Noise removal in extended depth of field microscope images through nonlinear signal processing

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Abstract: Extended depth of field (EDF) microscopy allows for real-time 3D imaging of live cell dynamics. EDF is achieved through a combination of point spread function (PSF) engineering and digital image processing. A linear Wiener filter has been conventionally used to deconvolve the image, but it suffers from high frequency noise amplification and processing artifacts. A nonlinear processing scheme is proposed which extends the depth of field while minimizing background noise. The nonlinear filter is generated via a training algorithm and an iterative optimizer. Biological microscope images processed with the nonlinear filter show a significant improvement in image quality and signal-to-noise ratio (SNR) over the conventional linear filter.

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References and links
1. Introduction

Real-time 3D microscopy is an essential tool for biologists and medical researchers investigating live-cell dynamics. Microscope modalities such as confocal microscopy and wide field deconvolution can provide 3D imaging, but require a lengthy multi-focal-plane acquisition process that limits real-time imaging [1-3]. By comparison, extended depth of field (EDF) microscopy simultaneously records information from multiple planes within the specimen in a single image, without the need to adjust focus [4-6]. This readily achieves biological imaging at video rates or faster. Extended depth of field microscopy also has the potential to expand into new, quantitative 3D imaging modalities [7,8].

The EDF microscopy system described here relies on the technique of point spread function (PSF) engineering. Specifically, it makes use of a specially engineered phase function, fabricated into an optical element that is inserted at the back aperture of the objective, shown in Fig. 1. The phase function creates a focus invariant PSF over approximately 10 times the standard depth of field (DOF) [4-6]. In principle, a single deconvolution filtering operation (such as a linear Wiener filter) [9] is all that is needed to restore the information from the entire depth in the sample back to diffraction-limited resolution in the resulting EDF image.

![Fig. 1. Diagram of an EDF microscope. A phase mask is placed at the exit pupil of the imaging objective. The phase mask creates a focus invariant PSF that blurs the signal through an extended depth. Resolution is restored through a deconvolution filter, creating an image with up to 10 times the depth of field.](image-url)

To illustrate the concept of extended depth of field microscopy an example is shown in Fig. 2 with a phase plate that has a cubic profile in both x and y [4-6]. The top row, Fig. 2(a), shows a simulation of a traditional PSF from a 0.5 numerical aperture (NA) objective, with
the far left being best focus. As defocus increases the PSF blurs significantly. The middle row, Fig. 2(b), simulates the effect of inserting a cubic phase plate at the back aperture of the microscope objective. The same cubic phase function was used for all simulations and experimental results presented due to it being readily available. However any of a number of phase functions can be used to extend the depth of field, and each one offers unique advantages and disadvantages [7,10]. The simulated cubic phase function has a peak delay of 20 waves measured across the x-y diagonal. The PSFs now exhibit a distinct blur, but their transverse profiles remain largely invariant through focus. This invariance allows for a single, digital deconvolution filter to be applied to the captured images of the encoded PSF to remove the blur introduced by the cubic phase plate. The bottom row, Fig. 2(c), shows the filtered images that have all maintained a sharp focus throughout an extended depth.

Fig. 2. Illustration of EDF microscopy with a cubic phase mask. The top row (a) shows a simulated traditional PSF with best focus at the left. As defocus increases the diffraction limited spot quickly blurs. The simulated cubic PSF simulated in the middle row (b) is largely invariant through focus. A single linear deconvolution filter can then be applied to all of the images of the engineered PSFs to remove the effect of the cubic phase plate. The bottom row (c) shows the filtered PSFs from varying focal planes that have all been restored to a sharp focus thus extending the depth of field.

