with the bottom slot presenting the highest magnifications and the top slot offering the least.

Exploring Caterpillars with the Holoscope

Introduction:

Our goal in this experiment is to closely observe a live caterpillar using the Holoscope. Caterpillars, with their intriguing shapes and movements, offer a captivating subject for microscopic exploration. By capturing their holograms and turning them into microscope images, we aim to explore the intricate details of the caterpillar's movement and structure.

Materials and Methods:

A live caterpillar is picked up and carefully placed on a glass slide to provide a closer look under the holographic microscope. The glass slide is inserted into the middle slot of the Holoscope. The adjustment knobs are used to center the caterpillar within the image frame, ensuring optimal viewing. The caterpillar's movements are observed in real-time on the preview monitor in the software interface. The hologram of the caterpillar is captured using the Holoscope, and transformed into a microscope image using the software, allowing us to observe the caterpillar's features in detail.

Results:

Qualitative observations highlight the effectiveness of the Holoscope in revealing intricate details of the caterpillar. The caterpillar's shape is very clear, and its legs and other components are easily distinguishable. Caterpillar is anotomically divided into three main parts: the head, thorax, and abdomen. The thoracic legs on the thorax facilitate walking, while prolegs on the abdomen’s segments assist in gripping surfaces as the caterpillar moves. The live observation through the Holoscope provides a genuinely cool experience. What's even more exciting is that since the Holoscope can measure holograms in a series, we can create a 3D video of the caterpillar in motion. Imagine watching the caterpillar moving around in 3D.

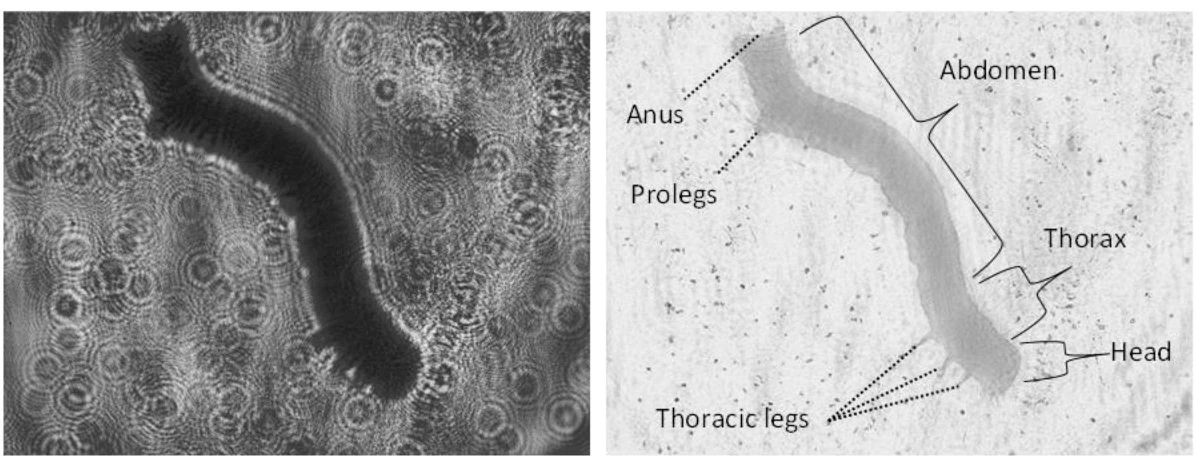


Figure 3. Hologram and reconstructed images of the caterpillar; (left) hologram; (right) reconstructed image with each component labeled.

Conclusion:

This experiment exhibits the Holoscope's potential in offering detailed insights into the world of live organisms. The clear visualization of the caterpillar's structure and the prospect of creating a 3D video underscore the exciting applications of advanced microscopy techniques.

A Close-Up Look at Insects

Introduction:

The goal of this experiment is to explore the microscopic world of commonly encountered insects, such as ticks, ants, and others, using the Holoscope. Insects are fascinating creatures with interesting structures. In this experiment, we aim to utilize the Holoscope to measure and reconstruct insects and gain a detailed understanding of their structures.

Materials and Methods:

Microscope slides featuring a variety of insects are prepared for measurement (Don't Bug Me Slide Set, 293519, Carolina). The set of slides has various insects ready for microscopic analysis. They are placed in the middle slot of the Holoscope. To ensure accurate measurements, the samples are precisely located using the preview window within the Holoscope's software interface. Holograms of the insect samples are captured and subsequently reconstructed using the software. The propagation depth is adjusted until sharp and clear reconstructed images are achieved. The determined propagation depth for the holograms captured from the middle slot is measured at 17.3 mm.

