WHAT'S THE BIOBUZZ?
A journal for students by students

Holographic Microscopes

Key Terms

Colloidal sphere:
A clear plastic ball created by the lab used in nanometer scale measurements.

Holographic microscope (H.M.): Type of microscope used to locate particles as small as droplets of fats in milk by looking at the way they alter light.

Interference:
When two different waves combine to create a new wave.

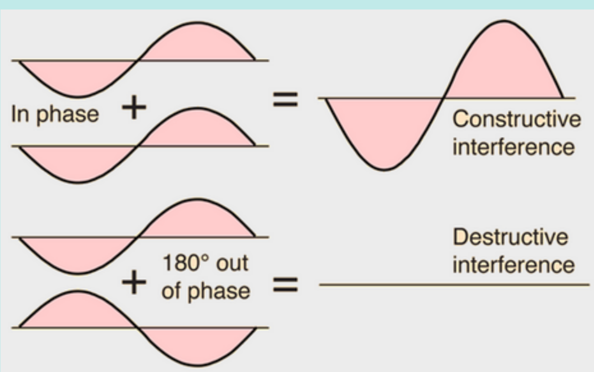
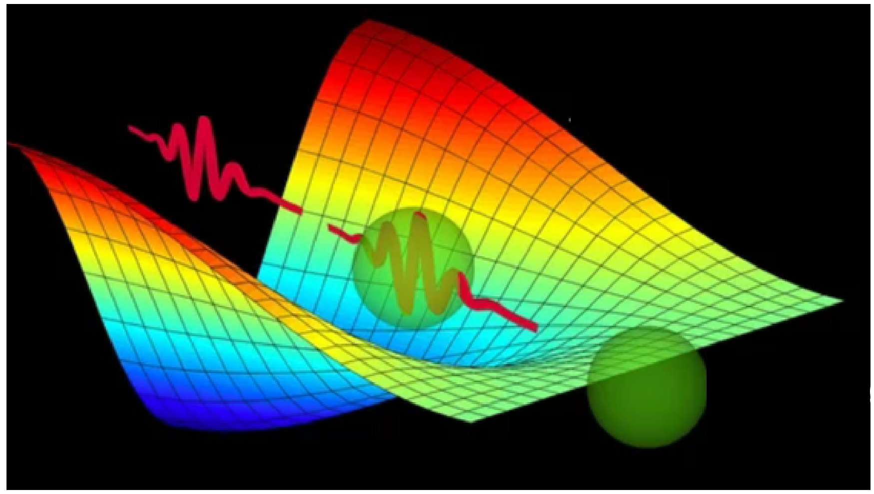


Figure 1. Image of destructive and constructive wave interference.

In Phase: When both peaks (highest point) and valleys (lowest point) in two different waves are aligned perfectly as in figure 1.

Out of Phase: When peaks and valleys of waves are not aligned as in the bottom of figure 1.

Trajectory: A pathway that a particle travels through in space over time.



OBJECTIVE

Solids, liquids, and gases have particles that are always in motion even though we cannot see them with our eyes alone. These particles move because everything has energy. The particle movement varies based on the state of matter and the amount of heat.

Physicists tried to understand this energy by studying individual particles and how those particles behave. The physicists were able to develop a new powerful **holographic microscope (H.M)**, which allows them to identify a particle's properties (like its size or what it may be made of).

Imagine visualizing particles one million times smaller than a raindrop! With laser technology, we can create images of light patterns that are impossible to obtain using the best traditional microscopes to see these tiny particles moving.

HOLOGRAPHIC MICROSCOPES

Light microscopes work by using white light (regular light), which has light waves that spread out to magnify the specimen. On the other hand, H.M. captures an image of light patterns using a laser beam. A laser is a field of focused light waves resulting in high energy light. The H.M. has a resolution a thousand times more powerful than light microscopes! This resolution helps us examine particles the size of DNA to discover their properties.

