

Limitations of Digital In-Line Holographic Microscopy

Holographic microscopy offers imaging capabilities beyond what is expected from conventional microscopy by capturing information from the entire sample volume with each frame, or hologram. However, there are limitations to this capability and these stem from the physical limitations of light and current technological capabilities. This document outlines **four of the major points of interest that often cause confusion.**

Information from 3 dimensions

Information from the sample volume is captured in holography. The reconstruction of the hologram to create an image is currently done in 2 dimensions, **so the images from holograms are in 2D.** Two dimensional images can be stacked to form a 3D **representation** of the volume; the image is still 2D. There are no commercial forms of 3D display functions which compliment the information gathered with holographic microscopy.

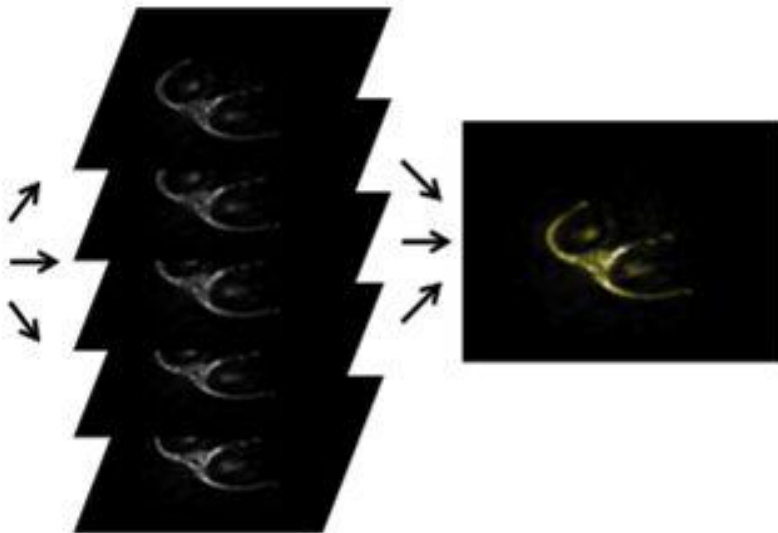


Figure 1: Several 2D reconstructions added to develop a 3D representation of *C. tripos*.

3 dimensional images of objects

Objects larger than a few microns and not highly **translucent create a shadowing effect.** There is no information in the hologram of the backside of the object that is facing the sensor camera. The position of the object in the volume is known, and the structure of the sides of the object not facing the sensor is known. This shadow effect prevents a proper 3D image of the object from being visualized. The real solution to this limitation lies with tomography techniques that are currently being developed to overcome this effect.

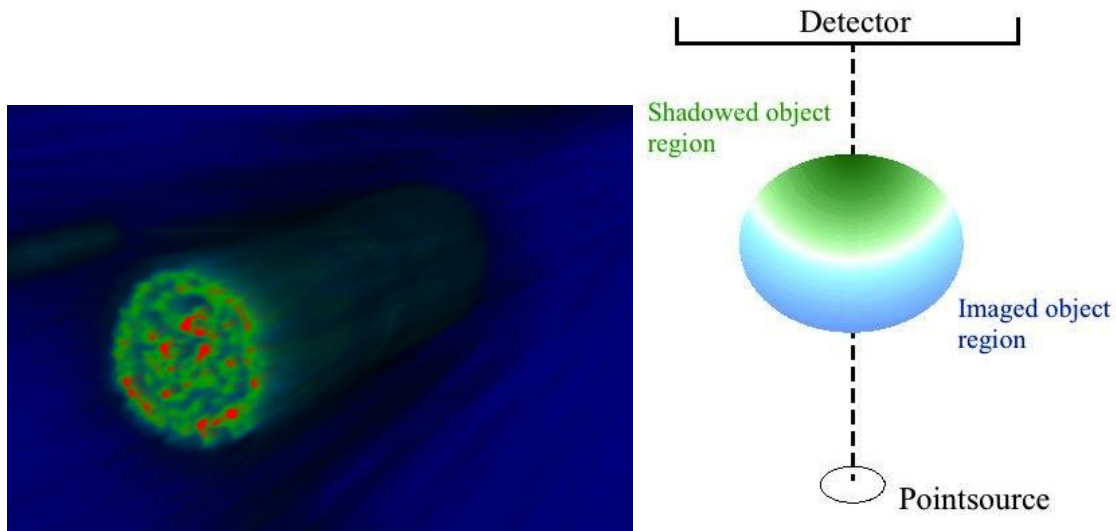


Figure 2: 3D representation of a 50 micron spherical object, showing the shadow (left) and an illustration showing the shadowed region of an object in green (right).

