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DeconvolutionLab2: An open-source software for deconvolution microscopy

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ABSTRACT

Images in fluorescence microscopy are inherently blurred due to the limit of diffraction of light. The purpose of deconvolution microscopy is to compensate numerically for this degradation. Deconvolution is widely used to restore fine details of 3D biological samples. Unfortunately, dealing with deconvolution tools is not straightforward. Among others, end users have to select the appropriate algorithm, calibration and parametrization, while potentially facing demanding computational tasks. To make deconvolution more accessible, we have developed a practical platform for deconvolution microscopy called `DeconvolutionLab`. Freely distributed, `DeconvolutionLab` hosts standard algorithms for 3D microscopy deconvolution and drives them through a user-oriented interface. In this paper, we take advantage of the release of `DeconvolutionLab2` to provide a complete description of the software package and its built-in deconvolution algorithms. We examine several standard algorithms used in deconvolution microscopy, notably: Regularized inverse filter, Tikhonov regularization, Landweber, Tikhonov–Miller, Richardson–Lucy, and fast iterative shrinkage-thresholding. We evaluate these methods over large 3D microscopy images using simulated datasets and real experimental images. We distinguish the algorithms in terms of image quality, performance, usability and computational requirements. Our presentation is completed with a discussion of recent trends in deconvolution, inspired by the results of the Grand Challenge on deconvolution microscopy that was recently organized.

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1. Introduction

The widespread development of fluorescent-labeling techniques has rendered fluorescent microscopy one of the most popular imaging modalities in biology. An epifluorescence (a.k.a. widefield) microscope is indeed the simplest modality for observing cellular structures: After labelling with a fluorescent dye, the biological specimen is illuminated at the excitation wavelength. The fluorescence emission is used to form the image. A 3D acquisition of the cell is built as a *z*-stack of 2D images, by moving the focal plane through the sample.

Unfortunately, the resolution of 3D micrographs is intrinsically limited by the diffraction of light; structures closer than the Rayleigh criterion cannot be distinguished. For a popular fluorophore (DAPI, emission wavelength $\lambda = 470$ nm) and for the standard numerical aperture NA $= 1.4$ and diffraction index $n_\mathrm{i} = 1.51$ nm, the Rayleigh criterion predicts that it is impossible to observe details smaller than about $0.61\frac{\lambda}{\mathrm{NA}} \approx 200$ nm in the lateral sections and $2\frac{n_\mathrm{i}\lambda^2}{\mathrm{NA}} \approx 700$ nm along the optical axis [1]. Thus, the resolution is anisotropic, *i.e.*, the resolution along the depth axis is lower than the resolution in the lateral dimensions. Moreover, this resolution is usually insufficient to satisfy the current demands of biological research for the visualisation of intracellular organelles. The impact of diffraction is perceived as a blur, where fine details are obscured by the haze produced by out-of-focus light. The acquired blurred image can be mathematically modeled as the result of convolving the observed objects with a 3D point-spread function (PSF). This PSF is the diffraction pattern of the light that would be emitted from an infinitesimal point-like object and collected by the microscope. In other words, the PSF sums up the effects of the imaging setup on the observations.

There are two approaches to improve the resolution: (i) changing the microscope design to improve the shape of the PSF (e.g. confocal, multiphoton and most super-resolution microscopy modalities), (ii) numerically inverting the blurring process to enhance micrographs: the deconvolution. The ultimate goal of deconvolution is to restore the original signal that was degraded by the acquisition system (see Fig. 1). It relies on methods that have to be carefully optimized to preserve biological information. We present these methods in Section 3.

Deconvolution is a versatile restoration technique that has been found useful in various contexts such as biomedical signal processing, electro-encephalography, seismic signal (1D), astronomy (2D), or biology (3D). It performs well in 1D or 2D, but its results are the most impressive for 3D volumetric data, especially when the PSF is large axially. In this case, 3D deconvolution has the capability to combine lateral and axial information when restoring the original signal.

Deconvolution has multiple advantages. It is applicable to even the simplest optical setup, reducing financial costs and streamlining the acquisition pipeline. In addition to the resolution improvement, indirect benefits of deconvolution are contrast enhancement and noise reduction. As it mitigates the effect of noise, it can be used in low-light condition. The dim excitation light lowers bleaching probability of fluorophores and is therefore beneficial in terms of photo-toxicity in living cells. Not surprisingly, deconvolution is used routinely by microscopists and has become a popular pre-processing tool to further image-analysis steps such as segmentation and tracking. Unfortunately, without a proper tuning of the algorithms parameters, the deconvolved volume can be corrupted by artifacts that might prevent sound biological interpretation. Among such possible degradations, the most notable ones are noise amplification, ringing (known as Gibbs or Runge phenomenon) and aliasing (both spatial and spectral).

The deconvolution of micrographs was first investigated by Agard and Sedat [2]. Many variations and improvements have been proposed since then [3–7]. Some of these "deconvolution microscopy" methods led to various commercial and open-source software implementations [8,9]. The typical cost of a commercial package varies between USD 5000 and USD 10,000. At the time of writing this paper, the most popular ones are: `Huygens` (Scientific Volume Imaging); `DeltaVision Deconvolution` (Applied Precision, GE Healthcare Life Science); and `AutoQuant` (MediaCybernetics). Some of these commercial solutions (e.g., `Huygens`) specialize in the processing of large data and are capable of running unattended deconvolution in batch mode [10].

Meanwhile, several open-source deconvolution solutions exist too, often taking the form of an `ImageJ`[1] plugin. One of the first such platform that was made available is the popular `DeconvolutionLab` software developed at the Biomedical Imaging Group (EPFL) and detailed in the present paper. Freely distributed, `DeconvolutionLab` hosts various algorithms for 3D microscopy deconvolution and drives them through a user-oriented interface. Other open-source softwares also exist, including Nick Linnenbrügger's `DeconvolutionJ`, Bob Dougerthy's `Iterative Deconvolve 3D`[2] which implements a deconvolution approach for the mapping of

[1] http://imagej.nih.gov/ij/.
[2] http://www.optinav.info/Iterative-Deconvolve-3D.htm.