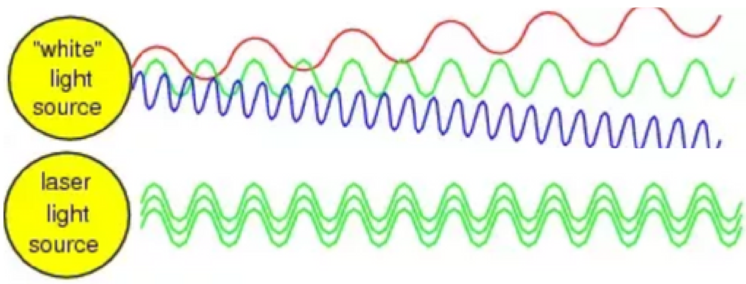


Figure 2. White light contains different wavelengths that travel **out of phase** with each other - waves of a laser travel **in phase** in a uniform direction.

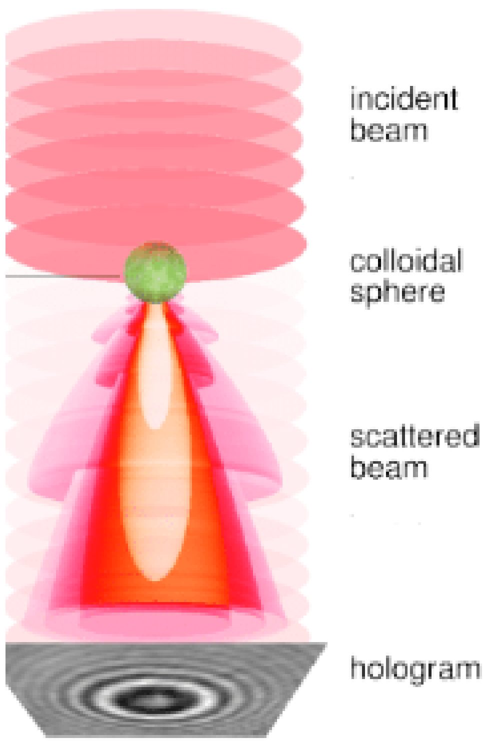


Figure 3. Breakdown of a H.M. demonstrating the interaction of the laser with the **colloidial sphere** and the resulting interference (hologram).

Wave Model

Did you know that light is a wave? The interaction of two light waves that differ **in phase** creates one overall wave.

The sum of the peaks and valleys from both waves creates a new wave through i**nterference**. Two types of **i**nterference exist, destructive and constructive.

Have you ever thought of how a massive ocean wave is made?

Two small ocean waves interact with one another, combining energy to create one resulting wave. If the two waves are perfectly lined up with one another, or **in phase**, the wave forms a much larger wave through constructive interference.

Destructive interference occurs when two waves are perfectly **out of phase** and their energies cancel each other - depicted in **Figure 1**.

PROPERTIES GATHERED FROM H.M.

H.M. allows us to examine an individual particle that is invisible to the human eye.

H.M. produces two different beam paths using the half mirror (beam splitter), as shown in **Figure 4**. One beam encounters the object while the other does not. The object beam is directed toward a colloidal sphere, allowing light to pass through it, creating a different light wave (shown as yellow in **Figure 5**). This yellow light has been slowed down and forced out of phase by passing through the particle. This wave meets with the unaltered laser (reference beam) creating a specific interference pattern as shown in **Figure 4.** This light pattern is known as a hologram.

The information of the hologram is fed through mathematical software to obtain various properties, including position, size, composition, and orientation. This can be used to obtain a 3D image of the particle, as well as to plot the **trajectory** and determine the heat energy.

