

# Background of Deconvolution

## AutoQuant Technical Note 1

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### *Deconvolution*

Generally speaking, outside of the field of light microscopy: *Deconvolution* is an engineering discipline, which refers to the retrospective improvement of fidelity in electronic signals such as voice, music, radar and pictures. In light microscopy, deconvolution is used for 3D widefield fluorescence imaging, which uses an ordinary microscope and optical sectioning techniques [Holmes, 1992, 1995]. *AutoDeblur*<sup>®</sup> is AutoQuant's deconvolution software product. It is used not just for 3D widefield fluorescence. Applications include resolution improvement of 2D images, improvement of confocal micrographs (2D and 3D), transmitted light brightfield (2D and 3D), spinning disk (2D and 3D) microscopes such as the Yokagawa and Perkin-Elmer microscopes, and multiphoton microscopy (2D and 3D). Deconvolution improves the clarity of images by improving resolving power, removing out-of-focus haze and eliminating noise such as that caused by low light levels and electronic thermal noise from video cameras.

Deconvolution is based on the equation:  $g(x) = f(x) * h(x) + n(x)$ , where  $x$  represents the 3D spatial coordinate,  $*$  represents convolution,  $f(x)$  represents the ideal image stack of perfect fidelity,  $h(x)$  represents the optical point-spread function (PSF) [Holmes] (diffraction pattern) of the microscope,  $n(x)$  represents noise due to electronics and quantum photons and  $g(x)$  represents the image stack. Deconvolution recovers an estimate of  $f(x)$  from  $g(x)$ .

### *Types of deconvolution and deblurring*

#### *Iterative Constrained*

These methods iteratively update the recovered image. This is done by reblurring the picture(s) according to  $f(x)*h(x)$  and comparing this blurred picture against  $g(x)$ . The difference is computed and then used to update the estimated  $f(x)$ . The reblurring process is then repeated. These methods are *constrained* in that the solution of  $f(x)$  must be positive, since  $f(x)$  represents a light intensity and intensities are positive by definition. The earliest Iterative Constrained deconvolution was introduced to light microscopy by Agard [1989] who used the Jansson-van Cittert algorithm which was used originally for deconvolution of spectra.

#### *Maximum Likelihood Deconvolution*

Maximum likelihood deconvolution is a more recent improved subset of Iterative Constrained algorithms. The iteration is designed based upon a probability model. The mathematical solution is the  $f(x)$  which has the highest probability of being correct. The mathematics of this algorithm is based upon the behavior of quantum photon emissions and diffraction. Among all known approaches, the Maximum Likelihood approach has proved to provide the best quality images [Verveer]. T. Holmes (Cofounder and CEO of AutoQuant) was the first to introduce Maximum Likelihood Deconvolution to optical imaging while at the U. of Missouri [Holmes, 1988]. *AutoDeblur* is the first known commercial product to use this method.

#### *Blind deconvolution*

Blind deconvolution is a subset of Iterative Constrained algorithms which produce an estimate of  $h(x)$  concurrently with  $f(x)$ . It does not need the PSF  $h(x)$  to be measured. Other iterative constrained algorithms require  $h(x)$  to be measured by acquiring data from subresolution fluorescent beads.

Blind deconvolution was first introduced to the imaging community, outside of light microscopy by Ayers and Dainty [Ayers] and it was introduced to light microscopy by T. Holmes while at the Rensselaer Polytechnic Institute [Holmes, 1992]. *AutoDeblur* uses blind deconvolution and maximum likelihood estimation (MLE) together. AutoQuant Imaging Inc. provides the first and only blind deconvolution product. *AutoDeblur* uses Blind Deconvolution with MLE. Vendors sometimes make inaccurate claims that they are using a blind deconvolution, when they are really using a conventional

Iterative Constrained deconvolution having a theoretically calculated PSF rather than a measured one. It is a blind deconvolution only if the algorithm is producing the PSF from information within the data set  $g(x)$ . This is done by first assuming an  $h(x)$ , then (1), estimating which  $f(x)$  could have caused  $g(x)$ . This calculation is followed by (2), estimating which  $h(x)$  could have caused  $g(x)$  from the estimated  $f(x)$ , and then steps (1) and (2) are repeated again and again. It is believable that the PSF information is in the data because one often sees the light spreading from fine point or line structures in the data, and this spreading makes up the PSF.

### Linear methods

#### Inverse Filter

Linear methods run quickly on a computer because they are not iterative. The Inverse Filter uses an approximate direct linear inversion of the equation:  $g(x) = f(x) * h(x)$ . This inversion is carried out according to:  $\hat{f}(x) = g(x) * h^{-1}(x)$ , where  $h^{-1}(x)$  is the inverse-filter impulse response and is designed by a minimum means-square-error criterion [Castleman, Ch. 14]. This criterion implies that among all possible Inverse Filters it provides the smallest difference between the perfect  $f(x)$  and the estimate  $\hat{f}(x)$ . It was first used for light microscopy by Agard [1989].

#### Nearest Neighbors and No Neighbors Deblurring

The Nearest Neighbor algorithm falls under the category of a *deblurring* algorithm and by definition is not really a deconvolution because it is not based upon an estimation of  $f(x)$ . Instead, it is based upon improving the picture by sharpening edges of structures. It is a specific type of sharpening filter called an *unsharp mask* [Russ]. It works by reblurring the image and then subtracting a fraction of the reblurred image from the blurry one. In AutoDeblur it works according to:

$\hat{f}_k(x) = g_k(x) - c[g_{k-1}(x) * h_{k-1}(x) + g_{k+1}(x) * h_{k+1}(x)]$ , where the subscripts indicate the optical slice number. One 2D slice is sharpened at a time. The two slices that are immediately above and below it are reblurred and a fraction  $c$  of these reblurred slices is subtracted. With *No Neighbors* deblurring the  $k$ -th slice is reblurred and subtracted from itself according to a similar formula.

The early ideas of this approach were introduced to light microscopy by Castleman [Castleman, Ch. 17] and brought into fruition by Agard [1984].

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