BLIND SPECTRAL UNMIXING OF 3D M-FISH IMAGES
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Keywords: Blind unmixing, M-FISH, spot counting, 3D segmentation and quantification.

Multi-colour Fluorescent In-Situ Hybridization (M-FISH) is a widespread and diversely applied technology which enables selective staining of various DNA sequences in cell chromosomes. Given the progressive use of FISH to image many distinct targets at once, images with more and more spectral components are recorded for data analysis. Such multi-colour approaches have their own limitations, including differential intensity of fluorophores, and spectral overlap causing cross-talk between the fluorescent emissions.

When cross-talk is a problem that cannot be alleviated with a bandpass filter around each fluorophore emission peak, automatic unmixing can be used. The main models, concepts and methods for linear unmixing have been inherited from the remote sensing field. The standard Linear Mixing Model (LMM) considers that the total detected signal for every channel can be expressed as a linear combination of the reference spectra. The latter account for the relative contribution of each fluorophore to each detection channel and is previously measured in well-defined regions of interest where only the emission of one fluorophore is present. They can be assembled into a matrix of fluorophore specific weighting factors that is subsequently used to determine the contribution of each fluorophore to each image pixel. Our initial approach for this task was to use a least squares inversion algorithm constrained to the non-negativity of each fluorophore contribution, which is called Non-Negative Least Squares (NNLS), as the solution adjusts better to reality than a standard quadratic error minimization.

However, the spectral characterization of the fluorophores is time consuming and no fully reliable as the intensity levels could change in time (i.e., due to sample degradation or bleaching). We hypothesize that a blind unmixing method that estimates the reference spectra should be able to perform proper and robust spectral unmixing. In this work, we propose to use a recently developed algebraic technique, called Non-negative Matrix Factorization (NMF) [1], for the blind unmixing of M-FISH images. Closely connected with the well-known Independent Component Analysis (ICA), it is based in the decomposition of the sensed spectrum as the product of two non-negative matrices: the true fluorophore spectrum and the weighting factors. This method is well suited for cross-talk reduction as the spectra can not have negative components and because only additive—not subtractive- combinations are allowed. Moreover, our implementation recovers a spatially sparse description of the image while minimizing the quadratic squared error.

We have validated this novel NMF method in terms of mixing reduction power and spot counting reliability using artificially created 4-FISH colour images. Then, its performance was compared against the NNLS method, showing that both are statistically equivalent in the spot counting task. Finally, its usefulness for blind umixing of 4-FISH samples of a spread of a cancer cell line stained with the commercial kit LAVysion (Vysis, Downers Grove, IL) was shown.