Z-Stacking vs Deconvolution for Microscopy

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Introduction

Despite continuous technological advancements, optical systems continue to be flawed. Defocus and aberrations inherent to a system affect the images produced by these systems. To combat this, a variety of techniques can be used and this project compares two of these: Z-stacking and deconvolution. Both are intended to be part of a larger system containing features like object size detection. As Z-stacking is a far faster method, one of the objectives is proving that it is possible to rely on a simpler method without sacrificing quality. Additionally, the combination of Z-stacking and deconvolution may provide even greater clarity than either method can provide on its own.

Deconvolution

A “true” image when captured through a microscope suffers quality loss due to optical imperfections. We can combine these imperfections in a function called the point spread function (PSF). The captured image can be thought of as a convolution of the true image and PSF. Hence to extract the true image we can apply deconvolution. As the name implies, deconvolution is the opposite of convolution, which can best be explained as multiplication in the frequency domain.

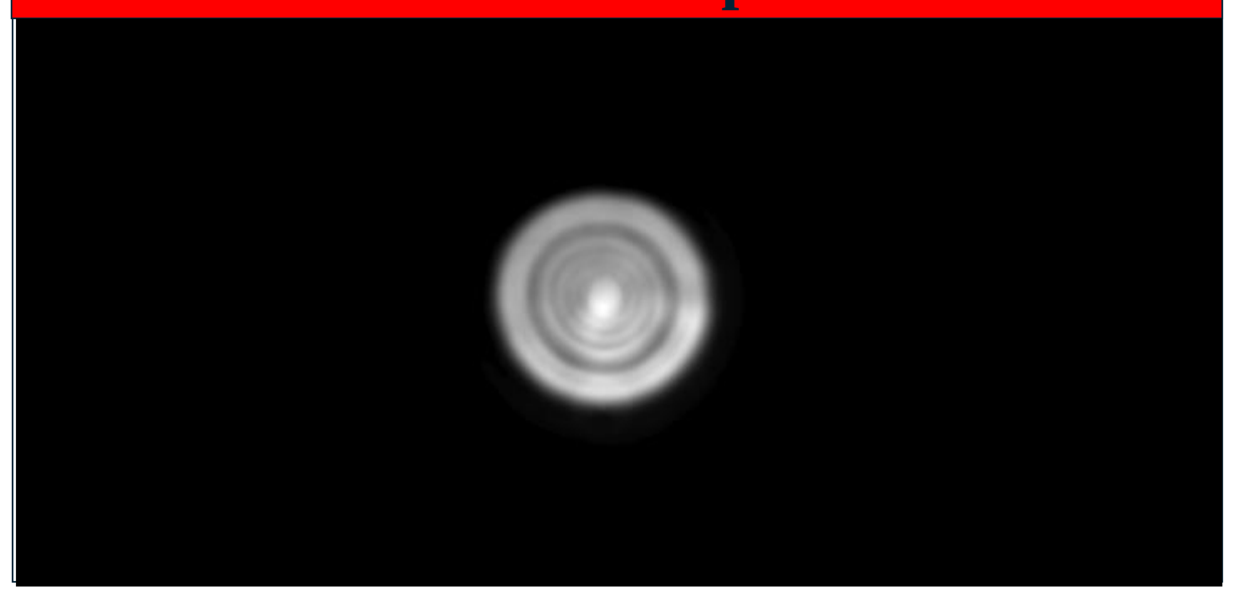
$$(f * g)(t) = F(t)G(t)$$

* denotes convolution and F(t) and G(t) are the Fourier transforms of arbitrary functions. Deconvolution is the inverse of this; division in the frequency domain.

In practice, simple deconvolution by division leads to computational problems. A basic approximation of a PSF would be a Gaussian distribution; as values get close to 0, this would lead to near-infinite values (or infinite if rounding occurs) when the calculation is made. As such, Wiener deconvolution is used. This computes a Wiener filter which adds a small value to the PSF, which relates to the signal-to-noise ratio.

