NMF in LM

1 Introduction

Localisation microscopy (LM) is a conceptually simple and accessible technique providing superresolution fluorescent images.[2,4,6,11] The structure of the sample is reconstructed by localising individual fluorophores with precision surpassing the calssical resolutioin limt.[13] LM make use of the fluorophore transition between bright (ON) and dark (OFF) states. Standard LM techniques (fPALM,[2] STORM[15]) control ON-OFF transition by photo-switching of the fluorophores as they require only one source to be ON within a diffraction limited area at a time. This is achieved by driving the sample into a state where only a small subset of the fluorophores are in ON state. The superresolution image is composed from the repetitive localisation of the different subsets of individual fluorophores. An optimal number of the ON sources in each acquisition frame must be experimantally estimated as a small density lead to a long acquistion time whereas a high density of the ON fluorophores results in overlapping sources that cannot be localised.[16]

Quantum dots (QD) are an order of magnitude brighter compared to the organic dyes used in the standard LM.[5,14] Under a continuous excitation the QDs exhibit a stochastic blinking between ON and OFF states.[7,12,17] An excellent photo-stability, low cytotoxicity and unique spectral properties[14] make QDs very attractive for biological research. However, the stochastic blinking makes QDs impractical for the standard LM as the rate of ON-OFF transition is difficult to control. This results in highly overlapping sources that cannot be localised with standard LM techniques.

Some methods exploiting the blinking behaviour of the QD have been proposed. Maximum aposteriori fitting of the positions and the intensities of known individual point spread functions (PSFs) into a movie of blinking QD (QD data) has been proposed in.[1] Independent component analysis (ICA) of the QD data was sugggested in[10] and a resolution improvement by a statistical analysis of the intensity fluctuation (SOFI) has been demonstrated.[?]

2 NMF

Non-negative matrix factorisation (NMF)[8] is a natural model for QD data. It decomposes spatio-temporal data - a movie of the blinking QDs - into a spatial component - images of the individual sources - and a temporal component - their fluctuating intensities. Unlike ICA, NMF imposes a natural non-negativity constraints on the images of the inidvidual sources (point spread function PSF) and their intensities. Moreover, we used a NMF algorith[9] that maximizes the likelihood of model for data corrupted with Poisson noise which makes it a method of choice compard to ICA.

NMF does not put any constraints on the shpae or blinking behavior of the individual sources (apart from being non-negative). Therefore NMF can separate images overlapping fluorophores each having different shape and blinking behavior. This can, for example, arise in a 3D sample where sources can be in different focal depth. This results in variety of point spread function

3 Model comparison

NMF requires a prior knowledge about the number of sources (K) to be separated. Principal component analysis (PCA) can be used as a simple method for dimensionality estimation. However, for noisy data the estimation of K is difficult.

4 Out of focus PSF

5 Notes

Fitting of the multiple PSF into the STORM data is in.[3]

Supplementary materials

Spatial temporal data (movie) of blinking quantum dots can be ragarded as a $N \times T$ data matrix $\boldsymbol{D}(x, t)$ where N is a number of pixels and T is a number of time frames in the movie. Each frame in the movie is transformed into a colmun of the matrix $\boldsymbol{D}$ by concateneting columns of the 2D image into a $N \times 1$ vector. Non-negative matrix factorisation (NMF) makes an approximative decomposition

$$\boldsymbol{D} \approx \boldsymbol{W}\boldsymbol{H}, \tag{1}$$

where the $N \times T$ matrix $\boldsymbol{D}$ is expresssed as a multiplication of the $N \times K$ matrix $\boldsymbol{W}$ and $K \times T$ matrix $\boldsymbol{H}$ subject to the non-negativity constraints on the entries $w_{xk} \geq 0$ and $h_{kt} \geq 0$. Each column $\boldsymbol{w}_k$ of the matrix $\boldsymbol{W}$ ($N \times 1$ vector) then represents a kth image of one source and each row $\boldsymbol{h}_t^T$ of the matrix $\boldsymbol{H}$ ($1 \times T$ vector) represents the time profile of hte kth source blinking.

The NMF algorithm[9] makes the decomposition such that the likelihood function of the model is maximised under assumption of the Poisson noise. The approximative factorisation(1) can then be written as

$$\mathbb{E}\left[\boldsymbol{D}\right] = \boldsymbol{W}\boldsymbol{H},$$

where $\mathbb{E}\left[.\right]$ denotes expectation value of the noisy data.

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