# Deconvolution

An image is only a *representation* of the object that it is formed from. All imaging systems are imperfect: lenses suffer from manufacturing defects, an objective lens only collects a small proportion of light emanating from the specimen, and diffraction – the very means by which an image is formed – also limits image fidelity.

As the size of the object approaches the wavelength of the illuminating radiation, diffraction becomes particularly acute. Every recorded image is blurred, since the instrument used to obtain it has a finite resolving power. The process of how a lens, or objective, forms an image of any object is called *convolution*.

A microscope objective is often characterised by its PSF: a 2-D or 3-D description of how a single point of light (in practice a sub-resolution fluorescent bead) in the specimen is transferred into the image. The PSF of an objective lens depends upon its Numerical Aperture (NA), design, the illuminating wavelength and the contrast mode (brightfield, phase, DIC, fluorescence). A widefield PSF is shown below.

Knowing the structure and resulting image of a well-defined object (a sub-resolution fluorescent bead), the convolution process can be rigorously mathematically defined. Applying the reverse process: *deconvolution*, to the image allows us to 'clean up' the image to be a better representation of the object from which it originated.
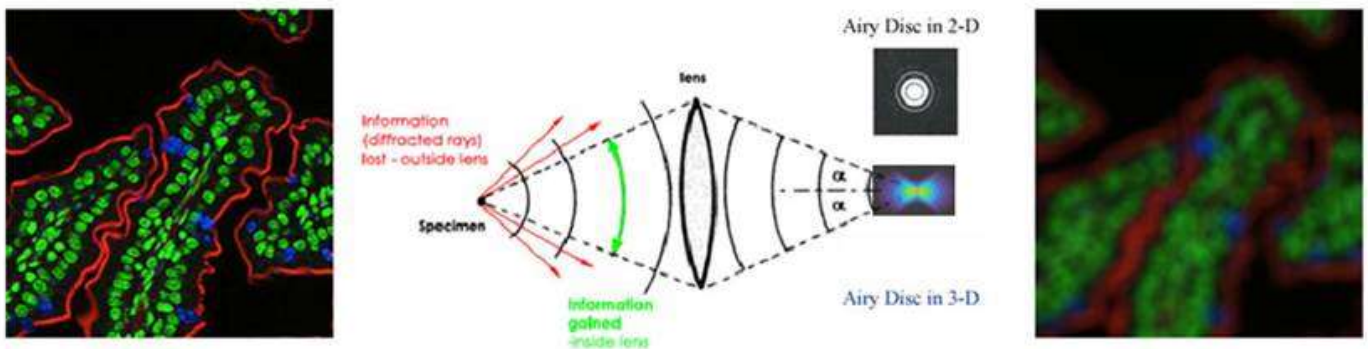
The image is the sum of all the PSFs, each centered at, and scaled in intensity, according to the corresponding point source in the sample. This is true for every image, however complex. The object convolved with the PSF forms the image, hence knowing the PSF by calculation or measurement allows a good approximation of the original object.

Fluorescence images are hazy and blurred because (being self-luminous) out-of-focus information from those parts of the specimen not seen by the objective, both above and below the focal plane, spills into the focused field of view. This degrades the contrast, dynamic range and resolution of the image. If a lens behaved perfectly, and points in the object were not smeared into a point spread function (PSF), then a defocused image of beads would appear black. Deconvolution algorithms are used to reassign this out of focus light to improve the contrast in the image.

Deconvolution microscopy (also known as computational optical microscopy) takes account of the distortion by the optical train of the microscope, and the out-of-focus signals are either:

• (a)   subtracted from the image or
• (b)   'reassigned' or 'placed back' to the points from which they came ('photon reassignment processing').

While the term reassignment is consistent with the effect of their use, obviously these algorithms cannot physically replace photons to their source. However, iterative restorative deconvolution will shift and concentrate the majority of the out-of-focus fluorescence back to the focal plane of origin from which it emanated. The result of both methods (a) and (b) is an improvement in image contrast, making it easier to resolve and distinguish features in the image.