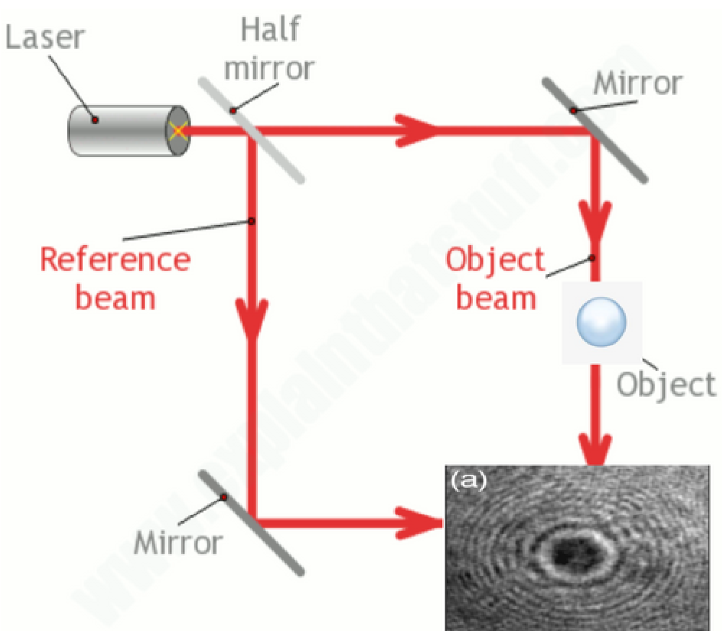


Figure 4. Holographic microscopes manipulate light to gather patterns at nanometer scale**.**

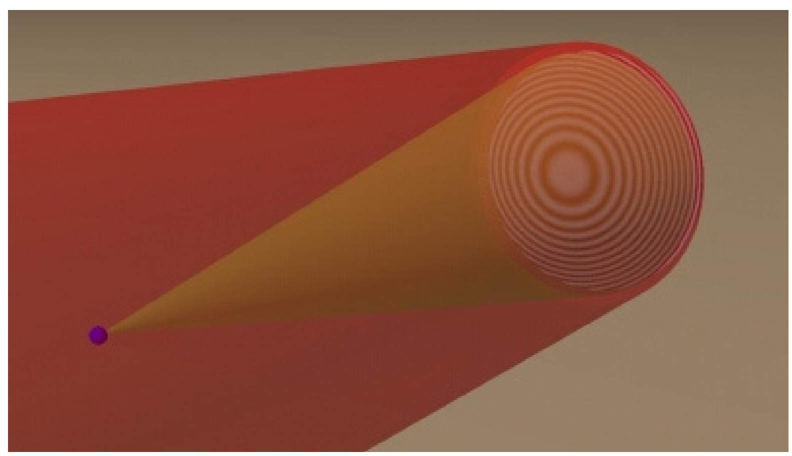


Figure 5. Red light is the original laser(reference beam) while the yellow light represents the object beam.

H.M. views nanometer-sized particles to discover their essential properties, including the composition of the particle. This is determined by calculating the particle's optical density which is what alters the object beam laser. Optical density is how much light is absorbed by a material like a colloidal particle. The more intensity the light wave loses, the more optically dense a material is. This measurement lets physicists figure out what the material could be.

DISCUSSION

- Holographic microscopy is used to create a real-time map of the movement of a colloidal sphere by measuring the sphere's coordinates in time and space with nanometer precision.
- Holographic microscopy allows scientists to also describe the physical properties of colloidal spheres, giving hints about what they're made of! These measurement can confirm predictions made by physicists about how matter works.
- Looking forward, holographic microscopy is an inexpensive method that can be used to detect and track particles in 3D, image living things in motion, map 3D surfaces, and record a time-lapse of microscopic growth and movement.
- Using the holographic microscope, we can examine individual particles like fat globules in dairy products, identify their properties, and regulate the product quality.

