A high resolution, holographically corrected microscope with a Fresnel lens objective at large working distances.

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Abstract: We present details of a microscope which incorporates an inexpensive, high numerical aperture Fresnel lens objective. The system aberrations are corrected by the use of an image hologram of the lens recorded using a point source of coherent illumination. This device gives high resolution, real time imaging while maintaining a large working distance. The same microscope can be used for micromachining and photolithography in situations where close proximity to the sample is impossible or undesirable.

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References and links

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1. Introduction

The design of high numerical aperture objectives for microscopy has traditionally required expensive multi-element systems with small apertures and very small working distances. In previous work [1] we introduced an alternative method using a single, inexpensive, low-quality lens which was holographically corrected to give diffraction limited imaging. This correction included over 1900 waves of spherical aberration as well as several more due to the poor surface figure of the lens itself. While this scheme was shown to be successful for a single element objective with a moderate numerical aperture, it becomes impractical as the numerical apertures are increased. This is due to the fact that the spherical aberration produces a beam that is too large to make it through the imaging lens, so the full extent of the wave aberration cannot be recorded. In this paper we extend our work to the use of Fresnel lens objective elements, making it possible to construct a simple microscope with much larger numerical apertures while maintaining working distances an order of magnitude greater than conventional objectives.

The holographically corrected microscope is constructed as shown in Fig. 1. A laser is spatially filtered to form a point source of light which illuminates a Fresnel lens objective (Fig. 1a). The pinhole in the spatial filter must be small enough that the objective is evenly lit. The Fresnel lens will then focus the light to a high-quality collimating lens which images the objective onto the plane of the hologram. The imaging condition increases the field of view when using a thin aberrated objective, by recording the specific phase shift associated with each point on the lens for any field point [2]. A hologram is created by recording the interference pattern produced between this object beam and a diffraction limited, plane wave reference beam incident from an angle.

Once the hologram is exposed and processed, it is returned to its original position. If the spatial filter illuminates the objective once more, with no change in the set-up, the diffraction limited reference beam will be reconstructed (Fig. 1b). The spatial filter is now removed and replaced by an object which is illuminated (in either transmission or reflection) by the same wavelength of light used to record the light. Light from a point on the object where the pinhole was will pass through the system to reconstruct the reference beam, *but with the object information retained*. If this reconstructed beam is focused down, a diffraction limited, magnified image of the object can be made (Fig. 1c). The hologram can also remove most of the aberrations of nearby object points (which have similar aberrations to those recorded for the on-axis source point) giving rise to a finite field of view. In this way, it is possible to produce a high resolution, diffraction limited image of an object with a simple low-quality Fresnel lens objective over a narrow bandwidth.
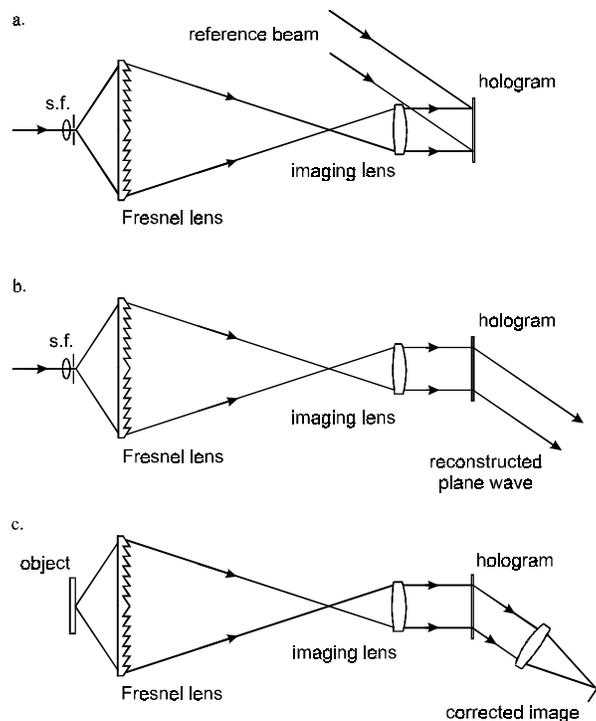

Fig. 1. a. Recording. A spatial filter (s.f.) illuminates the Fresnel lens objective to form the object beam, which interferes with a collimated reference beam to create the hologram. b. Reconstruction. If the set-up remains unchanged, the object beam will reconstruct the original reference beam. c. Imaging. An object replaces the spatial filter, and light from a point on the object reconstructs a diffracted beam which is focussed to form an unaberrated, magnified image of the object.