The main advantage of EDF in biological microscopy relies on the principle that a single filter can be generally applied to deconvolve a variety of specimens thus allowing for real-time, video-rate imaging. However, in practice, because some amount of noise is always present in the recorded image, a linear filter does not produce particularly good results. A given Wiener filter, shown in Eq. (1), can only be optimized to minimize noise at specific frequencies, \( \nu \), and magnitudes for a given object.

\[
F(\nu) = \frac{H^*(\nu)}{|H(\nu)|^2 + \frac{N(\nu)}{S(\nu)}}
\]

(1)

The Wiener frequency-domain deconvolution filter, \( F \), is found by taking the ratio of the complex conjugate of the PSF Fourier transformed into frequency space, \( H^* \), to the square of the PSF Fourier transformed into frequency space, \( H^2 \), plus a term often referred to as the Wiener parameter. This parameter is defined as the mean power spectral density of the noise,
N, over the mean power spectral density of the signal, S, and is used to optimize the filter for a specific signal-to-noise ratio (SNR) power spectral density. As a result it will amplify noise for any SNR distribution in frequency for which it is not properly tuned. This severely limits its ability to be generally applied to EDF microscopy where the signal and noise characteristics are not always well known, and can be difficult to estimate. In live-cell applications the signal is often rapidly changing making it difficult to create an optimal filter for the complete data set. In addition, the Wiener filter is also based on a least squares approximation that produces ringing artifacts which obscure fine detail and reduce lateral resolution [9]. These properties become even more problematic in EDF systems which are well-known to reduce image SNR by the very nature of their PSF engineering process [4-6].

To demonstrate this in experimental data, a moss leaf is imaged in traditional fluorescence, Fig. 3(a). The limited depth of field causes significant blurring. The leaf was then reimagined with a cubic phase function, Fig. 3(b), to extend the depth of field. The linearly filtered EDF image, Fig. 3(c), shows a significant amount of processing noise. This is due to the fact that the object and noise power spectral densities cannot be truly known, so the Wiener parameter cannot be tuned optimally. Ringing artifacts are also seen around the outer edges of the leaf cells and these can also be attributed to the linear 2D deconvolution filtering process.

Fig. 3. Linear noise amplification and processing artifacts in a biological sample. (a) Fluorescence image of a moss leaf taken with a traditional microscope. The limited depth of field causes most of the cells to appear out of focus. (b) Fluorescence image of same leaf encoded with a cubic PSF (before processing). Now features throughout the leaf volume appear uniformly blurred. (c) EDF image obtained by applying a linear filter to (b). The depth of field has clearly been extended as cell walls are now visible throughout the leaf. However, the linear filtering process has introduced significant background noise and ringing artifacts (i.e. parallel lines at leaf boundaries).

While this does produce the best mathematical approximation, the ringing artifacts are easily detected by the human eye and thus make the linearly filtered EDF images unsuitable for analysis by biological and medical researchers.

1.1 Frequency domain analysis of the Wiener filter for EDF imaging

The basic function of the linear filter in the frequency domain is to restore an EDF encoded optical transfer function (OTF) to the ideal diffraction limited OTF. The linear filter is effective at deconvolving noiseless images, or images for which it has been tuned to a specific noise level and signal distribution, but it can’t be generically applied with optimal performance. This results in the distinct, patterned background noise commonly seen in linearly filtered EDF images. Figure 4 illustrates this concept with a simulated cubic modulation transfer function (MTF), which is the modulus of the OTF. Increasing amounts of Gaussian white noise were added to simulate the physical, system frequency response (SFR). For this paper, all simulations of noisy data are done with additive Gaussian white noise (AWGN). The cubic system frequency response can be perfectly restored to diffraction-
limited (ideal circular pupil) performance in the noiseless case, Fig. 4(a). When the noise and signal frequency characteristics are not well known, as is often the case in live-cell biological observations, the Wiener filter cannot be tuned for optimal performance, and even 5% background noise is enough to significantly compromise the reconstruction, Fig. 4(d). Here values of the linearly filtered SFR above the ideal SFR curve represent noise amplification, while values below the ideal SFR curve represent lower than ideal contrast. Similar noise amplification and artifacts are seen regardless of whether the filter is applied in the space or frequency domain.

