SharpStack®

Deconvolution Plug-In Module for Image-Pro® Plus

Overview

Obtaining clear images from a Z-stack is a challenge for microscopists. Confocal technology has made great strides in improving the situation, but it comes at a premium price. The SharpStack plug-in 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 Filter algorithm functions are employed to sharpen one or all planes from a Z-stack.

Elucidating 3D Structures

Cells and tissues are three-dimensional structures, so 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 other parts of 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.

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 2D 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.

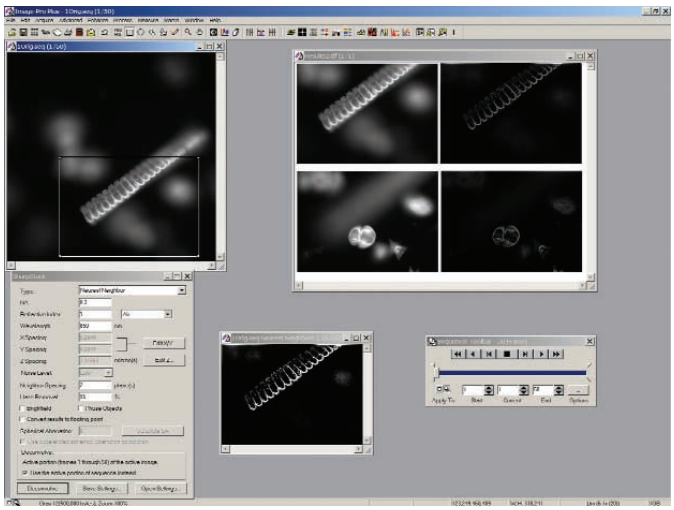


Figure 1. One frame with an AOI selected in a Z-stack on the left with two AOIs and their deconvolved counterparts; de-focused light contribution from other planes has been eliminated.

A typical fluorescence microscope image can be:

[measured image] = [PSF] * [desired image] where '*' symbol represents the mathematical operation of convolution. The deconvolution (*-1), the mathematical inverse of convolution can be represented as

[desired image] = [measured image] (*-1) [PSF]
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 pointspread 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 pointspread function. The inverse filter then accomplishes image restoration by dividing the Fourier transform of the image by the OTF.

The **Nearest Neighbor** algorithm works by deblurring 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.

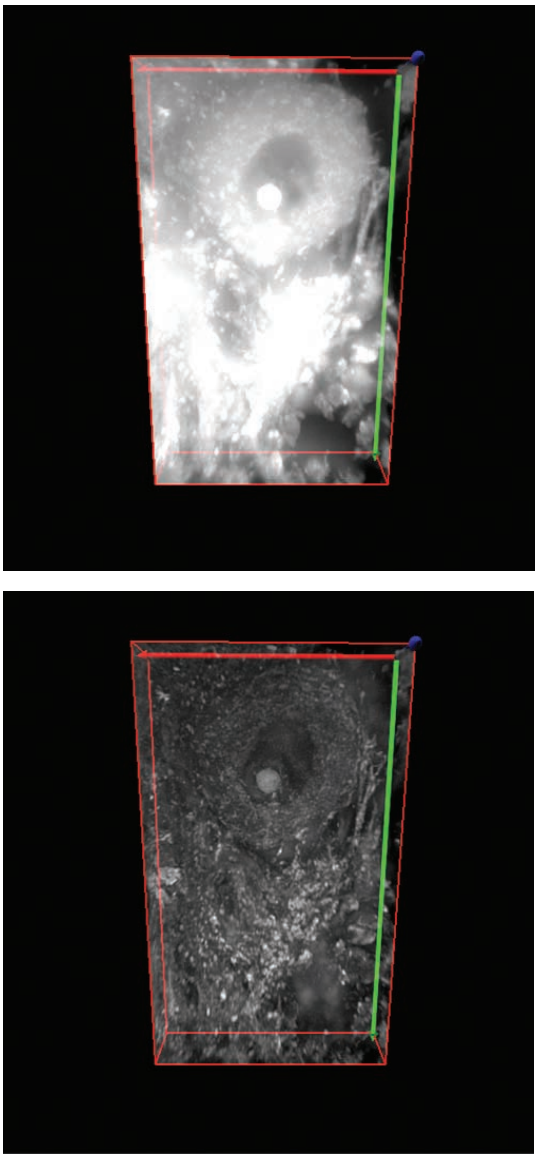


Figure 2. Pig cerebellum image stack deconvolved and rendered (bottom image) with SharpStack and 3D Constructor plug-in modules.