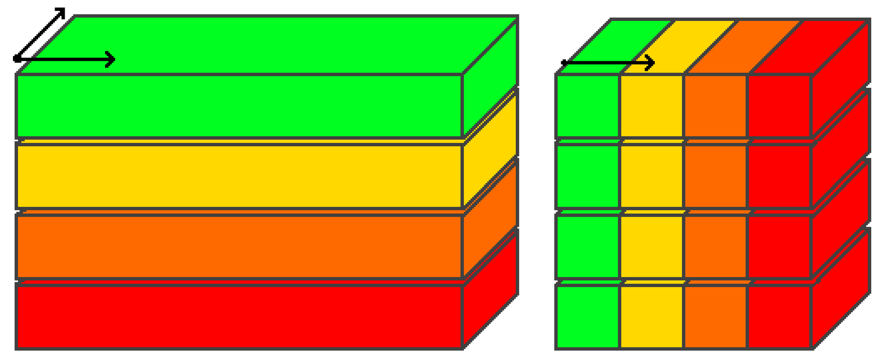
$$G(f) = \frac{PSF}{|PSF|^2 + 1/SNR}$$

PSF Example



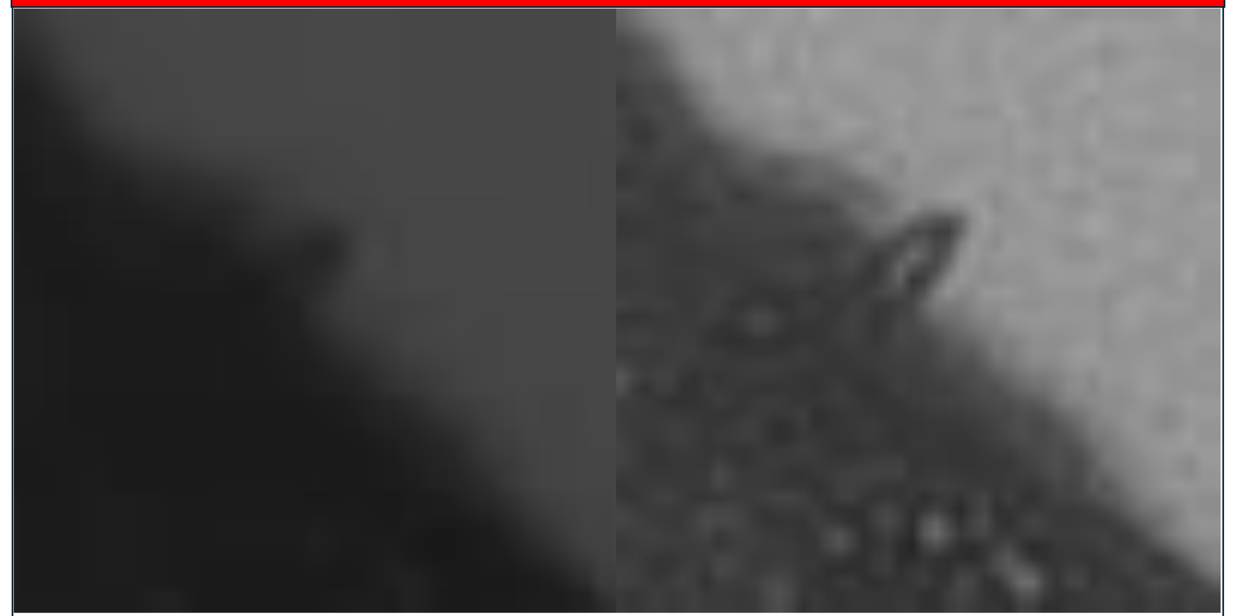
2-Dimensional vs 3-Dimensional

Using images captured at different focal planes, it’s possible to take advantage of the 3-D data using 3-D deconvolution to produce outputs of greater quality. Wiener deconvolution is not restricted to only 2 dimensions; it’s a general technique that works in any number of dimensions, including 3. To do this, a 3-D Discrete Fourier transform (DFT) is needed. The simplest way to perform one is through the row-column algorithm; a multidimensional DFT can be broken down into a set of 1-D DFTs in each direction. For 3-D, it can also be re-phrased to be a set of 2-D transforms followed by a set of 1-D DFTs in the perpendicular direction. This is called slab decomposition, and is used in the implementation.



