Reducing noise in extended depth of field microscope images by optical manipulation of the point spread function

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ABSTRACT

This work describes improved methods and algorithms for implementing extended depth of field (EDF) microscopy through point spread function (PSF) engineering. It utilizes adaptive optics to create a test bed on which to evaluate new phase shapes for EDF. Being able to quickly and cheaply design novel PSFs is essential to overcome limitations of EDF that have prevented the technology from reaching mainstream use. Further improvement is made by reducing the noise normally seen in EDF images. Computational optics principles are used to first encode the noise with an identifiable pattern and a specially-tailored non-linear algorithm then removes the noise. This approach improves a microscope's imaging capabilities in photon-starved applications such as live-cell fluorescence and object tracking.

Keywords: Extended Depth of Field, PSF Engineering, 3D Microscopy, Adaptive Optics, Neural Networks, Noise Removal

1. INTRODUCTION

Extended depth of field microscopy achieved through PSF engineering has the ability to significantly impact biological imaging. However, the technology suffers from major limitations which prevent its mainstream utilization. The use of costly phase plates to perform the PSF engineering hinders the design and evaluation of potentially more efficient phase shapes. Also, amplified noise, generated through the digital signal processing necessary for EDF, degrades the fine detail to the point where biologists often cannot use the images. These drawbacks are addressed herein through novel approaches in system design and digital signal processing.

2. BACKGROUND ON EXTENDED DEPTH OF FIELD (EDF) MICROSCOPY

The depth of field of a microscope objective decreases as the square of the numerical aperture increases. As a result of this relationship, a system which approaches the diffraction limit (~200nm) only maintains a plane of best focus that is approximately 600-800 nm thick. Features outside of this thin region cannot be accurately resolved by the microscope.

A number of approaches have been employed to overcome this trade-off. A technique commonly used is to acquire a stack of images moving through depth and then reconstruct the image using a digital signal processing algorithm. Scanning through focus does result in a sharp extended depth of field image, however this approach is ill suited for live cell biology applications. If a sample alters its position at any point throughout the scan it becomes impossible to accurately reconstruct the image. This process also requires relatively slow and inconvenient reconstruction algorithms.

An alternate method to extending the depth of field, proposed by Dowski and Cathey, combines engineering of the PSF and linear signal processing. An aspheric phase plate is inserted at the back aperture of the microscope objective. The phase plate has two distinct effects on the system: it encodes the PSF with a new shape and modifies the PSF so that it
maintains a constant intensity profile through a depth approximately 10 times that of a conventional system. The encoded point spread function is then returned to a diffraction limited spot using a linear deconvolution algorithm. Since multiple planes have the same PSF, they are all simultaneously shown in focus in a single image. A number of various phase shapes have been proposed and implemented for EDF. The most commonly used shape to date, however, is a two-dimensional cubic phase function. This paper will focus solely on EDF systems which utilize a cubically encoded PSF.

EDF microscopy through point spread function engineering has the benefit of requiring acquisition of only a single image frame. The technique therefore allows for video recording of a live specimen in which object features appear in focus throughout an extended volume. To date, the major drawback of this EDF approach for microscopy has been its tendency to suffer from a drop in signal-to-noise-ratio (SNR) in the recorded images. The noise is further enhanced during the linear deconvolution step, making the resulting EDF images unusable by biologists due to artifacts and background noise patterns that often mask the tiny biological structures of interest. Modifying and improving the EDF microscope system to overcome this drawback is the main focus of this paper.

### 3. ADAPTIVE OPTICS IMPLEMENTATION OF EDF

While the cubic has been the most successful phase altering shape for EDF microscopy, it is far from ideal. Other shapes may exhibit properties more ideally suited for the task of EDF through point spread function engineering. It is therefore essential to have a test bed that can quickly and accurately assess the effectiveness of new phase shape designs. The current established method for modifying the PSF is to insert a specifically machined polymer phase mask into the back aperture of the microscope objective. This approach suffers from fundamental limitations which make it ill suited for evaluating new designs. Specifically, the cost and fabrication time required for each new phase mask make the testing of these new designs infeasible.

![Figure 1. Setup of Extended Depth of Field microscope utilizing adaptive optics to implement PSF engineering.](image-url)
As an alternative, this paper describes the use of adaptive optics, specifically a deformable mirror (DM) and Shack-Hartmann wavefront sensor (WFS), to allow fast evaluation of phase shapes for EDF microscopy. Figure 1 shows the experimental setup for implementing EDF with adaptive optics. The DM and WFS were taken from the Thor Labs Adaptive Optics Kit. The mirror, made by Boston Micromachines Corporation, consists of 140 actuators with a maximum mechanical stroke of 3.5 μm. A collimated light source is used to illuminate a biological sample in between a condenser and standard microscope objective. The back aperture of the objective, where the EDF phase plate would typically be placed, is then imaged onto the deformable mirror through a 4-f relay. If necessary this 4-f relay also acts as a beam reducing telescope so that the mirror aperture does not further diffract the beam. The mirror plane is then relayed again and split into two paths. One path goes to the Shack-Hartmann wavefront sensor and the other path is imaged onto a CCD camera.

The system was initially tested to ensure that the deformable mirror has the sufficient transverse and lateral resolution to properly implement the cubic phase shape. A 0.5 μm pinhole, which was smaller than the diffraction limited spot size of the 20x/0.50 Zeiss objective, provided a PSF that was then encoded. The mirror was set to a cubic shape by use of a reference arm (not shown) and the closed loop software system provided with the mirror. The resulting PSF was captured on the CCD camera. Fig. 2(a) below shows a PSF generated from an ideal cubic phase shape simulated in MATLAB®. The PSF generated by the DM setup, Fig. 2(b), clearly shows that the mirror has sufficient resolution and smoothness to mimic this shape.