2. Experiment

We constructed a microscope based on the scheme outlined in Fig. 1, using an acrylic Fresnel lens (D = 150mm, f = 76mm) illuminated by a spatial filter with a working distance of 85mm. The objective in this arrangement is operating at a numerical aperture of 0.66. It should be noted here that conventional microscope objectives with similar numerical apertures have working distances around 5-8mm, which is an order of magnitude less. The focussed light from the objective was collimated by a diffraction limited lens (D = 36mm, f = 100mm) placed at the circle of least confusion. This lens formed an image of the objective, 31mm in diameter onto the plane of the holographic film 116mm away. The diffraction limited reference beam was incident on the plate from an angle of ~25°.

The phase holograms were recorded on Agfa 8E56 silver halide plate film using a CW, frequency-doubled, Nd:YAG laser (λ = 532nm), which were bleached to achieve a typical diffraction efficiency of 50-70%. This efficiency is marginally lower than that obtained in previous experiments with conventional refractive lenses due to some scattering from the sharp groove edges of the Fresnel lens, which have an infinitely high spatial frequency, and cannot be fully recorded. In our experiments we recorded transmission holograms, though a similar set-up to produce a reflection hologram (with the reference beam incident from the other side of the plate), would work equally well.

After exposure, the processed hologram was returned to its original recording position. With the spatial filter once more illuminating the Fresnel lens, the reference beam was reconstructed at the hologram. The focal spot of the reconstructed beam is shown in Fig. 2a. The fidelity of the reconstruction was tested by interfering the diffracted reference beam with the original reference beam used to write the hologram. The interferogram is shown in Fig. 2b., indicating a difference between the two beams of less than $\lambda/4$. In order to obtain some information about the field of view, the illuminating pinhole was moved laterally to simulate an off-axis source point, with the resultant focal spot and interferogram as shown in Figs. 2c. & d. The increase in wavefront error seems to suggest a diffraction limited field of view slightly smaller than ±5µm. Given the expected resolution of 0.4µm, and the fact that most objectives rarely achieve true diffraction limited imaging over large fields of view, this is not seen as a limitation. If required, the sample could still be scanned under the on-axis point for maximum resolution over a larger field of view in much the same way this is done for confocal microscopes. The depth of focus was not measured but seems to be close to the expected value of 0.6µm, as expected from simple theory [3].

a.

b.

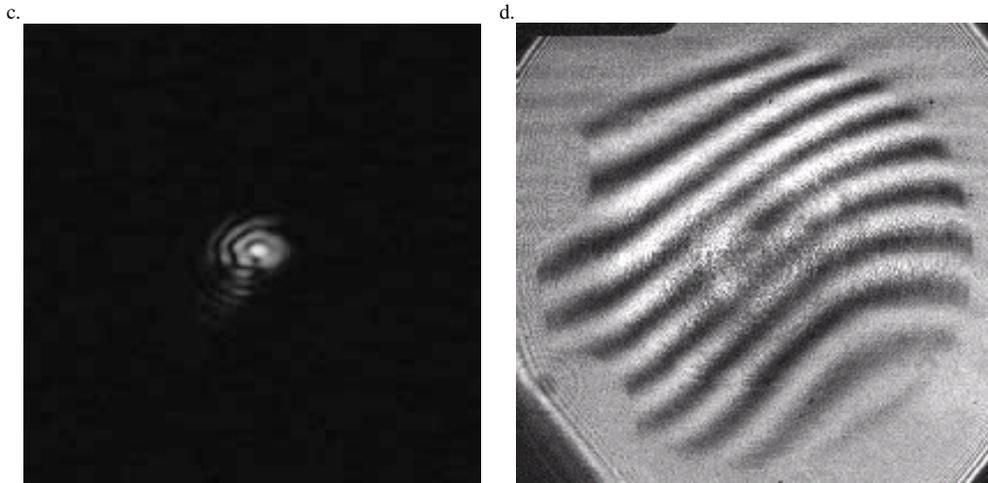c.   d.
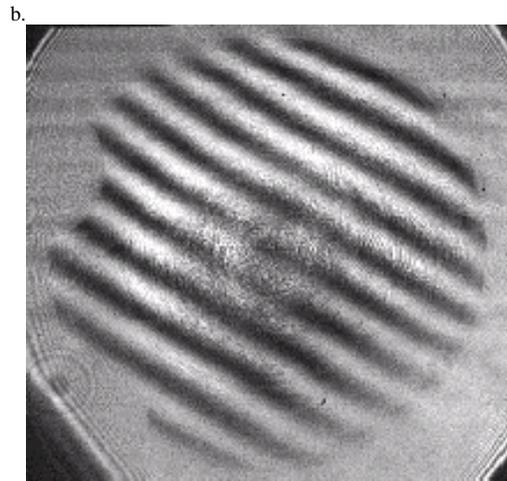
Fig. 2.a. The focal spot of the reconstructed beam. b. On-axis reconstruction to better than diffraction limited quality. c & d. The corresponding focal spot and interferogram for reconstruction with the pinhole moved laterally by 5µm.

