

poissonNMF – blind source separation of fluorescence microscopy data

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PoissonNMF is an ImageJ plugin to decompose spectrally resolved fluorescence microscopy data into the contribution of the labels under conditions where the spectra are not or only inaccurately known. It employs non-negative matrix factorization ?, suitably modified to shot-noise dominated data ?.

Installation

To install the plugin, copy the `PNMF_.class` and `PNMF_$1.class` files as well as the into folder `PNMF_SpectralLibrary` the plugins directory of your ImageJ distribution.

Input Data

PoissonNMF expects a 32 bit image stack, where each slice of the stack corresponds to the image in one spectral channel.

Parameters & initial conditions

Upon invoking pNMF from the plugin menu, a dialog opens, prompting the user for the number of sources expected in the sample. This dialog is followed by a second dialog allowing the user to provide additional input. The dialog is preset to the values of the previous run of PoissonNMF. The dialog is shown in Fig. 1. Several choices in this dialog invoke additional dialog after the *Run* button has been pressed.

Parameter Dialog

First and last wavelength:

Number of iterations: The total number of iterations the algorithm is run. Depending on the subsampling scheme (see below), the first iteration are only applied to a subset of the data.

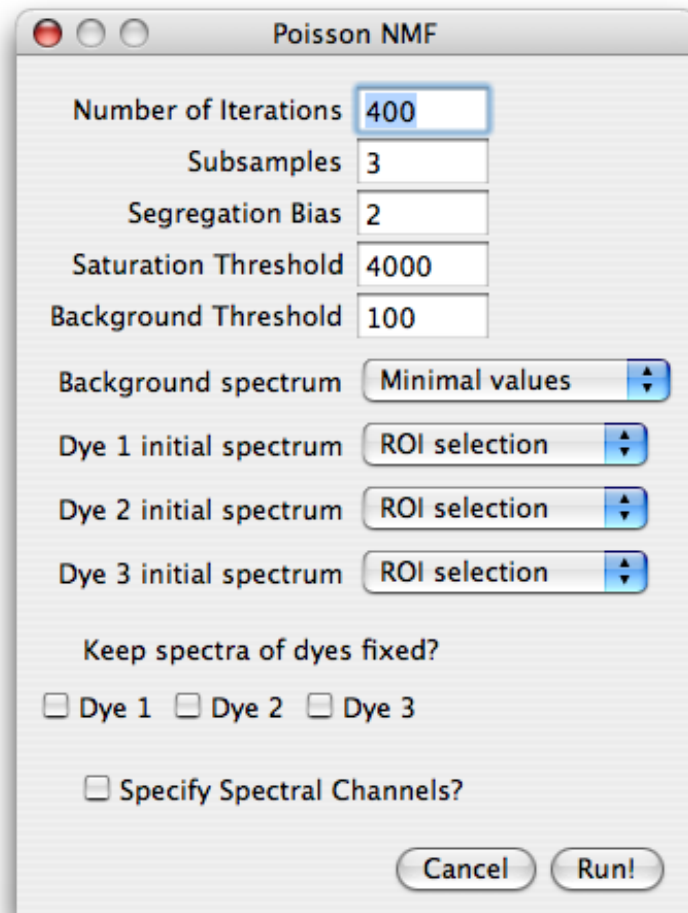


Figure 1: The parameter dialog of pNMF with default values.

Subsampling: To speed up the calculation, the algorithm can be initiated on a subset of the data and then successively uses the estimate for the spectra for larger subsets of the data. The parameter subsampling determines the number of subsamplings of increasing size to be made. Successive subsamples are larger by a factor of 10, while the last is the entire data set (above the background threshold). The number of iterations for larger subsamples is smaller (a factor 2 between successive subsamples).

Segregation Bias: A parameter determining the degree, to which overlap of estimated label distribution is penalized. Suitable weights of the segregation bias are around 1 or lower. Too high segregation bias will yield in a faulty decomposition.

Saturation threshold: Saturated or nearly saturated pixels have distorted emission spectra and therefore have to be excluded from the analysis. If the signal in any channel at a certain pixel is above this parameter value, the pixel is excluded.

Background threshold: Very faint pixels carry little information and are likely dominated by noise, autofluorescence and the like. It is therefore advisable to limit the spectra estimation to reasonably strong pixels. Any pixel, where, after background subtraction, the intensity is below this threshold in every channel is therefore excluded.

Background: Any constant background has to be subtracted before the image is processed. This background is either estimated using the minimal value in each spectral channel. Alternatively, the background can be estimated by specifying a ROI that contains nothing but background signal or the background can be entered manually.

Start spectra: Convergence is faster if the initial spectra are good guesses of the actual spectra. PoissonNMF provides several options to specify the start spectrum for each dye, which can be chosen from a pull-down menu. The simplest choice for the initial spectra are equally spaced Gaussians. Other options to specify start spectra are ROI selection, manual entry of the spectra and the choice of spectra from the spectra library.