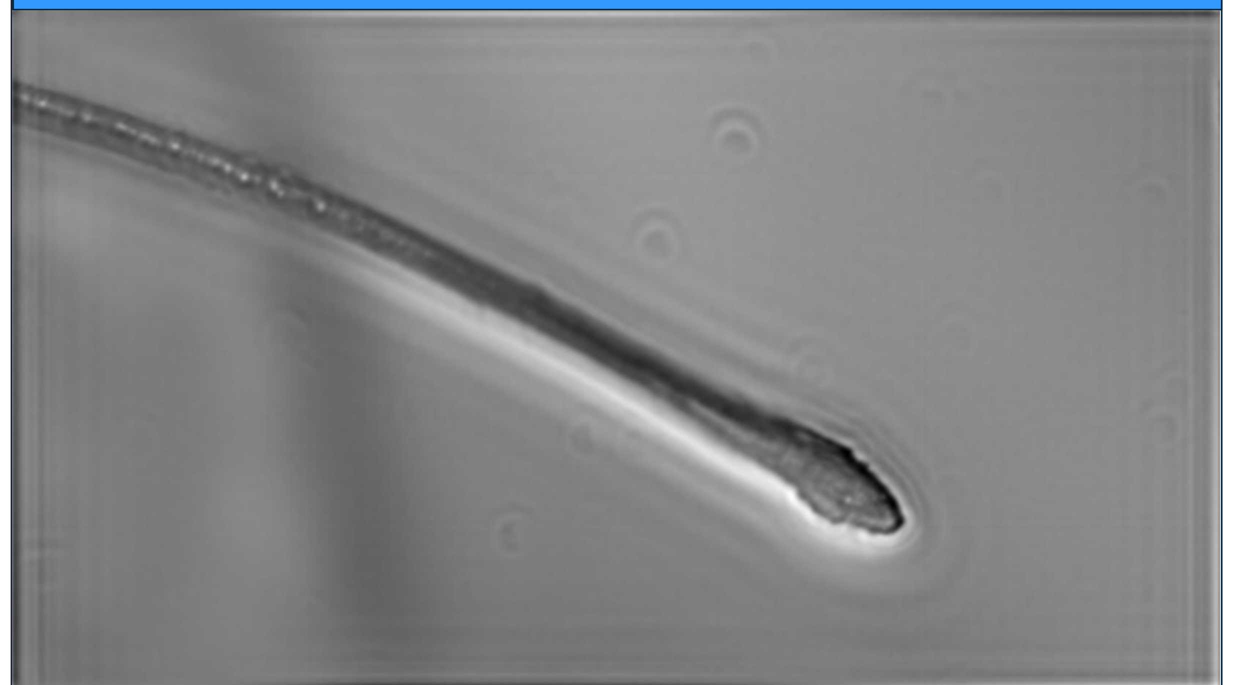
Testing Data at different focal lengths

2-D Deconvolution Result (Magnified)



