Holographic microscope enables 3D surface acquisition

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Using a simple point-source hologram and a grating in a grazing incidence configuration can yield a field-of-view that is unaffected by magnification.

Holography has proven somewhat impractical because of its complexity, and there are further complications when applying it to microscopy. Moreover, a hologram does not directly provide the numerical data needed for computer processing. Because of these limitations, holography is rarely used to acquire surface coordinates in three dimensions, even though it is intrinsically a 3D medium. Triangulation and stereoscopy are more common techniques. However, these older methods suffer from perspective foreshortening that minimizes, rather than magnifies, distances, resulting in depth data that is coarser than the two corresponding planar dimensions of width and height.

A more effective process uses a simple point source in which a laser directly produces an image of its own beam rather than one of any other subject. When this ‘vanilla’ point-source hologram is played back by viewing a surface illuminated by a laser beam, the illuminated point on the surface appears to float in space. (Where that point appears depends on the distance between the illuminated surface and the hologram.) A camera connected to a computer can then provide positional data suitable for interpretation by 3D surface-coordinate software. In this optical device, the hologram serves as the primary objective, and the camera and its lens are part of the secondary one.

When we described this diffraction method in 1987,1 it used co-axial illumination relative to the surface of a plane grating. The technique did not overcome perspective foreshortening but had useful features for microscopy.2 In 1997, we used the technique for the first time with a holographic primary objective rather than a plane grating.3 These range readings had no perspective foreshortening, but the depth dimension was not magnified.

We recently demonstrated a magnification feature4 (see Figure 1). It uses a holographic grating in a grazing incidence configuration in which the narrow aperture, \( a \), of light from the subject illuminates the considerable length, \( L \), of the hologram (see Figure 2). The magnification, \( M \), is the ratio of \( L \) to \( a \) (\( M = L/a \)). A laser stripe intersecting the aperture interrogates the volume for 3D surface acquisition.5

Our method differs significantly from conventional microscopy because the field-of-view is not affected by an increase in magnification power. The tradeoff is that, as the magnification goes up, the efficiency of the primary objective goes down (see Figure 3). This differs from the universally understood microscope paradigm in which field-of-view narrows as magnification increases.

Because the surface groove profiles of the primary objective hologram affect efficiency, part of our research focused on analyzing diffraction grating surfaces. Field emission scanning electron micrograms (FESM) shown in Figure 4 reveal that grating groove depths that are roughly 20% of the wavelength of incident radiation produce the most efficient reconstruction at

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Figure 2. Laser beam passing through aperture (a) is seen along hologram of length L.

Figure 3. Magnification is determined by angle of grazing incidence (i). As magnification (M) increases toward infinity, the efficiency (e) of the primary objective grating decreases toward zero.

3D surfaces over a 200mm wide field-of-view by embossing the hologram into an inexpensive polycarbonate film.

We demonstrated a new method of 3D microscopy. Rather than make a hologram of each object, the new approach uses a ‘vanilla’ hologram to record all objects and a grazing incidence configuration to achieve magnification. The inexpensive primary objective can be replicated in plastic. The microscope enjoys an unprecedented field-of-view and depth-of-field. In the next iteration, we plan to increase the resolving power of the microscope by an order of magnitude through a very slight modification to the primary objective hologram.

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References

5. Description of the holographic microscope. Credit: Derek Sweeney-Kesler and Tom Ditto. http://spie.org/documents/newsroom/videos/1846/SPIE_HolographicMicroscope_Figure2.mp4