

# Applications of extended depth of focus technology to light microscope systems

Sara Bradburn, W. Thomas Cathey, Edward R. Dowski, Jr.  
Imaging Systems Laboratory, Department of Electrical Engineering  
Campus Box 425, University of Colorado, Boulder, Colorado, 80309  
phone: (303) 492-3153, fax: (303) 492-3674

## Summary

Previous research has demonstrated the success of using a cubic phase mask in optical systems to perform wavefront coding which, along with digital post-processing can extend the depth of focus of standard optical imaging systems [1, 2]. Experimental images have demonstrated an increase of six to eight times the depth of focus of standard systems when using the extended depth of focus technology. This technology is now being extended to high magnification or light microscope systems. Several challenges have arisen due to the special nature of light microscope systems but extended depth of focus (EDF) technology is still found to be useful in such systems.

Due to the high numerical apertures and magnification of most light microscope systems, the depth of focus of the systems are generally small. Increases in magnification and numerical aperture will decrease the depth of focus of the microscope as will a decrease in the wavelength of the illumination used [3]. When imaging thin sections of specimen for details, a small depth of field is acceptable, but when details of a thicker object are sought, the low depth of focus is unacceptable. Even confocal microscopes suffer from limited depth of focus. The process for extending depth of focus in standard imaging systems can therefore be beneficial to microscope imaging systems. Placing a cubic phase mask in the aperture stop of the microscope will have the effect of optically encoding information about the specimen. Once the information about the object has been encoded and passed through the optical system, a CCD camera can then capture the coded information, and pass it along to a chip or into software where the information is recovered through digital post-processing. The cubic phase mask has a phase delay function with respect to spatial coordinates according to:

$$P(x, y) = \exp(j\alpha(x^3 + y^3)). \quad (1)$$

Where  $\alpha$  is a parameter which determines the amount of increase in depth of focus. A diagram of the phase mask is shown in Figure 1. In previous experiments, a cubic phase mask with  $\alpha = 20\pi$  has been used. When a cubic phase mask is placed in an imaging system, the MTF and therefore the PSF of the system are coded. Due to linearity and paraxial space-invariance, information about the entire object is coded. The coded MTFs and PSFs are essentially invariant to defocus. Using the information about a single PSF taken when calibrating the system, an inverse digital decoding filter (usually a least mean squared error (LMSE) filter [5, 6]) is built. When applied to the image, the decoding filter will restore the information about the entire object and produce an image with an extended depth of focus.

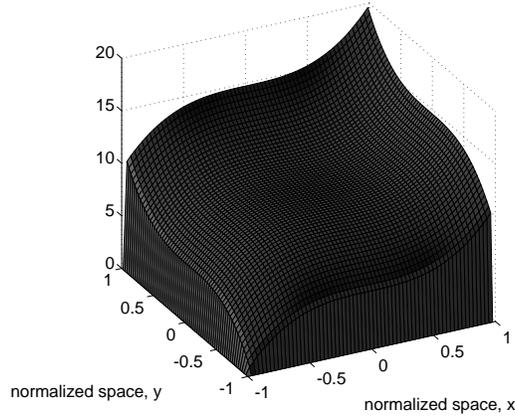


Figure 1: Diagram of cubic phase mask function

Depth of focus, according to Rayleigh, can be defined as twice the axial distance within which the radius of an image beam is within  $\sqrt{2}$  of its minimum value [4] or:

$$z_{dof} = \frac{2\pi r^2}{\lambda} \quad (2)$$

where  $r$  is the radius of the minimum size of the beam or the "spot size" of the optical system. It has been demonstrated that the depth of focus can be extended up to eight times the original Rayleigh distance with a cubic phase mask parameter,  $\alpha = 20\pi$ .

An example of a digital decoding filter that is often used in these EDF procedures is a two-dimensional, frequency domain filter,  $\mathbf{F}$  calculated by:

$$\mathbf{F} = (\mathbf{H}^* \cdot \mathbf{H})^{-1} \cdot \mathbf{G}, \quad (3)$$

where  $\mathbf{H}$  is the two-dimensional frequency domain version of the PSF data,  $\mathbf{G}$  is the ideal two-dimensional frequency domain target PSF, and the "." symbol represents point wise product multiplication between the data matrices.

