Critical comparison between holographic & conventional microscopy

The Focal Plane:

Conventional microscopy only captures object information in the focal plane. It is the compound lens structure which gives conventional microscopy its magnification that limits the depth of focus which the microscope can achieve; what is essentially a plane of view. By contrast, digital in-line holographic microscopy (DIHM), with a single hologram, records object information throughout the whole volume element that has the camera chip as its base and the point source as its apex. Holographic microscopy is a lens free system, achieving magnification from the geometry of the device. The limitation of the depth of focus is not present and images of objects can be extracted from any part of the volume within the field of view.

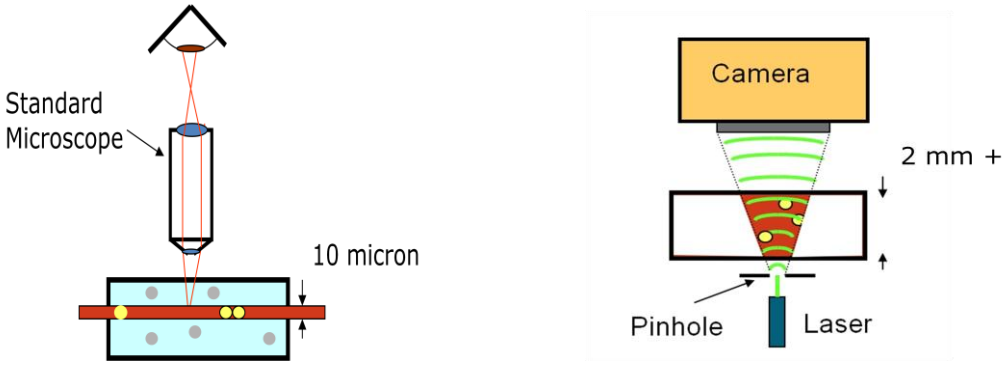

Figure 1: Comparison of the focal depth (red) of a conventional microscope (right), which is typically on the order of 10 microns versus that of a holographic system (left) which has a depth of view of several millimeters.

Specimen sampling and preparation:

In order to get good images, specimens must be within the focal plane. In conventional microscopy, this entails constricting the sample and often some sort of sample preparation such as staining or fixing. With holography, the sample can be less constrained, allowing for normal movement of specimens and requires no sample preparation. With a less complicated set up, and the ability to capture information from the entire volume, holographic microscopy is highly functional underwater. Conventional microscopic systems cannot perform underwater and require samples to be collected and returned to a laboratory for optimal imaging functions.

Color imaging:

Holographic microscopy uses monochromatic lasers to produce holograms, and currently

reconstructed images are intensity based greyscale. The greyscale intensity values from reconstructed holograms, however, can be interpreted with false color to enhance contrast in the image (see figure 2). Conventional microscopy uses white light, and can produce true and vivid color images.

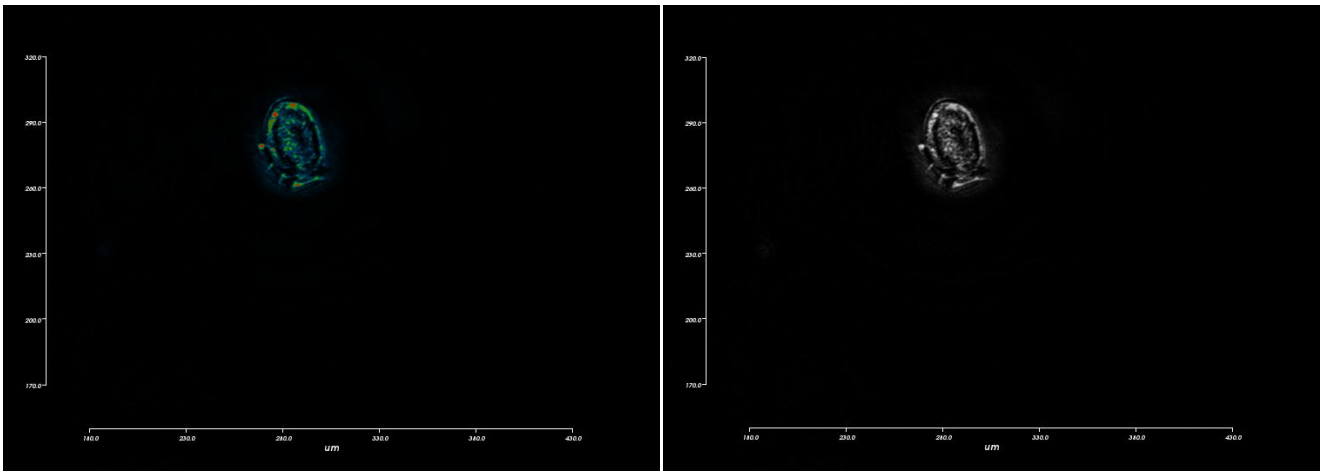

Figure 2: Gray scale image of zooplankton (right) and the same image in false color (left).

Object tracking

The limited focal plane and depth of view of conventional microscopy makes it very difficult to track objects and image dynamic movements (ex. microfluidics). Conventional microscopy requires objects to be constrained to a plane, allowing for movement tracking in only two dimensions. Holographic microscopy captures the movement of objects in three dimensions; it can track objects used as markers in fluid dynamics tests, and the complex free swimming motions of microscopic organisms such as dinoflagellates).

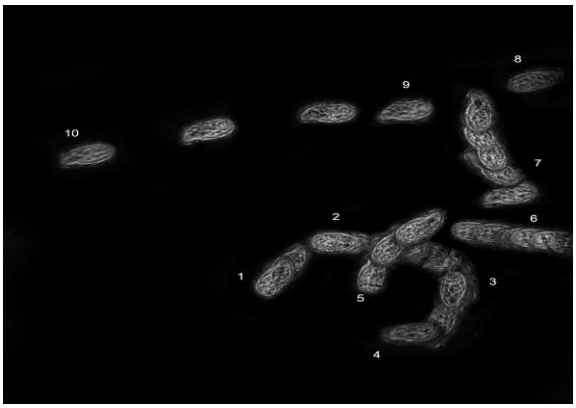

Figure 3: Tracking the swimming pattern of a Rotifer.

Phase imaging

Phase information is easily obtained using phase reconstruction of holograms. It is possible with conventional microscopy techniques but it is expensive and requires specialized methods and equipment to capture phase changes.

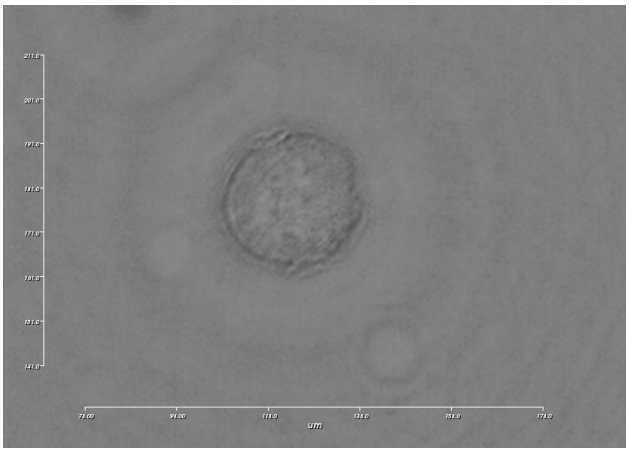

Figure 4: An example of an *Alexandrium* phase reconstruction from a holographic microscope.