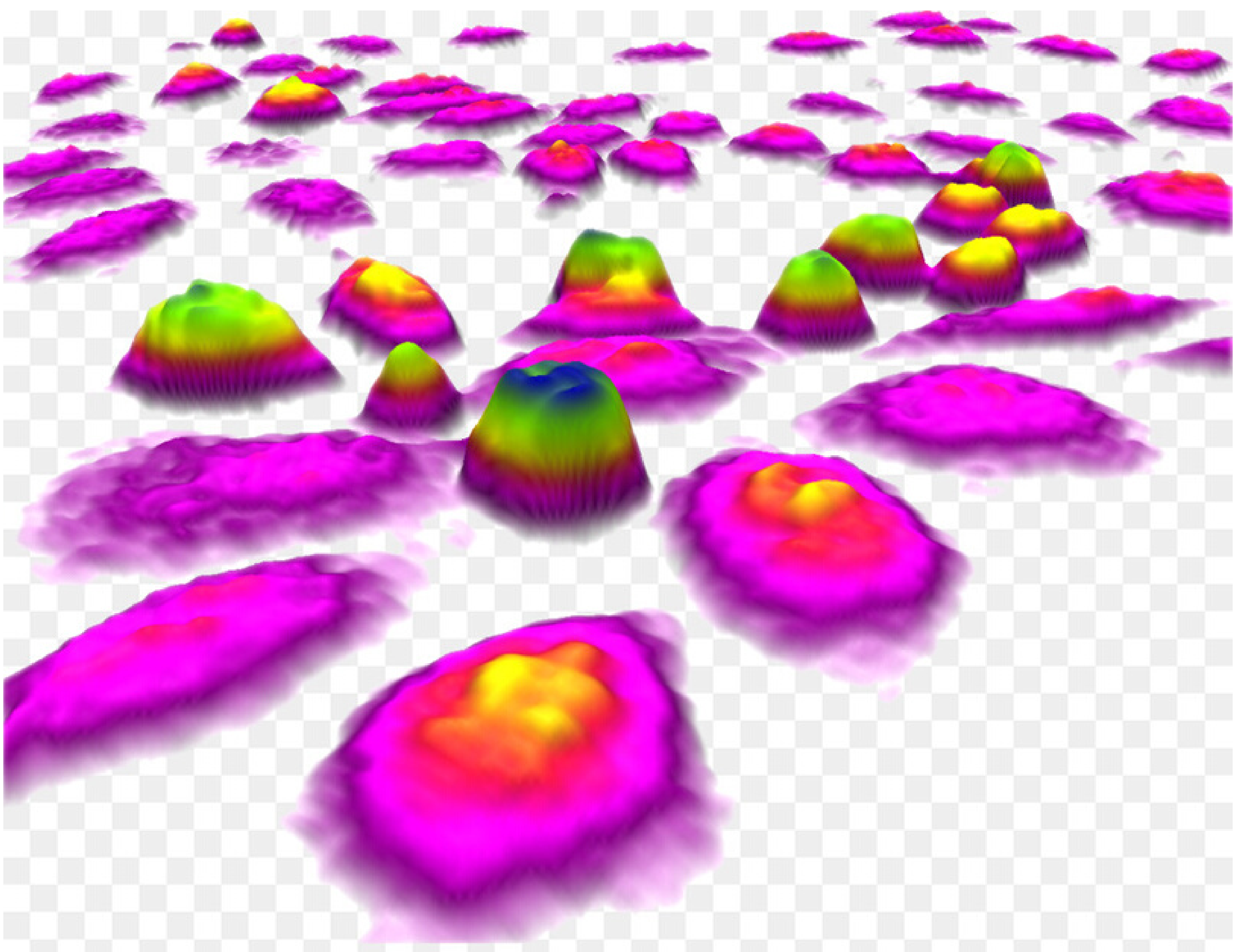


Figure 5. Digitial holograpic microscopy of cells.

Adapted by Bryan Campos and Christine Kuang from Measuring Boltzmann's Constant through holographic video microscopy of a single colloidal sphere by David G. Grier et al., *American Journal of Physics, Vol 82. No. 1* (2014).