Before After

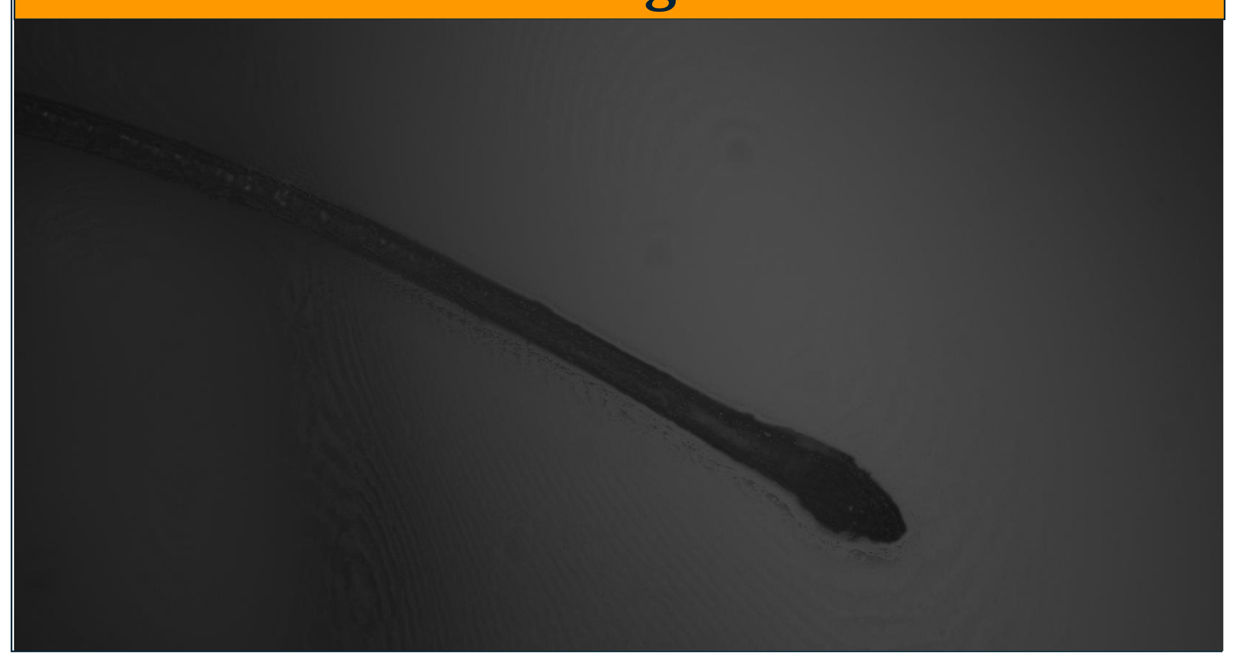
3-D Deconvolution Result



Z-Stacking

Unlike deconvolution, Z-stacking does not improve upon existing data. It seeks to combine the sharpest parts of a set of images into one cohesive image. In this implementation, it is done through one of two differential methods; Laplacian, or Sobel operators. These are 2nd and 1st derivatives respectively. The steeper the gradient in a region, the brighter the relevant pixel after the image has been through the differentiation. These “sharpness maps” can then be compared, and the sharpest parts can be combined into a final image. Should a tie occur, the results are averaged.

Z-Stacking Result



Combined 2-D Z Result

Combined 3-D Z Result

Performance

Table below shows the performance metrics of each technique for 5 and 10 layers. The time is shown in milliseconds and averaged over 10 measurements.

Technique	5 Layers	10 Layers
2-D Deconvolution	3410	6448
3-D Deconvolution	5434	13515
Z-stacking	341	934

Conclusion

The advantages of each method can be seen in the relevant results. 2-D deconvolution shows great promise for increasing the acuity of the input images, whilst 3-D deconvolution appears to marginally improve the quality of the images. Z-stacking excels in combining the sharpest elements of each image into one cohesive output, and the combined Z-stacking and 2-D deconvolution technique shows increased clarity when compared to the output of just Z-stacking. It appears that Z-stacking produces better results than 3-D deconvolution, however, this may partially be due to inaccuracies in the captured PSF. Finally, a combination of Z-stacking and 2-D deconvolution appears to have the most potential as a light-weight alternative to 3-D deconvolution.