In order to test the imaging capabilities, the spatial filter used to record the hologram was removed, and replaced with the sample to be viewed. Initially, the objects chosen were transmissive, lit from the rear with laser light passed through a rotating diffuser to remove speckle. Fig. 3a. shows the image of a 1951 USAF resolution test chart, with bars of 228 line pairs/mm (Group 7, Element 6) easily resolved. The second image (not reproduced to the same magnification) shows a sinusoidal intensity grating fabricated in-house. The spatial frequency of this grating is 1145 line pairs/mm which implies a separation between maxima of just 0.44 µm.
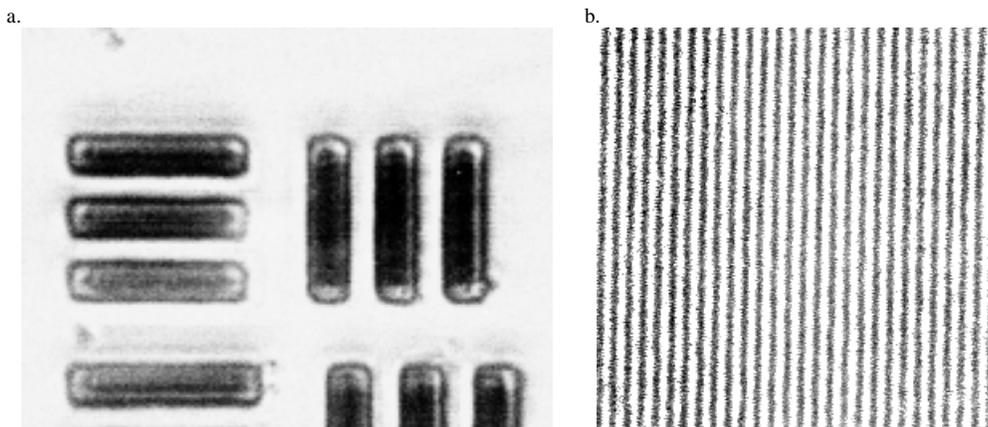
a.   b.

Fig 3. a. Group 7, Element 6 of a 1951 USAF resolution test chart with bars and spaces 2.2µm wide. b. A sinusoidal grating with a spatial frequency of 1145 line pairs/mm. The two images were slightly contrast-enhanced for clarity and are not shown to the same magnification.

Fig. 4a. shows an image of human blood cells having an average individual diameter of ~5µm. The effect of a decrease in resolution with field of view is not apparent in this image, most likely due to the fact that there are no obvious details near the diffraction

limit for this subject. The second image is under a different magnification and shows a microchip viewed in reflection, with 0.7μm-wide tracks clearly visible. In order to light a subject in reflection, a slightly different recording scheme had to be used, as shown in Fig. 5. A 50% beamsplitter was placed at an angle to the beam, just before the imaging lens during recording. On reconstruction, light could be directed onto a rotating diffuser, to expand backwards along the optical axis of the microscope using the full numerical aperture of the objective to illuminate the object. This same set-up can also be retained for imaging transmissive objects. It should be noted that the system aberrations have been recorded as a phase shift at a given wavelength. Reconstruction at other wavelengths will result in some residual aberration remaining, leading to a degradation in image quality, which is why the original laser is used to illuminate the subjects. We are currently investigating ways that an incoherent light source may be used instead.
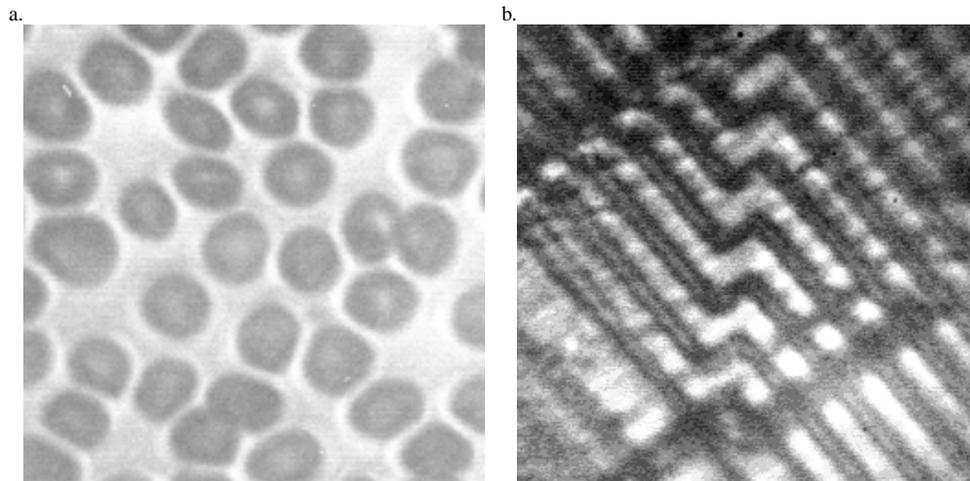

