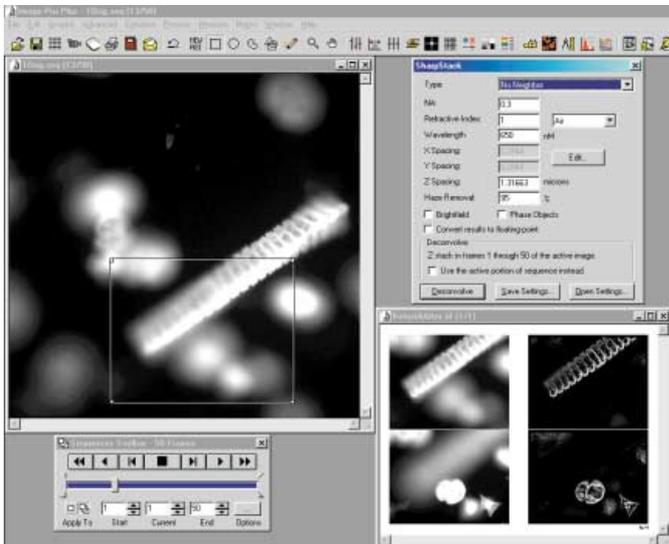# SHARPSTACK™ DECONVOLUTION

Plug-In Module for Image-Pro® Plus v4.5

One frame with an AOI selected in a Z-stack on the left with two AOIs and their deconvolved counterparts; de-focused light contribution from other planes has been eliminated

Overview

Obtaining clear images from a Z-stack is a challenge for microscopists. The SharpStack add-on module for Image-Pro Plus extracts clear, sharp images from a stack of hazy planes. Unlike other deconvolution solutions, SharpStack integrates seamlessly with image capture, 2D processing, analysis, and reporting within the world's most popular image analysis software, Image-Pro Plus. Nearest neighbor, no neighbor, and inverse algorithm functions are employed to sharpen one or all planes from a Z-stack.

Elucidating 3D Structures

Cells and tissues are three-dimensional structures. The observed image at the focal plane contains information from the plane on which the microscope is focused as well as out-of-focus contributions from the specimen above and below the plane of focus. Thus interpretation of the 3D structure of the biological specimen is hampered in optical imaging. The image at a given focal plane is a poor representation of a true section through the thick specimen. To remedy these problems and produce more reliable 3D data, digital deconvolution and confocal microscopy are widely employed.

Advantages over Confocal Microscopy

A confocal microscope uses a pinhole aperture to restrict the out-of-focus flare reaching a single detector, the photomultiplier tube (PMT). Because of the pinhole aperture, all the fluorescence signal collected by the objective lens is not used. In order to increase the signal, increased excitation laser intensity is used, but this will often introduce photobleaching and photodamage. Another disadvantage of confocal microscopy is its relatively high cost.

Digital Deconvolution

Unlike the confocal configuration, digital deconvolution microscopy uses the entire fluorescence signal collected by the objective lens without using pinhole to deliver the emitted light to high-sensitivity CCD cameras. The out-of-focus flare introduced into the imaging at different optical sections with its subsequent image degradation is reversed by computer deconvolution through the use of a pointspread function (PSF) of the imaging system. By modeling the microscope optics as a linear and shift-invariant system, the PSF can be used to describe the transformation of any image by the microscope. A typical fluorescence microscope image can be

[measured image] = [PSF] * [desired image]
where '*' symbol represents the mathematical operation of convolution. The deconvolution, the mathematical inverse of convolution can be represented as

$$[\text{desired image}] = [\text{measured image}] * [\text{PSF}]^{-1}$$
The goal of deconvolution is to solve the equation for the desired image.