Results:

The use of the Holoscope, along with the provided software, results in a super close-up view of the insects. These images show each part of the bugs with fine resolution, allowing for precise examination. Particularly noteworthy is the ability to easily distinguish various components of aphids and the mosquito head in the images, highlighting the effectiveness of the holographic microscope system in capturing intricate details.

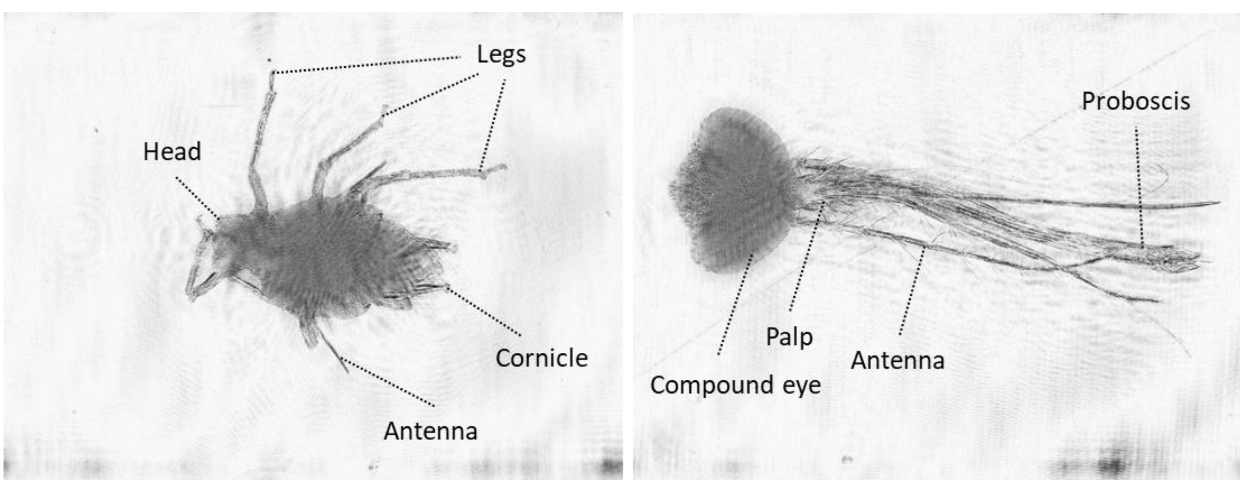


Figure 4. Reconstructed images of bugs on glass slides, where each component is labeled; (left) aphids and (right) mosquito head.

Every leg on aphid is easy to see, and there are also these small tube-like things called cornicles found near the posterior end of the aphid's body. When aphids are threatened by predators, they release a defensive fluid through these cornicles. This fluid contains chemicals that deter or repel attackers. Looking at mosquito's head under the microscope, its eyes, palps, and a needle-like thing 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.

Conclusion:

The experiment with the Holoscope demonstrates its effectiveness in providing a detailed and high-resolution view of commonly encountered insects. The images reveal the structural details of the insects such as aphids and mosquito heads. The Holoscope, with its innovative holographic microscopy system, proved to be a valuable tool for exploring and understanding the anatomy of insects.

Exploring Pond Microorganisms

Introduction:

Pond ecosystems are vibrant habitats hosting diverse microorganisms like copepods and worms. In this study, we aim to provide a qualitative understanding of these organisms through holographic and microscopic examinations, giving us a peek into the biodiversity of pond environments.

Materials and Methods:

Live organisms are carefully collected from a local pond. Glass cuvette is utilized as a container for the live organisms. Using adapter caps, the cuvette is securely inserted sideways into one of the slots of the Holoscope. The adjustment knobs are used to locate and center an organism within the image frame. This step is crucial to ensure precise observations. Afterward, with the help of the software tool, holograms of the live organisms are captured, which were then reconstructed to enable deeper observations.

Results:

The initial findings of this study show the biodiversity of pond microorganisms. Copepods and worms are successfully identified, and their movements are observed by looking at live holograms on the preview monitor. A series of holograms can be captured and recreated to microscope images, and then put them together in a video, which can visualize the cool movements of these organisms.