Spherical Aberration Correction

SharpStack also contains a method to correct for spherical aberrations. These result from the fact that the focal points of light rays far from the optic axis of a spherical lens are different from the focal points of rays of the same wavelength passing near the center (Figure 3). Depending on the severity and direction of the aberrations, it can result in PSFs similar to those in Figure 4, and images similar to those in Figure 5. Notice that spherical aberrations can cause a noticeable heavier tail on one side of the XZ projection of the image, depending on whether positive or negative spherical aberrations are found. Notice also that Figure 4 shows how spherical aberrations affects the convergence of rays, from converging to one point (Figure 3a; Figure 4c), to converging in multiple points (Figure 3b; Figure 4a,b; Figure 4e,f).

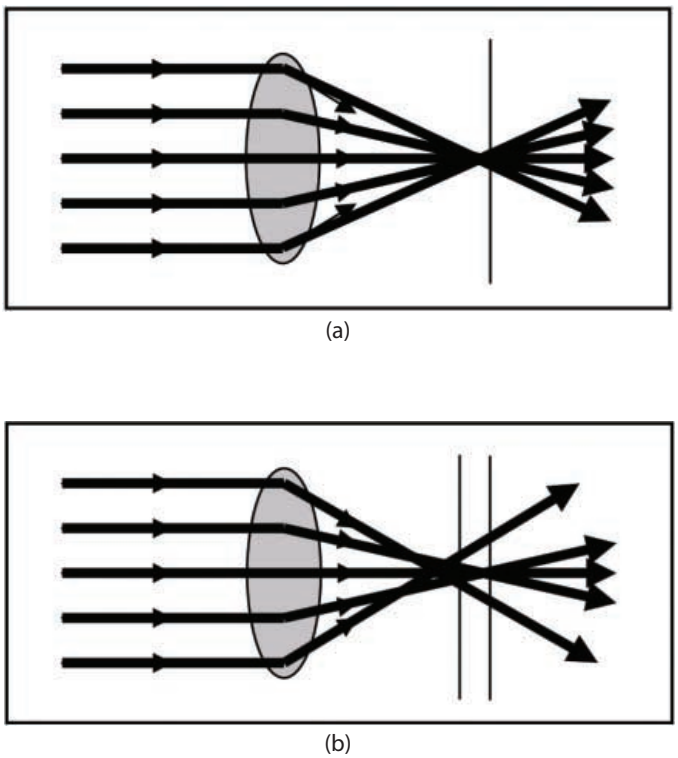


Figure 3. Optical system without (a) and with (b) Spherical Aberrations. Spherical aberration is caused by different focal points for rays far from and center and others closer to it (b).