Fig. 4. a. Human blood cells with an average diameter of ~5 μm. b. A microchip with track widths of 0.7μm. The two images are not shown to the same magnification.
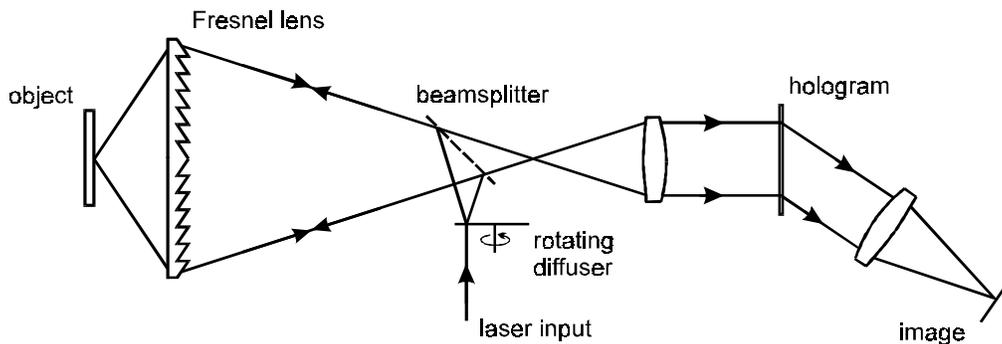

Fig. 5. Scheme for viewing reflecting objects. An angled beamsplitter is added to the system on recording which, on replay, can be used to illuminate a reflecting object as shown above.

3. Alternative modes/designs

While the emphasis of this paper has been to demonstrate high-resolution imagery, this same microscope can be used for image reduction, photolithography and micromachining. The principle is much the same as for magnifying images, but in this case the microscope is used

to project light in a reverse of the beam paths shown in Fig. 1c. We have already used this technique to micromachine 1μm diameter holes in metal foils under vacuum using a low-power pulsed, frequency doubled YAG laser [1]. Future work will be aimed at developing this device for high-resolution photolithography.

We have demonstrated that both simple refracting lenses and Fresnel lenses can be used as objective elements in our holographically corrected microscope. Previous work [4] with reflecting primaries for telescopes has shown that aberrated mirrors could also be used as objective elements. This would make it possible to extend the operation of the microscope into the deep ultra-violet for further increases in resolution. The only requirements would be that the laser used would have to have a sufficient coherence length to record a hologram and that the holographic medium is sensitive and operable at these wavelengths. Beyond the ultra-violet, many types of zone plates [5] can be adopted in order to achieve X-ray imaging, with the basic scheme being the same as that demonstrated for the Fresnel lens objective. Although X-ray holography suffers from very low efficiencies, the extension of this microscope design into wavelengths this short should make it possible to achieve resolutions of 10nm or better.

4. Conclusion

We have presented the design for a new type of microscope which uses an inexpensive high numerical aperture Fresnel lens objective. The large amounts of aberrations present in the system are corrected by the use of an image hologram recorded of a point source. This device has been experimentally shown to give diffraction limited imaging over a moderate field of view while operating at a working distance over an order of magnitude greater than conventional objectives with similar numerical apertures. Our current work is concentrating on numerical apertures beyond 0.9 while extending the operational wavelength into the ultra-violet with the aim of obtaining better than 0.1μm. resolution. Other research is also being carried out to evaluate the possibility of using holographic lenses and zone plates as objective elements.