The **Inverse Filter** is a one-step non-iterative approach based upon inverse filtering theory. The deterministic blurring as a convolution of the image with the point spread function can be modeled. In the frequency domain a convolution transforms into a multiplication of the Fourier transform of the sample with the optical transfer function. The optical transfer function (OTF) is the Fourier transform of the point spread function. The inverse filter then accomplishes image restoration by dividing the Fourier transform of the image by the OTF.
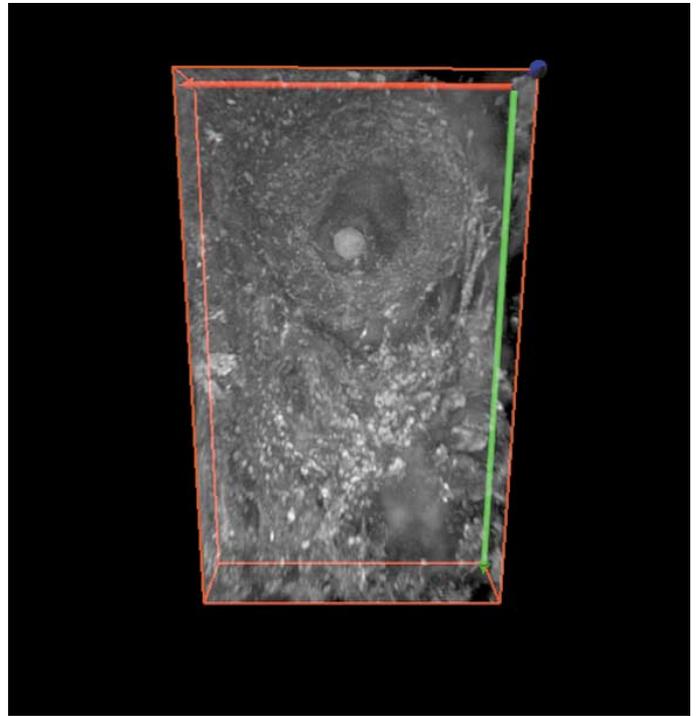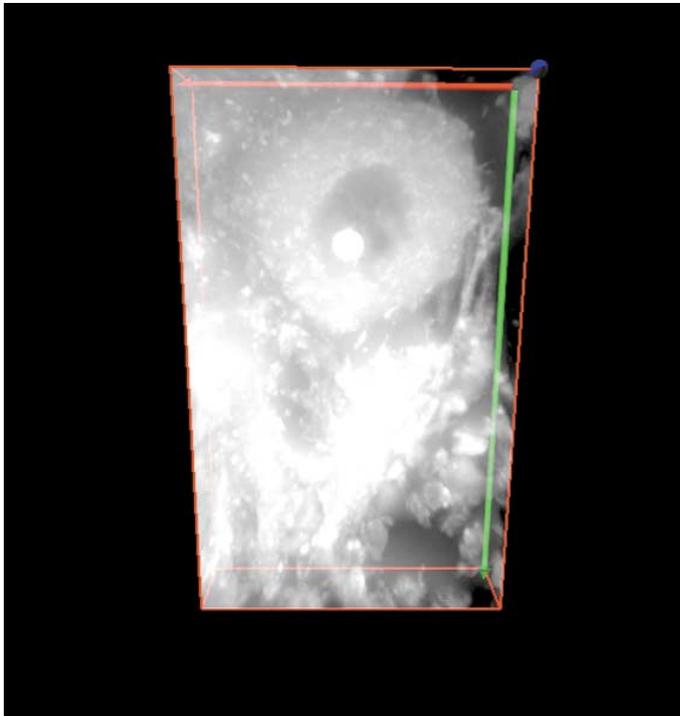
Pig cerebellum image stack deconvolved and rendered with SharpStack and 3D Constructor plug-in modules

The **Nearest Neighbor** algorithm works by deconvolving one image slice at a time. It uses information from image slices that reside above and below the image slice that is being processed. The precise increment slice position from the measured slice is user selectable. If the slices are chosen judiciously, this approximation will produce results very close to the inverse filter method but takes much less time.

The **No Neighbor** method uses the information from each single slice to construct a 2D PSF. This is the fastest but may not be as representative of the sample as the other methods.

SharpStack System Requirements
- Image-Pro Plus v4.5 or higher
- Pentium III or Athlon CPU, running at 450 Mhz or higher
- Microsoft Windows™ 98/ME/NT/2K
- 256 MB of RAM (512 MB recommended)
- 3 GB disk drive with available disk space for installation with additional space to accomodate four times the image size with four bytes/pixel
- Color monitor displaying 16-bit high color (24 or 32- bit color preferred)

SharpStack™
PLUG-IN
An Image-Pro® Solution

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From Images to Answers®

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