Fig. 4. Noise amplification from the linear filtering process for a cubic PSF. When no noise (a) is present, the cubic SFR is perfectly restored to a diffraction-limited (ideal SFR) condition. As the noise increases to (b) 1% and (c) 2.5% the reconstruction becomes less reliable. At (d), 5% noise, the linear filter has severely reduced the resolution of the EDF image. Note that the spikes and rapid fluctuations in these filtered SFR plots represent errors in the fidelity of signal (image) reconstruction at each spatial frequency in the image.

Noise and ringing artifacts that appear in linearly filtered EDF microscope images are the primary reason why this technology has not yet obtained widespread use in the biological microscopy community. To overcome the limitations of Wiener type filters, we have developed a nonlinear filtering scheme that minimizes the background noise and artifacts in the post-processed image. The nonlinear filter is implemented using a neural network in conjunction with a training algorithm and an iterative optimizer to find the best filter parameters.

2. Nonlinear filtering for deconvolution and noise removal

To overcome the aforementioned problems, a shift-invariant neural network was utilized to nonlinearly filter the EDF images. Nonlinear filtering offers a distinct advantage over linear processing in that it is capable of producing a null output for the noise while reconstructing the signal [11,12]. Neural networks have been successfully demonstrated in similar noise reduction and processing applications in biological imaging systems [13-18].

In this study, the neural network was combined with an iterative search algorithm, an optimizer, and training images, to determine the ideal filter parameters for a given EDF
engineered PSF. Numerous permutations of training and optimizing algorithms were explored. A detailed description of the processing schemes and training algorithms follows.

2.1. Structure of the shift-invariant neural network

The shift-invariant neural network consists of three distinct processing layers: the first layer is a weighted sum of the pixels in the input image, the second layer filters the weighted input pixels using a nonlinear, sigmoid activation function, and the third layer performs a weighted sum on the output from the filtering layer. The shift-invariance allows the weighted sums to be applied using the two-dimensional convolution operations shown in Eq. (2):

$$Z = W^{OUT} \otimes g(W^{IN} \otimes X),$$

where $Z$ is the filtered output image, $W^{IN}$ and $W^{OUT}$ are the input and output weights of the filter, $\otimes$ represents a 2D convolution, $g(\cdot)$ is the sigmoid activation function, and $X$ is the EDF encoded input image. The size of $W^{IN}$ and $W^{OUT}$, which are square weighting arrays, is determined by the size of the engineered PSF (in pixels) used to extend the depth of field. When the two arrays are convolved together their size should match that of the cubic PSF (or other EDF PSF used). This size limitation prevents pixels from beyond the extent of the encoded PSF from having an effect on pixels within the PSF extent throughout the filtering process. The shift-invariant configuration mandates that each pixel is weighted using a single input array and a single output array. This approach limits the number of variables that need to be optimized and provides a computationally fast way to find the ideal nonlinear filter (i.e. convolution operation). A simplified diagram of the entire three-layer neural network is shown in Fig. 5.

Fig. 5. Diagram of the three layer, shift invariant, neural network. This simplified example shows pixels from an input image, $X$, as they are weighted, summed, and operated on to create the non-linearly filtered pixels of the output image, $Z$. The entire input image, $X$, is convolved with the input 2D weighting array, $W^{IN}$, where the elements of the array are represented by lower case letters (a, b, c, etc.). The weighted input image pixels, $M$, are then operated on by a sigmoidal activation function, $g(*)$. The output of the activation function, $Y$, is then convolved with the output 2D weighting array, $W^{OUT}$, where the elements of the array are represented by the lower case letters (e, d, f, etc.). This generates the final non-linearly filtered image, $Z$.

The activation function, $g(\cdot)$, has a sigmoid shape, has a minimum value of 0, a maximum value of 1, and lies on the interval [0,1]. The functional form is shown in Eq. (3):

$$Y_j = g(M_j) = \frac{1}{1 + e^{-2r(M_j-c)}},$$
where $M_j$ represents a given pixel entering the second layer of the neural network, $r$ determines the steepness of the transition, $c$ sets the center point of the transition, and $Y_j$ represents a given output pixel of the second layer of the neural network. It has been shown that a three layer neural network structured using this general framework has the ability to approximate any nonlinear function \cite{11,12}. Thus, this filtering scheme has the capability to reconstruct high-resolution EDF images despite large amounts of background noise.