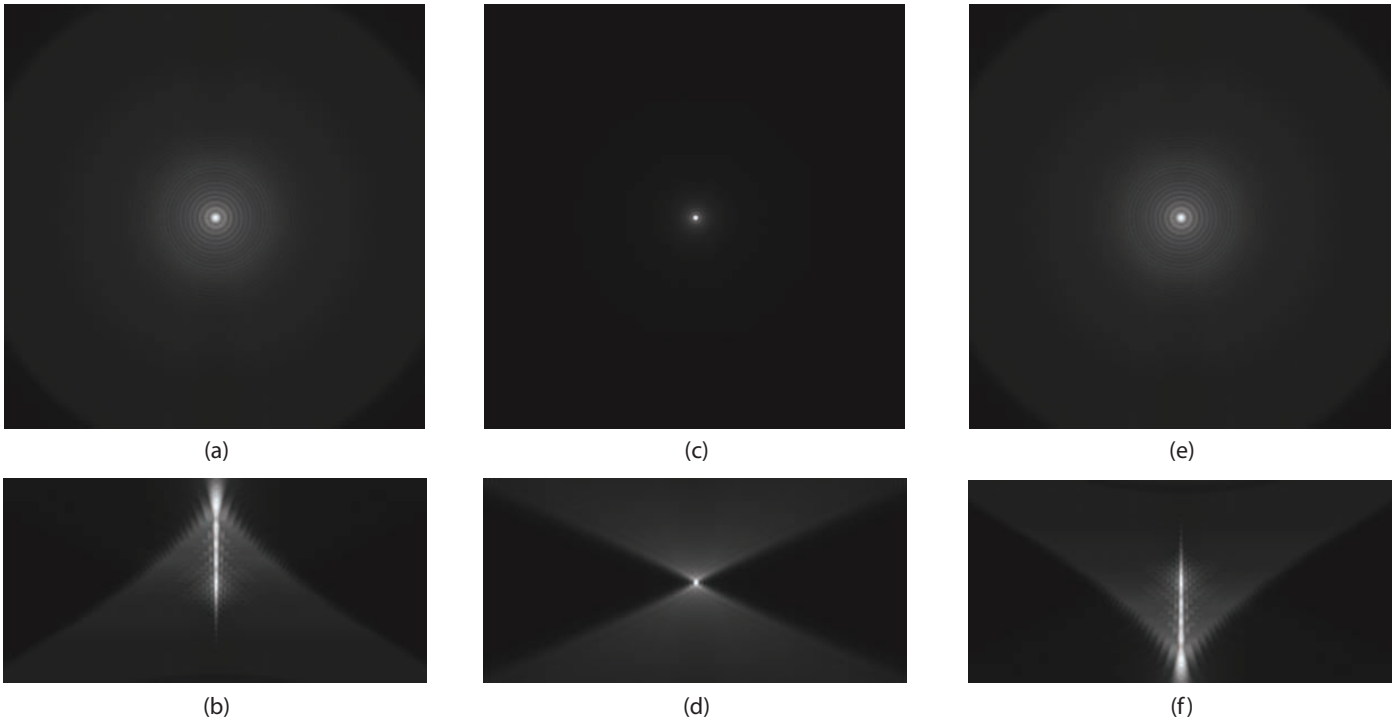


Figure 4. Spherically aberrated PSFs: a+b) XY and XZ maximum intensity projection of a PSF with negative severe spherical aberrations, c+d) XY and XZ maximum intensity projection of a PSF with no spherical aberrations, and e+f) XY and XZ maximum intensity projection of a PSF with positive severe spherical aberrations.