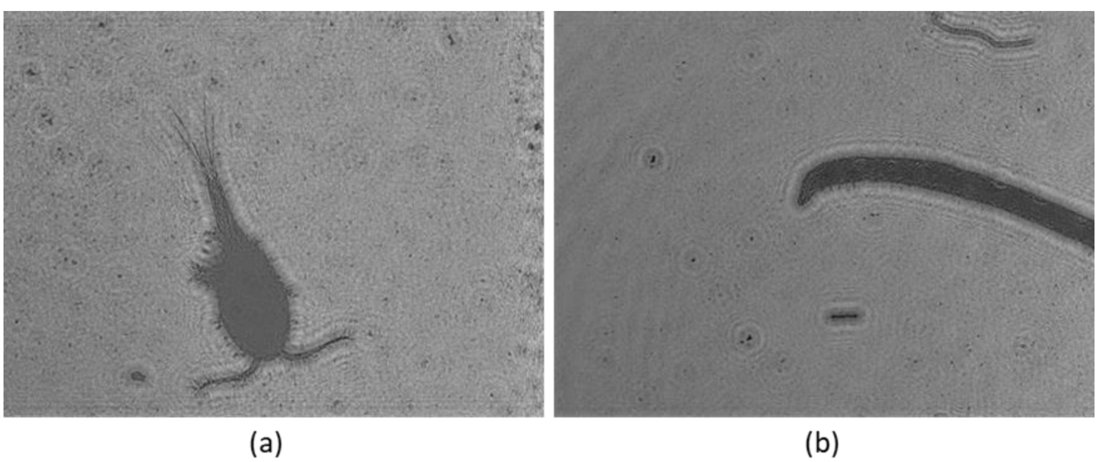


Figure 5. Reconstructed images of organisms in the pond; (a) copepod and (b) worm

Conclusion:

This research shows that the Holoscope can help explore the diversity of pond ecosystems through advance microscopic techniques. The holographic and microscopic examinations have opened a window into the world of tiny organisms, providing qualitative insights into their structures and behaviors.

A Three-Dimensional Exploration with the Holoscope

Introduction:

This study is about measuring dust particles suspended in water, utilizing the Holoscope to provide a three-dimensional analysis of particle density and position over time. By using the Holoscope, not only the dynamics of these particles but also three-dimensional position of the particles can be obtained by reconstructing the hologram at different propagation depths.

Materials and Methods:

A glass cuvette, filled with water and dust particles, is measured to track how the particles changed over time. Holograms of the water and dust particle mixture are captured at three different times: right after shaking the sample, 5 minutes later, and 10 minutes later. This temporal approach illustrates how particle density affects the quality of the electronically focused microscope image. In this study, the hologram of the cuvette with dust particles is reconstructed at three different depths, revealing distinct positions and characteristics of particles at various depths.

Results:

The initial hologram displays densely packed particles, representing overlapping dust particles' holograms. As time passes and the particles settles, holograms from later intervals shows clearer and more distinct particles due to a lower particle density.

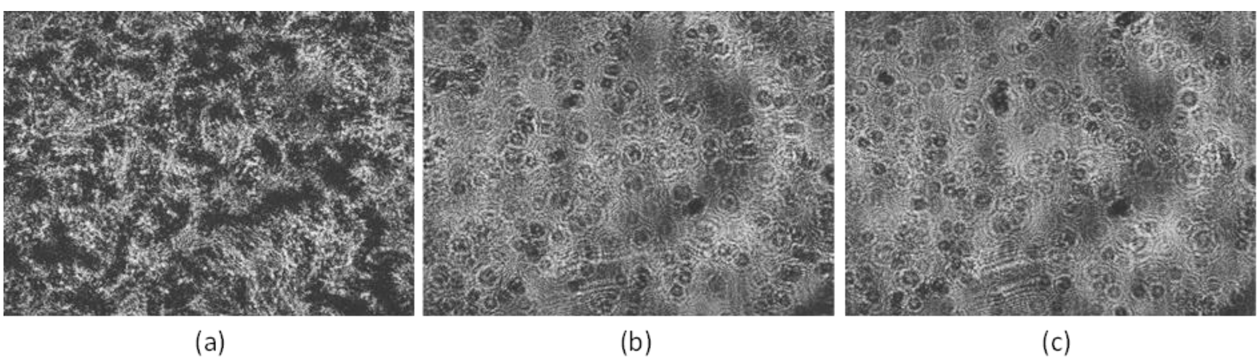


Figure 6. Holograms of dust particles suspended in water; (a) t = 0 mins; (b) t = 5 mins; (c) t = 10 mins.