An example of a high magnification system which uses the cubic phase mask is shown in Figure 2. On the left is an image of an EPROM imaged with a standard imaging system. The center image of the EPROM was taken using a standard system with a smaller aperture and increased exposure time. Note the loss in resolution when the small aperture is used. On the right is the decoded image taken using the cubic phase mask system. Note how the depth of focus has been extended but resolution has not been sacrificed except in a roll off of the filter to reduce the noise amplified by inverse filtering. There are, however, still residues of the coded PSFs throughout the image. Most of these residues are due to the high amount of specular reflection in the image and imperfect decoding of the coded image. Due to the smaller working distances in microscope systems, special lighting systems such as diffuse axial illumination need to be used in order to avoid characteristics such as specular reflection which hinder the decoding process.

It is believed that further experimentation with additional types of microscopic objects, all with different characteristics, will demonstrate the necessity and value of extended depth of focus imaging

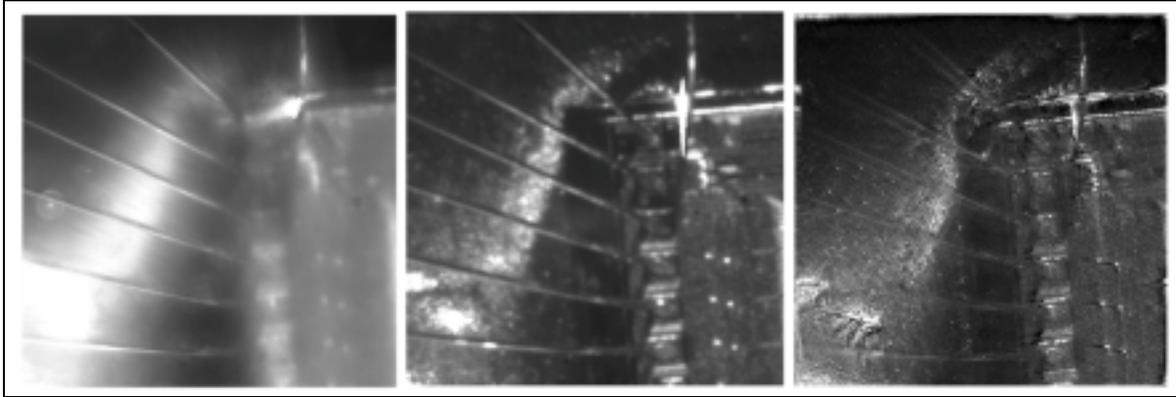


Figure 2: Images of EPROM with standard (left), small aperture (center), and EDF (right) imaging systems

to the field of microscopy. Additional work on the digital filters used in the procedure will also be performed to adapt the filtering processes to the specific needs of light microscope systems. Eventually, encoding the information about microscopic objects with a cubic phase mask and digitally decoding the information in a digital domain, will allow for higher magnification systems and observation of finer detail in objects, without the sacrifice of decreased depth of focus.

## References

- [1] Bradburn, Sara, Edward R. Dowski, Jr., and W. Thomas Cathey, "Realizations of focus invariance in optical-digital systems with wave-front coding," *Appl. Opt.* 36, (1997).
- [2] Edward R. Dowski, Jr. and W. Thomas Cathey, "Extended depth of field through wave-front coding," *Appl. Opt.* 34, 1859-1866 (1995).
- [3] Perlman, Phillip, *Basic Microscope Techniques*, (Chemical Publishing Company, New York, 1971), Chapter 1, pp 24-25.
- [4] Bahaa E. A. Saleh and Malvin Carl Teich, *Fundamentals of Photonics*, (John Wiley & Sons, Inc., New York, 1991). p. 87.
- [5] B. R. Frieden, "Image Enhancement and Restoration," *Topics In Applied Physics, Vol. 6, Picture Processing and Digital Filtering*, Editor: T.S. Huang, (Springer Verlag, New York, 1979), 177-248.
- [6] Richard A. Roberts and Clifford T. Mullis, *Digital Signal Processing*, (Addison-Wesley, Reading, 1987), 105-112, 129, 229-236.