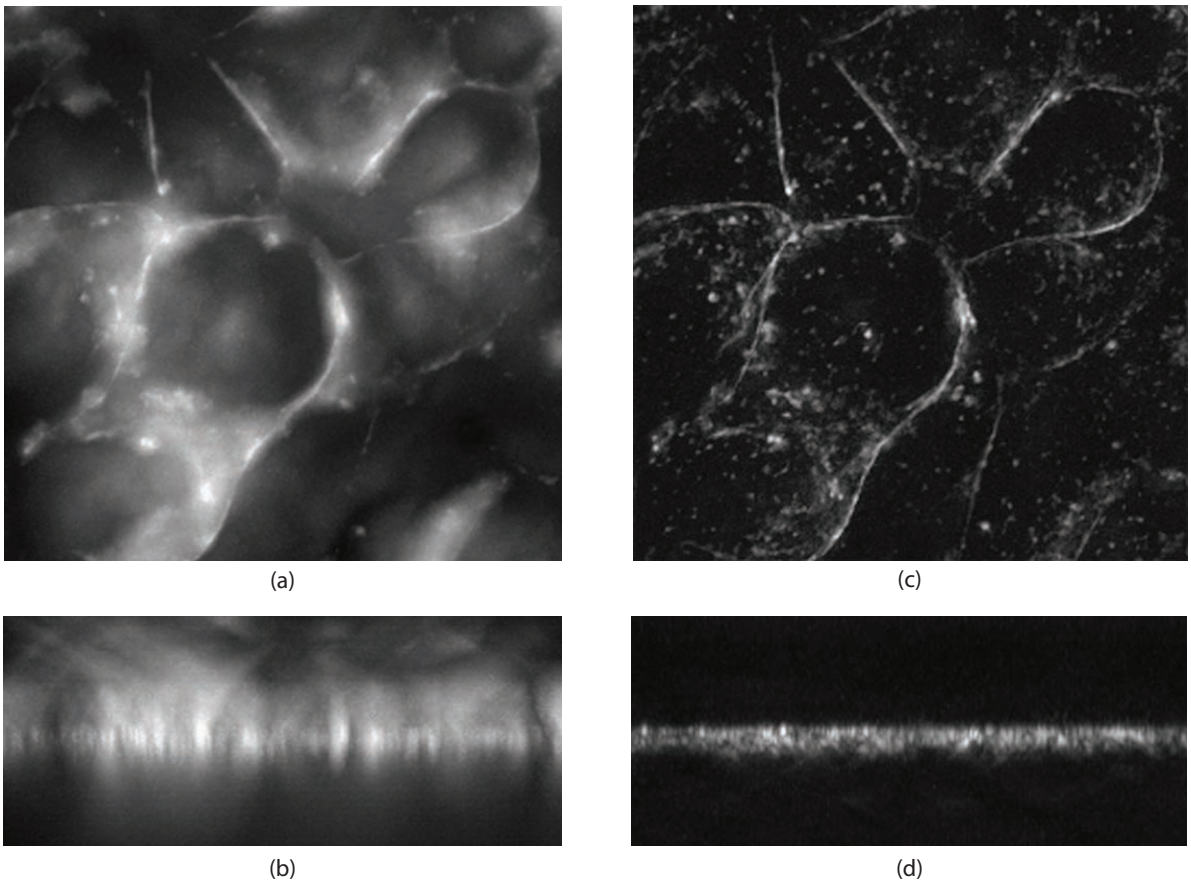


Figure 5. Example of spherically aberrated image: a) XY and b) XZ maximum intensity projection of the original image, c) XY and d) XZ maximum intensity projection of the deconvolved image with spherical aberration compensation (algorithm provided by AutoQuant Imaging, Inc.).*

*Courtesy of Diane Kube, Ph.D., Co-Director CF Imaging Core, CWRU Department of Pediatrics.

FROM IMAGES TO ANSWERS®

SharpStack System Requirements

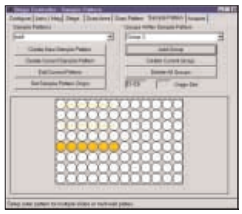
- Image-Pro Plus v4.5.1 or higher
- Pentium III or Athlon CPU, running at 800 Mhz or higher
- Microsoft® Windows 2000 and XP Professional
- 512 MB of RAM (1GB or better recommended)
- Disk drive for installation, images, and virtual memory (40GB or better recommended)
- Color monitor displaying 16-bit high color (24 or 32-bit color preferred

Image-Pro Plus Advanced Microscopy Software Suite

Image-Pro Plus offers the following seamlessly integrated plug-in modules for advanced multi-dimensional images:

Scope-Pro® PLUG-IN An Image-Pro® Solution

Scope-Pro enables you to control and program the movement of your automated microscope, stage, and peripherals in a simple, repeatable manner for reproducible results.



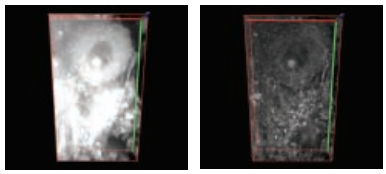
AFA™ PLUG-IN An Image-Pro® Solution

AFA allows you to manage all combinations of acquisition modes and image sets including:

- Time
- Channel (wavelength)
- Focus (Z-stack)
- Stage position

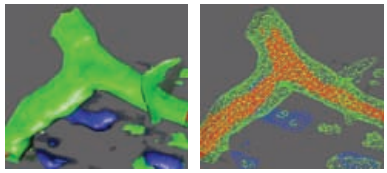
SharpStack® PLUG-IN An Image-Pro® Solution

SharpStack provides tools that allow you to extract clear, sharp images from a stack of hazy planes.



3D Constructor® PLUG-IN An Image-Pro® Solution

3D Constructor enables you to explore three-dimensional relationships within and among objects and includes interactive and automatic measurement capabilities in three and four dimensions.



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