2.2. Training the neural network

The best fit values for the input and output weighting matrices for a given PSF are found through the use of a training image and an optimizer. The training image used to determine the ideal value for each element in both weighting arrays was a resolution bar target with increasing frequency components. A sample set of these images can be seen in Fig. 6. The original image is seen in Fig. 6(a). It is convolved with an experimentally measured cubic PSF, imaged on a 20x/0.5NA Zeiss objective. The PSF used was averaged over 15 measurements to reduce the noise introduced by the experimental data. White Gaussian background noise is then digitally added to simulate an encoded EDF image, Fig. 6(b). The image is non-linearly filtered to create Fig. 6(c) and compared to the original. A figure of merit is assigned to each filtered image and the optimizer appropriately modifies the filter parameters to minimize the error as it iterates. The PSFs measurements were obtained by imaging a 0.5μm pinhole though a cubic phase plate with a peak delay of 20 waves along the x-y diagonal. An experimental PSF is used over a theoretical PSF because the aberrating effects of a given microscope objective cannot be incorporated into the simulation. The exact prescription of the imaging objective is a significant unknown. Also slight defects exist in the cubic phase plate that cannot be easily incorporated into the theoretical simulation. In both the linear and nonlinear filters the experimentally measured PSF has empirically produced more accurate results.

![Fig. 6. Training images used to determine the optimal value for the weighting arrays in the nonlinear filter. The original, diffraction limited resolution target (a) is convolved with a cubic PSF and background noise is added to simulate an encoded EDF image. The nonlinear filter is applied (c) and an optimizer adjusts the filter to minimize the difference from the original. A cubic PSF from a 20x/0.5 NA objective was used for the training.](image)

While the cubic PSF is largely focus invariant over an extended depth, there are slight variations in its profile through depth. To accurately represent the 3D evolution of the cubic PSF, an array of training images was used. Images in the array are convolved with experimentally measured cubic PSFs from 5 equally spaced planes in depth centered on best focus. The planes stretch evenly over the full extended depth (focus invariant) region of the engineered PSF, and each plane is given a weight in the merit function. PSFs closer to the estimated best focus position are given a higher weight than PSFs near the upper and lower boundaries of the extended depth of field. The same principle is also applied to training
images with 5 increasing amounts of background noise at 5 different levels. Ultimately, this creates a 5x5 matrix of images to train the nonlinear filter. The columns of the matrix represent cubic PSFs different focal planes evenly spaced from -10μm to +10μm about best focus. The rows represent increasing amounts of digitally added Gaussian white noise evenly spaced from 0-20%. This helps to generalize the filter for both varying amounts of noise and slight through focus variations in the cubic PSF.

2.3. Optimizer

The optimizer consists of two parts: a search algorithm and a merit function. The search algorithm iteratively perturbs the values in the weighting arrays in order to find the ideal values for a given PSF. The magnitude of the perturbations decreases with each iteration, eventually converging on a solution. The merit function determines the quality of each perturbation and directs the search algorithm towards weighting array values that minimize the error in the filtered training image from the original.

The particle swarm [19] and simulated annealing [20,21] algorithms were compared to find the ideal search algorithm for the neural network optimizer. Every solution space contains a unique layout that can create challenges for a given search algorithm. For the given solution space, simulated annealing proved to be the more effective algorithm of the pair. The optimizer was able to converge on a solution within a few thousand iterations depending on the size of the filter. Simulated annealing consistently produced a lower figure of merit in less time than the particle swarm algorithm. The simulated annealing algorithm was therefore used for all of the results shown in Section 3.