Fix Spectra: The initial spectrum of each dye can be kept fixed during the PoissonNMF run by checking the appropriate checkbox.

Channel boundaries: To display the spectra correctly and to read spectra from the library, the boundaries of the spectral channels have to be entered. To do so, check the check box and you will be prompted for the channel boundaries later.

Figure 2: The user is asked to specify the background and the initial spectra for the run of PoissonNMF.

While PoissonNMF processes the image, the current spectra are continuously displayed. At any point, the user can cancel the PoissonNMF or use the current spectra for least squares unmixing of the image.

Output

PoissonNMF displays an image stack with the estimated label distributions and opens a window with a number of buttons that allow to save the spectra, examine the background spectrum and the set of background pixels and construct RGB overlays of a triplet of dye distributions.

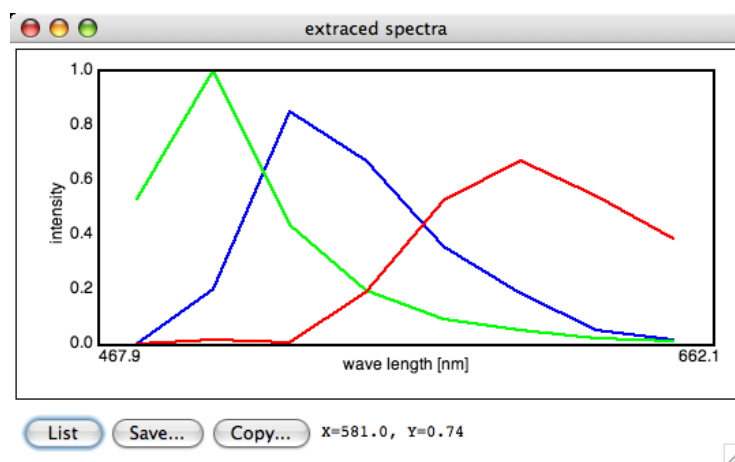


Figure 3: An example estimated spectra delivered by PoissonNMF.

Spectra Library

PoissonNMF can make use of literature spectra or spectra determined in previous runs, for example by using them as start spectra or keeping them fixed while optimizing other unknown spectra. For a spectrum to be available in PoissonNMF, it has to be placed in the folder `PNNF.SpectraLibrary` located in the plugins folder of ImageJ. The files are assumed to be text files with the wavelength in column one and the emission in column two, conform with the output format of PoissonNMF.

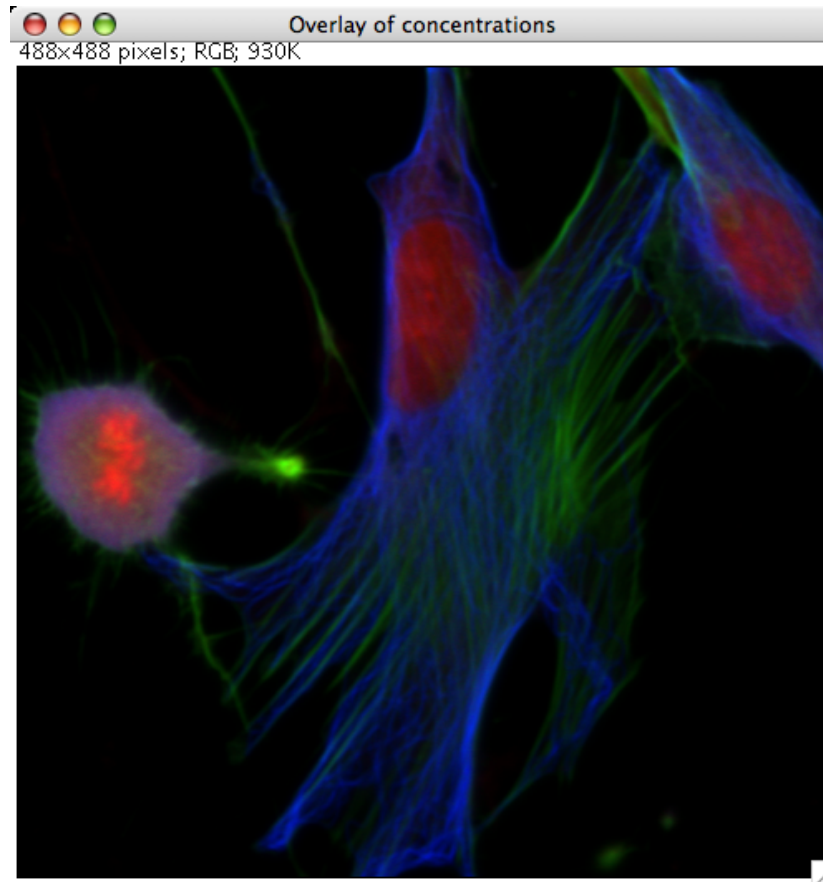


Figure 4: For three sources, PoissonNMF calculates a composite image with each source specifying one color channel.