

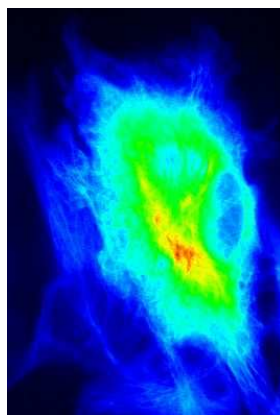


Deconvolution and TILLvisDECO package

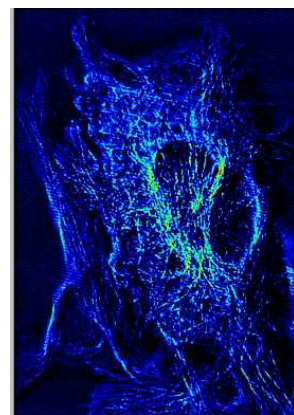
Background

Wide-field fluorescence microscopy can be used to acquire images (2D and 3D) of sub-micron resolution. However, convolution and out-of-focus effects blur the resulting images, reducing the ability to resolve the finer details of biological structures. These are the result of several factors:

- The image plane is convolved by the finite optical aperture in a way that can be described by diffraction theory. The diffraction limited resolution of the microscope is related to the wavelength of the light used, the numerical aperture (NA) of the lens and the refractive index of the milieu. This leads to the Rayleigh criterion that defines the minimum resolvable distance between two distinct points in two dimensions.
- Blurring due to slight positional errors in the focal distance to the imaging plane result in further convolution of the image. Blurring or noise is also caused by objects with low spatial frequency (i.e. objects which have a large depth of field) that contribute light to the image from neighbouring focal planes above or below that in focus. This results in fine details becoming smothered in “out of focus light” and thus lost, or at least visualized with much reduced contrast.
- Optical aberrations can also contribute to a degradation of image quality, which they affect in three dimensions. Aberrations are classified as five types: spherical, astigmatism, curvature of field, distortion, and coma. However, aberrations are greatly reduced through the use of modern, well-corrected, high quality objectives.



Cells before Deconvolution



Cells after Deconvolution



Equipment needed for high quality deconvolution results

TILL Photonics supplies all the components needed to set-up a system matching all of those requirements listed above. The centre piece of any TILL Photonics system is the latest generation of our groundbreaking Polychrome light source together with the integrated Imago CCD camera and imaging software TILLvisION. As an expansion for existing systems, or new customers wanting a complete single package solution, we offer a piezo controlled objective positioning device, together with the TILLvisDECO deconvolution software. This system allows one to set-up and run precisely coordinated imaging of specimens in 3D, at different wavelengths and as time series. The resulting data can then be deconvolved using the integrated TILLvisDECO package.

Requirements for optimal processing of image data in deconvolution experiments

To achieve the best possible result, the deconvolution of fluorescence images requires a high level of precision and accuracy at each stage of data acquisition and processing. Errors can be minimised by attention to detail (some general points of consideration):

- Microscope must be free of stage drift
- Coverslips of correct and consistent thickness
- Focal positioning with sub-resolution accuracy (piezo driven)
- Use of water immersion objective of highest NA (1.3) and quality. Oil immersion objectives are **not** recommended. This is because spherical aberrations are dependent on the nature of the optical path, which of course changes with focal position (as the objective front lens moves relative to the coverslip the thickness of the immersion fluid layer changes as well as the positional depth of the lens focus within the specimen). The change in spherical aberration (and hence PSF) is greatest when the mismatch in refractive index is maximised by using oil as the immersion fluid
- Use of high quality dichroics and filters
- Tube length errors caused by a non-parafoval position of the CCD detector (this again increases spherical aberration)
- Use of a high quality scientific grade CCD camera with high quantum efficiency, large dynamic range, and low dark counts (refer to the Imago range of CCD cameras on this site)
- Magnification must be matched to the individual pixel size of the CCD detector according to the Nyquist criteria; in general: required Mag. = $(2 \times \text{pixel size}) / [(0.61 \times \lambda) / \text{NA}]$