Deconvolution algorithms

There are two main types of deconvolution algorithm:
* **Deblurring/Nearest-Neighbour**   and
* **Restorative**

Deblurring algorithms are nearest-neighbour based and use an estimate of the image blurring to subtract blurred fluorescence from an image. These Nearest Neigbour algorithms are fast, but cannot be used quantitatively for measurements of intensities or calculation of ratios between fluorophores because they are non-linear, and are only limited to visualization and 3-D rendering. They use a calculated, rather than measured, PSF, and are best for thin samples. Nearest neighbor algorithms work best with a Z-stack of three slices: the blur in the centre slice is approximated from the blur inherent in the outer two slices, and is subtracted to leave the in-focus information. Because they are simplistic, they tend to use a theoretically-calculated PSF, assuming that it is circularly symmetric in the image plane, and mirror symmetric along the optical axis. This tend to increase contrast, but reduce the signal-to-noise (S:N) ratio.

Restorative methods use the PSF to 'guess' what the objective must have looked like. They are often iterative (changing the guess and repeating the deconvolution calculation), and use a measured or calculated PSF (derived from a sub-resolution fluorescent bead). Because the relationships between intensities is *preserved* using this method, quantitation is possible. The primary advantage to constrained iterative deconvolution is in boosting image contrast. Measured PSFs give increased resolution along the Z-axis. Blind deconvolution is similar to constrained iterative deconvolution, except that the PSF is not measured directly, but derived from the image data. This may seem counterintuitive, since the algorithm is trying extract a solution for both the image and the PSF. The PSF is not known beforehand, hence the term blind; it is adaptively estimated directly from the data set. The reasoning being that the best PSF that fits the actual data is obtained.

Practical tips

Do not over-process the dataset with too many iterations. This may result in false features being over-accentuated out of the noise inherent in the image. Initially the contrast should drop, and the resulting image should not look so sharp. These sharp 'features' are noise artifacts which are removed. The contrast can then be raised to match the characteristics of the display monitor.

Deconvolution can be combined for images acquired using transmitted-light brightfield, widefield fluorescence, or confocal fluorescence and multiphoton microscopes. Used properly, deconvolution can also clean up image blur caused by spherical aberration. Some investigators consider that all images, even those collected on the confocal microscope, should be deconvolved.

Finally, do note that deconvolution can reveal flaws in the specimen that may not be so obvious in the raw image. Fluctuations in illumination intensity and inadequate sample preparation may be much more obvious after deconvolution (reference 2). Applying deconvolution to 2-D images is necessarily not as effective as with a full 3-D stack of Z-sections, because the algorithm lacks information to take account of distortion in the z-axis.

The old adage "garbage in = garbage out" is never more true than when applying post-processing techniques, such as deconvolution. Do sample correctly in both X,Y and Z dimensions, according to Nyquist's sampling theorem, and focus sufficiently above and below the specimen when taking Z-stacks, and prevent image saturation on the CCD. Deconvolution will not make bad data good; but it will make good data better.

It is also possible to restore images acquired with a confocal or multiphoton microscope. The combination of confocal microscopy and deconvolution techniques improves resolution beyond what is generally attainable with either technique alone. Furthermore, low intensity signals contain a lot of high frequency Poisson, or shot, noise which can be erroneously sampled and digitised into non-existent single-pixel 'features' that could never have been resolved by the microscope. Deconvolution essentially averages the signal and removes these noise artifacts, which is more efficient than Kalman averaging, say, fifteen times! The contrast can always be raised to match the characteristics of the display monitor. However, confocal data tends not to be deconvolved in practice because it has already been 'sampled' with the pinhole to produce an optical section.

References

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3. Wallace W, Schaefer LH, Swedlow JR (2001) A Workingperson's guide to Deconvolution in Light Microscopy. *Biotechniques* 31 (5): 1076-1097.