The merit function is designed to reduce noise and minimize artifacts. Namely it works to suppress small ringing errors around image features with sharp edges, as these artifacts are easily detected by the human visual system. The figure of merit is calculated by taking the mean square error between the values of the nonlinearly filtered training image, Fig. 6(c) and the ground truth training image, Fig. 6(a), for all pixels. To prevent ringing artifacts, pixels located on either side of the edge of a feature in the training image (i.e. sharp light to dark transitions at the resolution bars) are weighted 12 times heavier than the rest of the training image. This makes the small ringing errors before and after the transition more important to the figure of merit -- hence they are more heavily suppressed. The relative strength of the weights (12:1) was found by iteratively adjusting their magnitude over multiple trainings to find the point where the ringing artifacts were no longer perceptible to the human eye. The mitigated ringing artifacts come at the expense of larger absolute errors on the transition itself, but these errors are masked by human visual processing. So even though it may not be the best mathematical fit, the images will be easier for the human eye to interpret. This is an important feature since biological and medical researchers will ultimately be evaluating these images qualitatively. Any slight loss in resolution is outweighed by the increase in ease of visualizing and interpreting features that have no distracting artifacts.

3. Results

A series of biological extended depth imaging experiments were conducted to compare the performance of the new nonlinear filter with that of the previously used 2D (linear) deconvolution approach. For these experiments a cubic phase plate made from diamond turned glass was mounted in a slider and inserted at the back aperture of a 20x/0.5 NA objective on an upright Zeiss microscope (in the same slot commonly used for differential interference contrast (DIC) prisms). The phase plate is same one described in Section 2.2 that was used for training the neural network.

The nonlinear filter was created using the optimizer and the simulated annealing search algorithm. The optimizer was allowed to run for 10,000 iterations and it trained the neural network using the matrix of training images described in Section 2.2. The parameters used in the activation function were \( r = 2 \) and \( c = 0.9 \). The values for these parameters were adjusted...
iteratively over multiple trainings and were found produce the best results. The input array was 11x11 pixels and the output array was 9x9 pixels. It should be noted that the size of $W_{IN}$ with respect to $W_{OUT}$ has a minimal effect on the filter as long as they are similar in size. Their relative sizes are largely determined by where the optimizer starts assigning weighting values near 0 in magnitude. The input and output weighting arrays, which correspond to $W_{IN}$ and $W_{OUT}$ from Eq. (2), are shown in Fig. 7. These arrays yielded the minimum error according to the merit function.

![Input and Output Weighting Arrays](image)

Fig. 7. Input weighting array, $W_{IN}$, (a) and output weighting array, $W_{OUT}$, (b) for the shift-invariant neural network.

The above solution was tested quantitatively with simulated resolution charts and qualitatively with live biological samples. The charts provided information on the resolution of the filter and the SNR. The biological samples demonstrate the filter’s effectiveness in producing high-resolution live-cell images with minimal noise and artifacts.

### 3.1. Spatial frequency and noise analysis

In order to test the quality and noise reducing properties of the nonlinear filter at high resolution a series of resolution targets were simulated with incrementally increasing amounts of white Gaussian noise. The resolution target starts with bars of a high spatial frequency, 4.29 cycles/µm, and incrementally decreases their width until the diffraction limit is reached. The cutoff frequency of the imaging system used was 1.40 cycles/µm. The original object, Fig. 8(a), was convolved with the experimentally obtained cubic PSF associated with a 20X/0.5NA. Figures 8(b) and 8(c) show the linear and nonlinear filter respectively in the noiseless case. The bandwidth of both filters has been matched resulting in the loss of the highest spatial frequency. The nonlinear filter shows superior contrast when compared to the Wiener filter. Figure 9 shows the same resolution chart with 7.5% additive Gaussian white noise both linearly, (a), and nonlinearly filtered, (b). The nonlinearly filtered image is faithfully reconstructed out to near the cutoff frequency and shows minimal effects from the noise. The linearly filtered image has amplified the background noise, corrupting the image and reducing resolution of the highest spatial frequencies even though the Wiener parameter has been tuned to minimize noise. The linearly filtered image also exhibits ringing artifacts at the edges, although it is difficult to distinguish this from the noise. The nonlinear filter does exhibit some edge artifacts as seen in Figs. 6(c), 8(c), and 9(b). These artifacts are significantly mitigated when compared to the ringing generated by the Wiener filter. A number of techniques exist to minimize the impact of the nonlinear filter artifacts further. Training the neural network over a larger number of iterations, altering the weights in the merit function, or modifying the scaling parameters of the activation function all have the capability to improve the performance of the nonlinear filter and is an ongoing subject of
study. The effort placed on minimizing these artifacts is largely a function of the resolution and image quality required for a given application.