The collected holograms are reconstructed using the Holoscope's software. As anticipated, the hologram featuring densely packed particles does not yield a clear microscope image. The challenge arises from overlapping particles, making it difficult to convert them into clear microscope images. This occurs because holographic microscopes need clear and distinct fringes for effective reconstruction. So, the second and third holograms, taken when the sample is less concentrated, produce better reconstruction with more clearly visible particles.

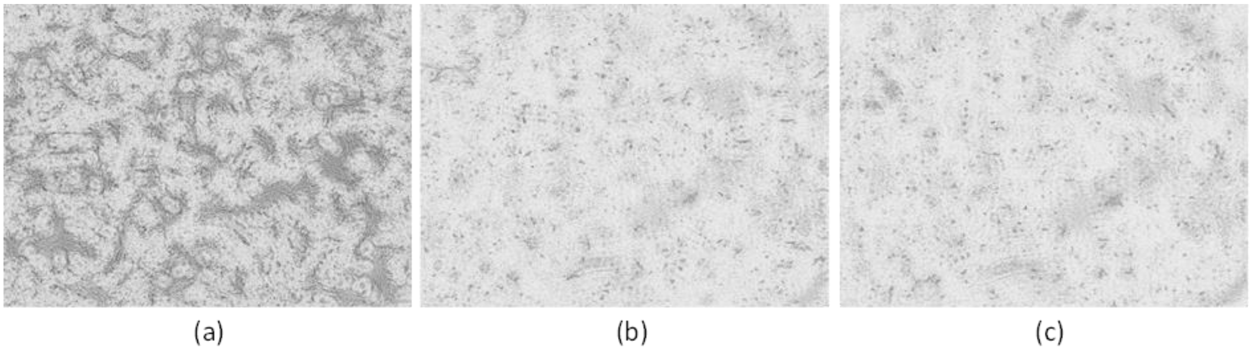


Figure 7. Reconstructed images of the dust particles in water; (a) t = 0 min; (b) t = 5 mins; (c) t = 10 mins.

Here's the cool part: a hologram contains three-dimensional info, and we can reconstruct it at different depths. Unlike regular microscopes that use lenses to focus, a digital holographic microscope does focusing electronically. The hologram of the cuvette with dust particles is reconstructed at three different depths, revealing different positions and characteristics of particles along the depth. Hence some particles can be detected at different depths.

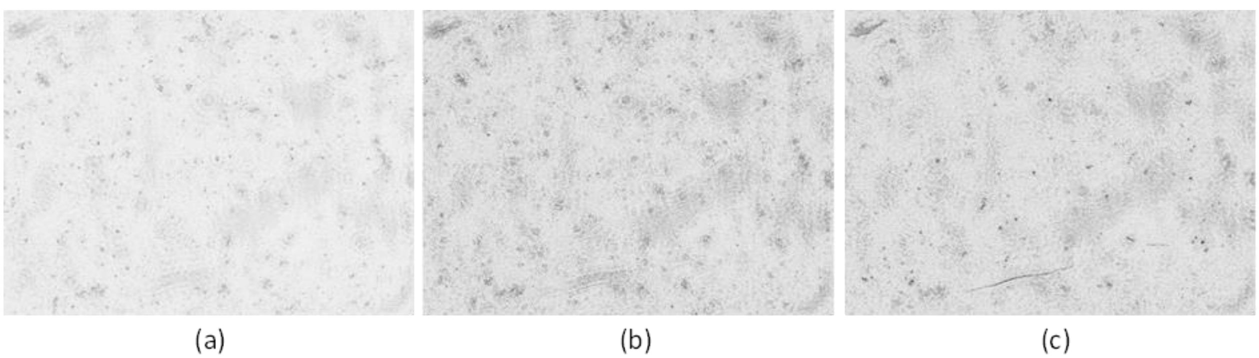


Figure 8. Reconstructed images of the dust particles in water with different propagation depth; (a) 12.0 mm, (b) 16.5 mm, (c) 21.0 mm.

Conclusion:

The study demonstrates the potential of the Holoscope in offering a dynamic and three-dimensional understanding of tiny particles in water. Capturing holograms at different intervals provides insights into how particle density affects the image quality of the holographic microscope. Furthermore, the Holoscope's capability to reconstruct holograms at various depths shows up its ability to provide three-dimensional information about particles.