![Figure 2. Comparison of (a) an ideal cubic PSF to (b) the cubic PSF from the deformable mirror shows similarity in shape.](image)

The final step in confirming the suitability of adaptive optics for PSF engineering was to actually capture and filter an encoded image with the setup. A plant sample under bright field illumination from a white light LED source, seen in Fig. 3(a), was used as the test object. The plane of focus is at the tip of the foremost object, while the other features are obscured. The same image was then recorded with the DM in a cubic shape and linearly filtered to create Fig. 3(b). The previously obscured features can now be resolved and the contrast has been enhanced. This demonstrates that adaptive optics can be successfully used to extend the depth of field of microscope images through engineering of the PSF. This system will serve as an essential test bed for the future design and evaluation of new phase shapes.

A fundamental consequence of the linear filtering process is the amplification of background noise, as seen in Fig. 3(b). This noise obscures high spatial frequencies and any textures present in a sample. Non-linear algorithms which can be applied to deconvolved EDF images to remove the noise and any additional artifacts will now be discussed.
Figure 3. Extended depth of field implemented with a deformable mirror under bright field illumination. Image (a) is a plant sample taken with a conventional microscope configuration. Image (b) is the same image after being cubically encoded and linearly filtered.

4. NON-LINEAR NOISE REMOVAL

By exploiting the properties of the linear deconvolution process described in Section 2 above and adding a non-linear filter, noise can be reduced in EDF images. The linear filtering process restores cubically encoded PSFs in the image back into diffraction limited spots. However, any noise present in the image arising from the CCD camera or its read out electronics does not have the cubic encoding, and as a result becomes patterned with the shape of the deconvolution filter. This patterning is the background noise commonly seen in EDF images obtained through PSF engineering. However, the image quality can be improved by training a non-linear filter to identify and remove the noise patterns via a neural network. Figure 4 illustrates the process.

Figure 4. Signal PSFs have a cubic shape while the noise remains unencoded in the unfiltered image. After linear filtering, the PSFs have been restored to diffraction-limited spots but the noise is now patterned with the shape of the deconvolution filter. The non-linear filter is trained to identify this pattern and remove the noise, leaving only the decoded signal.

The non-linear filter operates using a shift invariant approach and consists of three layers. The first layer is made up of the input pixels convolved with a matrix of weights. They are then operated on by a non-linear activation function which has a sigmoid shape in the second, or hidden, layer. The pixels are then convolved with another weighting matrix to produce the output layer. Mathematically, the non-linear filtering process can be described as:
\[ Z = W^{OUT} \otimes g(W^{IN} \otimes X) \]  

(1)

Where \( X \) is input image, \( W^{IN} \) is a matrix of input weights, \( g \) is the non-linear activation function, \( W^{OUT} \) is a matrix of output weights, and the circle inscribed with an \( x \) represents the 2D convolution operator. Using a shift invariant setup for the non-linear filter allows for the fast implementation and training of the neural network.

The weighting matrices are determined by a simulated annealing\(^7\) optimization routine. A noisy training image is run through the non-linear filter and registered against an ideal noiseless image. The solution space is searched by randomly perturbing the weights and storing the filter parameters if they create a better match between the training images. The current best solution becomes the new center of a now reduced perturbation, and this process repeats over thousands of iterations eventually converging on a minimum error solution.

The results of this process can be seen in Fig. 5. A conventional fluorescence image of a moss leaf is shown in Fig. 5(a) and the cubically encoded version can be seen in Fig. 5(b). The standard linear deconvolution is performed to generate Fig. 5(c), the extended depth image. While the depth of focus has been increased, there is a significant amount of background noise. Finally, Fig. 5(d) shows the moss leaf after the non-linear noise removal has been applied. Figure 6 shows a line profile of the moss leaf before and after the non-linear noise removal algorithm was applied. The signal appears approximately between pixels 100 and 300, while the rest of the profile shows the background. The contrast of the noise has been greatly reduced with little effect on the signal. The signal-to-noise-ratio for both the linear and the non-linear filtered images was computed as the mean value of the signal over the standard deviation of the noise. A 2.15 times improvement in SNR was observed after the non-linear algorithm was applied to the noisy image.

![Figure 5](image-url)

Figure 5. Fluorescence images of a moss leaf. The excitation wavelength is 546 nm and the emission is observed for wavelengths greater than 590 nm. The original image (a) shows a shallow depth of focus. It is then encoded (b) with a cubic phase mask and in (c) linearly filtered to extend the depth of field. This linearly filtered image was processed by the non-linear algorithm to generate (d) the reduced noise image.
5. CONCLUSIONS AND FUTURE WORK

The PSF engineering approach to extended depth of field microscopy was improved in two distinct ways. Firstly, it was shown that a cubic PSF can be achieved through the use of adaptive optics. The use of a deformable mirror in conjunction with a Shack-Hartmann wavefront sensor to implement EDF can provide a test bed critical to the future design and evaluation of phase shapes which more clearly separate the signal from the noise. Secondly, a 2.15 times improvement in SNR of PSF engineered extended depth images was observed through the use of a non-linear noise removal filter. The algorithm identifies and removes the patterned noise in linearly filtered EDF images. Future work will include modifying the neural network training process by experimenting with other optimization routines, such as particle swarms or genetic algorithms. The image training process will also be improved by utilizing merit functions which more closely reflect metrics associated with human vision. The non-linear filter will also be adapted to perform both the deconvolution and noise removal simultaneously, removing the need for the linear filtering step all together.

REFERENCES

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