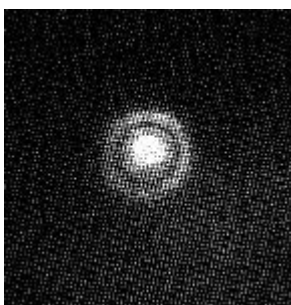
- A uniform field of illumination (optimised over the area visible to the CCD detector) limited by a field stop iris encircling the CCD detector field of view
- Background correction of all images prior to processing for background, light source instability (minimised in the TILL Photonics high stability Polychrome light source)
- Reduction of photobleaching (optimised in the TILL Photonics imaging system due to precise control over wavelength, illumination and CCD exposure)

Deconvolution by adaptive inverse modelling – the TILLvisDECO approach

The convolution of an imaged object by the PSF is in principle reversible – a process called deconvolution. The TILLvisDECO software (developed together with the research group of Les Loew) (<http://www.cbit.uchc.edu/>) uses an adaptive inverse modelling method to deconvolve image data. To do this, a three-dimensional finite inverse response (FIR) filter is used to adaptively model a measured PSF (the image of a sub-resolution fluorescent bead), visualised under the same conditions (wavelength, objective, z-step, etc.) as the specimen of interest, and which characterises the “corruption”.

The output of this process is compared to a desired response (defined as a low, medium, or high resolution virtual PSF model in TILLvisDECO) and the difference, or error, is used to update the FIR coefficients via a least mean squares (LMS) algorithm. Multiple iterations of this process eventually lead to a convergence of the coefficients, which fixes the values of the FIR filter. The adaptively modelled FIR can then be applied to the image data.

The point spread function

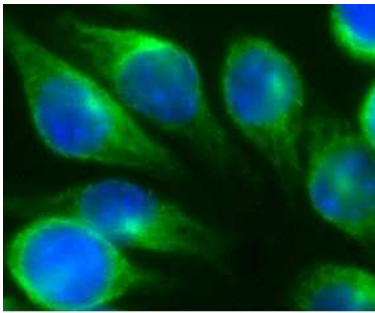


An object and its image are related to one another by a convolution of every point within it. That is to say, every point in the object is replaced by an appropriately blurred point, with the final image represented as the sum of these. The way in which each of these points is convolved can be described by the so-called point spread function (PSF), which is the image of a single point. The PSF is specific for a given set of conditions that include, for example, wavelength, objective NA, and system specific aberrations. Thus, the way in which a microscope images a single, sub-resolution object (such as a fluorescent bead) can be used to define a mathematical convolution relationship between object and image

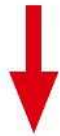


References & links

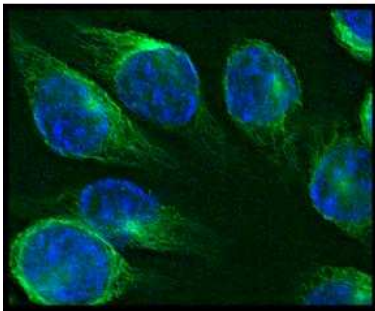
- Sapia, M., I. Greenshields, M. Fox, L. M. Loew, J. Schaff, and G. Coutu. (1997). Three-dimensional deconvolution of optical microscope images using adaptive inverse modelling. Proceedings of the 40th IEEE Midwest Conference on Circuits and Systems, Sacramento, CA.
- Loew, L.M., R.A. Tuft, W. Carrington and F.S. Fay (1993). Imaging in 5 dimensions: Time dependent membrane potentials in individual mitochondria. [Biophys. J. 65:2396-2407](#)
- Les Loew's lab homepage (Centre for Biomedical Imaging Technology [\(CBIT\) at the University of Connecticut Health Centre](#))
- General Interest: Peter J. Shaw, In "Handbook of Biological Confocal Microscopy", edited by James B. Pawley, 2nd edition ISBN 0-306-44826-2, (See chapter 23).
- The **molecular expressions web site** (<http://micro.magnet.fsu.edu/primer/digitalimaging/deconvolution/deconvolutionhome.html>) contains a number of articles on the topic of deconvolution in optical microscopy



Before Deconvolution



Deconvolution with TILLvisDECO Software



After Deconvolution