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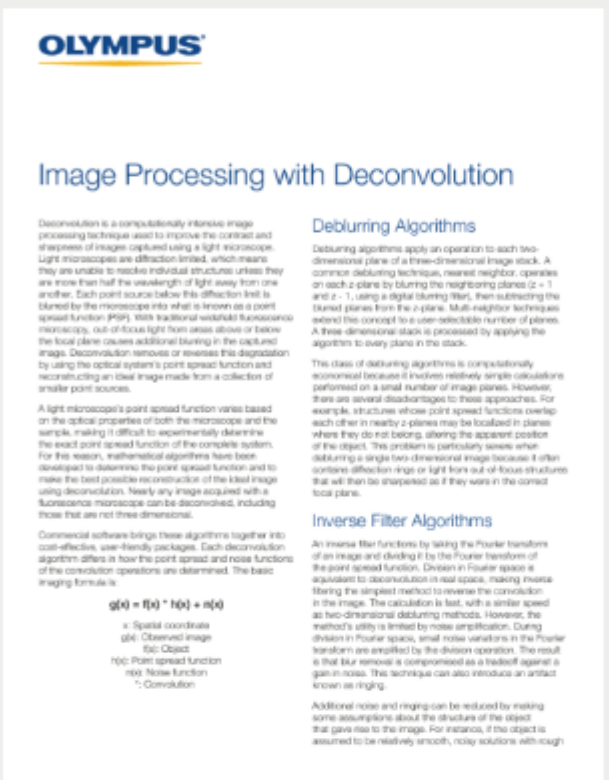
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Image Processing with Deconvolution

Deconvolution is a computationally intensive image processing technique used to improve the contrast and sharpness of images captured using a light microscope. Light microscopes are diffraction limited, which means they are unable to resolve individual structures unless they are more than half the wavelength of light away from one another. Each point source below this diffraction limit is blurred by the microscope into what is known as a point spread function (PSF). With traditional widefield fluorescence microscopy, out-of-focus light from areas above or below the focal plane causes additional blurring in the captured image. Deconvolution removes or reverses this degradation by using the optical system's point spread function and reconstructing an ideal image made from a collection of smaller point sources.

A light microscope's point spread function varies based on the optical properties of both the microscope and the sample, making it difficult to experimentally determine the exact point spread function of the complete system. For this reason, mathematical algorithms have been developed to determine the point spread function and to make the best possible reconstruction of the ideal image using deconvolution. Nearly any image acquired with a fluorescence microscope can be deconvolved, including those that are not three dimensional.

Commercial software brings these algorithms together into cost-effective, user-friendly packages. Each deconvolution algorithm differs in how the point spread and noise functions of the convolution operations are determined. The basic imaging formula is:

$$g(x) = f(x) * h(x) + n(x)$$

x: Spatial coordinate
g(x): Observed image
f(x): Object
h(x): Point spread function
n(x): Noise function
*: Convolution

Deblurring Algorithms

Deblurring algorithms apply an operation to each two-dimensional plane of a three-dimensional image stack. A common deblurring technique, nearest neighbor, operates on each z-plane by blurring the neighboring planes (z + 1 and z - 1, using a digital blurring filter), then subtracting the blurred planes from the z-plane. Multi-neighbor techniques extend this concept to a user-selectable number of planes. A three-dimensional stack is processed by applying the algorithm to every plane in the stack.

This class of deblurring algorithms is computationally economical because it involves relatively simple calculations performed on a small number of image planes. However, there are several disadvantages to these approaches. For example, structures whose point spread functions overlap each other in nearby z-planes may be localized in planes where they do not belong, altering the apparent position of the object. This problem is particularly severe when deblurring a single two-dimensional image because it often contains diffraction rings or light from out-of-focus structures that will then be sharpened as if they were in the correct focal plane.

Inverse Filter Algorithms

An inverse filter functions by taking the Fourier transform of an image and dividing it by the Fourier transform of the point spread function. Division in Fourier space is equivalent to deconvolution in real space, making inverse filtering the simplest method to reverse the convolution in the image. The calculation is fast, with a similar speed as two-dimensional deblurring methods. However, the method's utility is limited by noise amplification. During division in Fourier space, small noise variations in the Fourier transform are amplified by the division operation. The result is that blur removal is compromised as a tradeoff against a gain in noise. This technique can also introduce an artifact known as ringing.

Additional noise and ringing can be reduced by making some assumptions about the structure of the object that gave rise to the image. For instance, if the object is assumed to be relatively smooth, noisy solutions with rough

Image Processing with Deconvolution

In an effort to improve the contrast and sharpness of images captured with light microscopes, deconvolution has become a mainstay in the field of microscopy due to its ability to computationally achieve these goals without the need for additional optics or confocal pinholes. This white paper explains the differences between popular deconvolution techniques such as deblurring, inverse filtering, and constrained iterative methods to help determine which algorithms best suit your imaging needs.

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