BLIND SPECTRAL UNMIXING FOR THE DECOMPOSITION OF MULTIPLY LABELED FLUORESCENCE IMAGES

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Simultaneous fluorescent labeling is one of the most powerful ways to elucidate complex interactions at the molecular level. However, the emission spectra of the fluorescent labels typically overlap and their contributions have to be separated by 'linear unmixing'. Linear unmixing, in turn, is critically dependent on reliable emission spectra, which may be instrument-specific and may differ for the same label, when assayed in different environments, penetration depths etc. Precise and convenient methods for determining component spectra are therefore needed to fully tap the potential of fluorescence microscopy. In many cases however, it is not possible to obtain reference spectra with the required accuracy.

We present an algorithm that employs *Non-negative Matrix Factorization (NMF)*, a modern machine learning technique, to estimate the label distribution and the emission spectra directly from the raw data. Our algorithm correctly accounts for the Poisson noise characteristics of light signals and is therefore applicable to low light intensities. Furthermore, NMF can be augmented with partial information (such as approximate spectra or a subset of spectra). This allows the researcher to make optimal use of the acquired data, which can be crucial when the photon flux is limiting. We show that the algorithms can be generalized to handle data acquired in multiple exposures at different wavelength, which greatly increases the quality of the label separation and the maximal number of separable labels.

REFERENCE: R.A. Neher, M. Mitkovski, F. Kirchhoff, E. Neher, F. Theis, A. Zeug "Blind source separation techniques for the decomposition of multiply labeled fluorescence images" Biophys J (in Press)

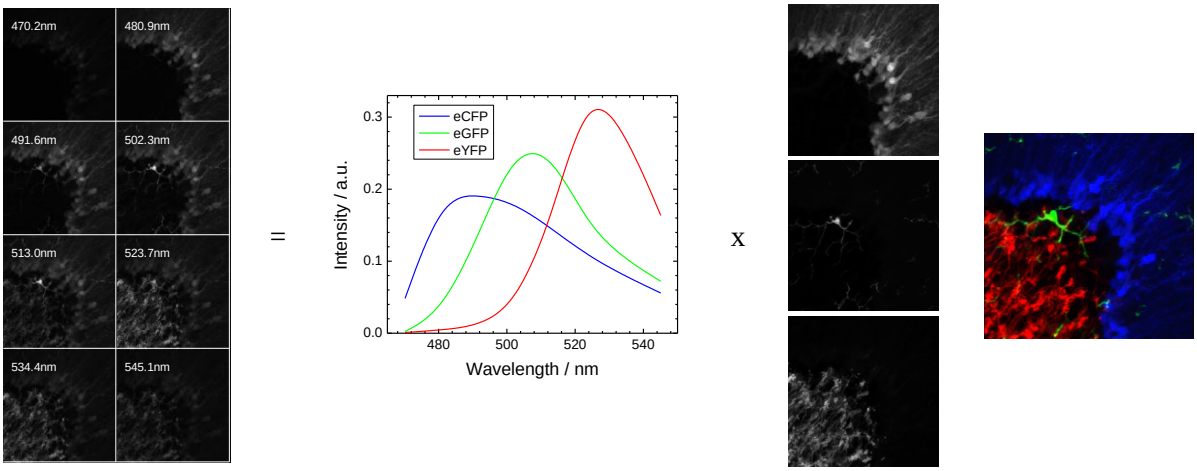

Figure: Cerebellum of transgenic mouse with spectrally strong overlapping eCFP (Bergmann glia, blue), eGFP (micro glia, green) and eYFP (axon terminals of mossy fibres, red). The left column shows the 8-channel recordings. The estimated source spectra (second column) and separated images (third column and false-colour image in the right column) were obtained from unmixing the image stack with sparse nonnegative matrix factorization without prior knowledge of the spectra of the individual VFPs.