Resolution along the optical axis

The resolution along the (XY) plane of a reconstruction is extremely good; between **0.5 and 2 microns**, depending on the configuration and utilization of the microscope. Along the optical (Z) axis, however, the resolution is typically less than $\frac{1}{2}$ the resolution in XY directions. This adds to the difficulty of producing 3D images of objects, and develops a **stretching of the objects reconstructed image in Z.**

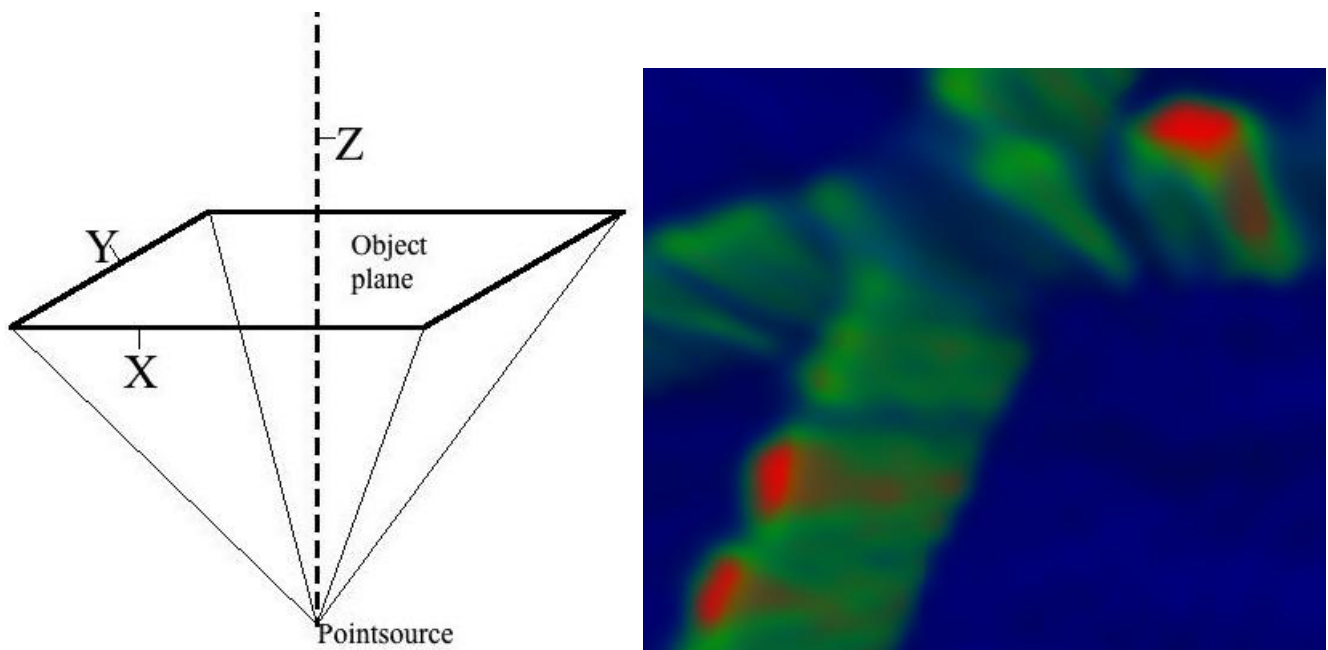


Figure 3: 3D representation of 3 micron objects (in red) stretched along the z-direction

(right), and the X, Y and Z-directions defined (left).

Lensing from spherical objects

Translucent spherical objects, such as oil drops or air bubbles in water, **can create lensing effects which concentrate light away from the boundaries of the object.** These concentrated points are often higher in intensity than the object in the image, and can create intensity peaks away from the object plane.

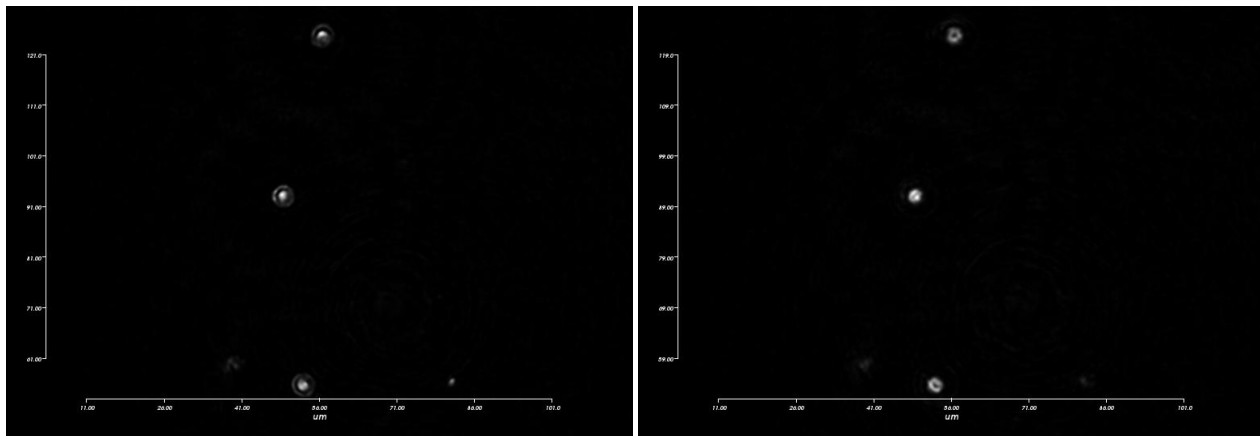


Figure 4: Centre of 5 micron diameter beads (left), and reconstructions of the same beads 6 microns away, showing the high intensity of the lensing (right).