

# MULTICHANNEL CELL SEGMENTATION AND CLASSIFICATION USING CONSTRAINED NONNEGATIVE MATRIX FACTORIZATION

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The segmentation of cells embedded in a mixture of biological material is a non-trivial problem in fluorescence microscopy. In the presence of autofluorescent material, in fact, direct segmentation using an intensity threshold in a single channel commonly leads to over-segmentation. By combining multiple fluorescent channels, we can potentially distinguish between objects that emit in a single channel from those emitting in multiple channels, i.e. autofluorescent objects. The ratios between the intensities in the different channels depend on a variety of factors, such as the effectiveness of the staining or the different transmittance of the filters in the light path. Therefore, the simple adjustment between the channels in the form of intensity ratios is not sufficient to correctly identify positive objects.

We have developed a blind algorithm for the separation of stained and autofluorescent objects. The algorithm uses nonnegative matrix factorization with sparsity constraints (NMFsc) to separate three input channels into two components, one containing the stained objects and the other autofluorescent objects.

We have tested the algorithm on synthetic and real images. Synthetic images were generated using a simplified model for fluorescence imaging, which takes into account the following factors: the fluorescent emission for three types of objects – two of which are autofluorescent in three channels and one that emits true fluorescence in one channel–, the transmission efficiency of the three channels, the presence of additive Gaussian noise and gain variability.

Real images were taken from a sample of broncho-alveolar lavage sprinkled with cells from a established lung cancer cell line, stained by immunofluorescence using a blue fluorescent marker conjugated to an antibody specific for the A1 protein, commonly overexpressed in lung cancer. One hundred fields were chosen at random within an area of the slide, captured in three fluorescent channels and each object within each field of view was manually classified as either containing or not containing a tumoral cell. The manual classification was considered as ground truth against which the automatic classification was tested.

Our results –which are shown in the table– show very good performance for both synthetic and real images.

	Synthetic		Real	
	Ratio	NMF	Ratio	NMF
Sensitivity	90%	97%	67%	81%
Specificity	100%	100%	65%	97%
Positive Predictive Value	90%	100%	15%	89%
Negative Predictive Value	97%	99%	95%	96%