Fig. 8. Comparison between the linear and nonlinear filter on a simulated resolution target in the noiseless case. The diffraction limited bar chart (a) was convolved with a cubic PSF. The target was then both linearly (b) and nonlinearly (c) filtered. Both filters have been matched in bandwidth (the highest spatial frequency is lost). The nonlinear filter shows higher contrast than the linear filter in the ideal imaging case. The scale bar shown in (c) is 5 μm.

Fig. 9. Comparison of the linear and nonlinear filter with 7.5% additive Gaussian white noise. The linearly filtered image (a) exhibits a large amount amplified noise. The nonlinearly filtered image (b) maintains resolution at higher contrast at high spatial frequencies.

The series of resolution targets with increasing amounts of background noise were also used to calculate the signal-to-noise ratio. The linear filter does have a higher SNR in the low noise case; however as the noise increases the advantages of nonlinear filtering quickly become evident. A complete comparison of the SNR can be seen in Table 1. The SNR was computed as the ratio of the expected value of the signal to the standard deviation of the noise. For this comparison the Rose criterion [22] was used. The Rose criterion (SNR above 5) serves as the threshold for defining features with 100% certainty. The linear filter falls below this threshold when the PSF engineered image is buried in 10.0-12.5% background noise. The nonlinear filter however can go up to 20.0-22.5% background noise before the SNR drops below the Rose criterion. The nonlinear filter effectively doubles the amount of noise that can be present in an image prior to processing. There is also no need to tune the nonlinear filter for specific noise levels as is the case with the Wiener filter.
### Table 1. Comparison of signal-to-noise ratio (SNR) between the linear and nonlinear filter.

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<th>Nonlinear SNR</th>
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#### 3.2. Experimental results

The nonlinear filter was applied to images of *Oscillatoria* algae as a test of EDF imaging of live-cell biological samples. The algae were imaged with a 20X/0.5 NA Zeiss objective. The depth of field of the objective under normal operation is approximately 2.2μm. The algae were imaged in both bright field, shown in Fig. 10, and fluorescence, shown in Fig. 11, using the same filter parameters. Figures 10(a) and 11(a) show the algae imaged under normal operation of the microscope. Large portions are beyond the objective’s depth of field and show significant blur. Figures 10(b) and 11(b) show the strand of algae imaged with a cubic PSF. The depth of field has been extended, but the cubic blur can be clearly seen. The image was then processed with both the linear filter, Figs. 10(c) and 11(c), and the nonlinear filter, Figs. 10(d) and 11(d). The linearly filtered image shows a significant amount of background noise and ringing artifacts at edges. The nonlinearly filtered image has much less noise and no ringing artifacts. The neural net does however exhibit a shadowing artifact that can be seen around the signal in the brightfield image, Fig. 10(d). We believe this artifact results from the orientation of the features in the training image with respect to the structure of the cubic PSF. Future work will be conducted with more sophisticated training images that take into account the unique structure of the EDF engineered point spread function. An advantage of this PSF engineering and nonlinear filter approach is that the same filter can be used for both brightfield and fluorescence modes of the microscope. In addition, because the filter is created by an initial training step that needs to occur only once for each different microscope objective and phase plate, it can be implemented in milliseconds using a look up table allowing a wide range of biological objects to be imaged without having to create a new digital filter. Figures 11(e-f) show an enlarged view of the cell walls of the algae. The nonlinear filter has preserved the low contrast, high frequency modulation from the cell walls, while this information has been lost in the linear filter. As a note, the traditional images, Figs. 10(a) and 11(a) are not the exact same frame as the EDF images, Figs. 10 and 11(b-d) since the samples were live cell specimens and exhibited some movement during the experiment. There was a small delay of a few seconds between frames while the cubic phase mask was inserted for EDF imaging.
Fig. 10. Comparison of conventional vs. EDF microscopy of *Oscillatoria* algae imaged in brightfield with a 20x/0.5 NA objective. (a) Shows the algae under traditional imaging. The limited depth of field causes significant blur. (b) The algae is then imaged with a cubic phase plate. The DOF has been extended, but there is still an overall uniform blur imparted by the cubic phase mask. The image is then filtered linearly (c) and nonlinearly (d). Both filters deconvolve the cubic PSF, but the linear filter has significant background noise and ringing artifacts. The nonlinearly filtered image extends the DOF without amplifying the noise and with minimum artifacts.
Fig. 11. Comparison of conventional vs. EDF microscopy of *Oscillatoria* algae imaged in fluorescence. Sub-figures a-d follow the same progression as Fig. 10 using the same weighting arrays. Again, the nonlinearly filtered image shows improved SNR and fewer artifacts when compared to the linear filter. The nonlinear filter has also better preserved the low contrast, high frequency modulation from the cell walls when compared to the linear filter, (e) and (f). Note that a portion of the algae is still beyond the depth of field so is slightly blurred.

It should be noted that other recent attempts to reduce the noise and ringing artifacts produced by linear filters (i.e. by 2D non-iterative deconvolution filters) have met with some limited success. For example, the linear filter can be truncated to produce low noise images
with virtually no artifacts. However, a significant tradeoff in resolution occurs since it is effectively low-pass filtering the signal. By comparison, the nonlinear filter described here is able to maintain the high spatial frequency information without amplifying the corrupting background noise. Figure 12 shows the resolution tradeoff in the truncated linear filter approach compared to the nonlinear filter.

![Comparison between filters](image)

**Fig. 12.** Comparison between the full bandwidth linear filter (a), the truncated linear filter (b) and the nonlinear filter (c) in a fluorescence image of the same Oscillatoria algae. The bandwidth of the linear filter was iteratively shortened until the intensity of the ringing artifacts was less than 10% of the peak intensity value. Truncating the linear filter mitigates ringing artifacts and background noise, but at the cost of degraded resolution. The fine detail has been lost in the linearly filtered image, while the nonlinearly filtered image maintains resolution without the ringing artifacts or noise. Imaged with a 20x/0.5 NA objective.

### 4. Conclusion

Nonlinear filtering of extended depth of field images implemented via a shift-invariant neural network has significantly improved image quality compared to the Wiener filter. Amplified background noise and processing artifacts associated with the linear filter have been eliminated while maintaining near diffraction-limited resolution. The neural network can be trained to filter an EDF point spread function in minutes to hours depending on the number of elements in the weighting arrays. The generated filter can then be quickly and generically applied (in milliseconds) to any image encoded with the given extended depth of field PSF.

The nonlinear filter was evaluated quantitatively using simulated resolution targets and experimentally with samples of live algae. The quantitative analysis showed that the nonlinear filter can tolerate approximately twice the initial background noise before the SNR drops below the Rose criterion when compared to the linear filter. The resolution charts also showed that resolution is preserved to very near the diffraction limit. The experimental images of the Oscillatoria algae, in both brightfield and fluorescence, confirmed the simulated analysis. The nonlinearly processed images did not amplify the background noise or show any ringing artifacts at high frequency edges. The new filter also preserved the fine structure of the algae cell walls. The nonlinear filter does exhibit a slight shadowing artifact, although it does not severely impact the image quality. We believe this artifact to be a function of the training algorithm and will be addressed in future work.

These advancements show that we can now overcome the image processing noise and artifacts that have hindered the widespread use of EDF imaging in live-cell biological microscopy. Dynamic processes over an extended depth can now be observed in high resolution. The nonlinear filtering approach it also opens the door to further advancements in EDF microscopy, such as real-time, quantitative 3D imaging.

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