Localisation microscopy with quantum dots using non-negative matrix factorisation

Chris Williams
joint work with Ondřej Mandula, Ivana Šumanovac, Rainer Heintzmann

Institute for Adaptive and Neural Computation
School of Informatics, University of Edinburgh

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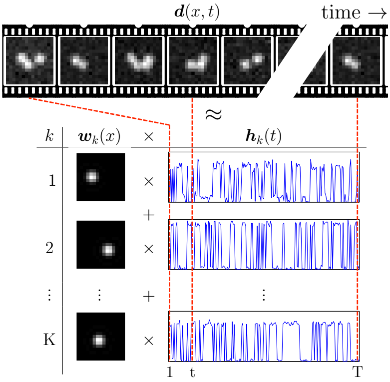
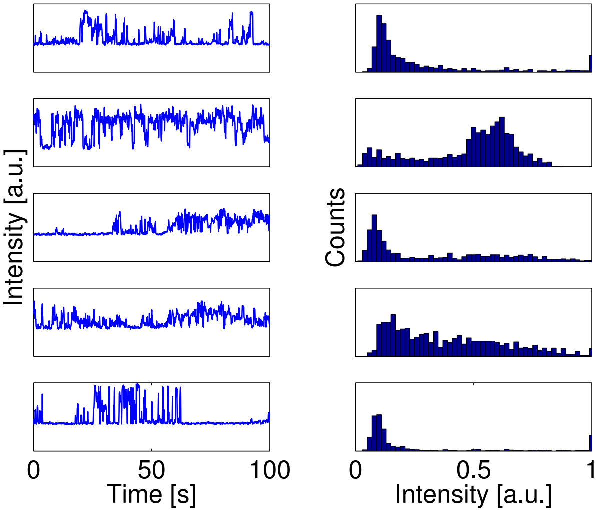
The Problem



Localization microscopy with highly overlapping sources using non-negative matrix factorization

- Want to analyse a time-lapse sequence of images of a specimen labelled with fluorophores switching between ON and OFF states, in order to localize the sources
- Conventional methods (PALM, fPALM, STORM, dSTORM) actively drive a large majority of the fluorophores into an OFF state
- This avoids overlaps between individual point spread functions (PSFs), but leads to low throughput
- Quantum dots (QDs) are brighter than alternatives, reducing acquisition times
- However, QD blinking cannot be controlled so we need to analyze overlapping sources

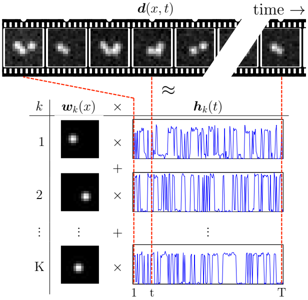
Quantum dots: blinking



Outline

- The NMF model
- iNMF enhancements
- Competitor methods
- Quantitative Evaluation
- Comparisons on Simulated and Real Data
- Localization in Depth
- Conclusions

The NMF Model



$$d(x,t) \simeq \sum_{k=1}^{K} w_k(x) h_k(t)$$

$$D \simeq WH$$

- D is $N \times T$, W is $N \times K$, H is $K \times T$
- Scale so that $\sum_j w_{jk} = 1$

Fitting the Model

- Poisson likelihood is the natural choice for microscopy

$$\log p(D|W, H) = \sum_{xt} \left(d_{xt} \log \sum_{k=1}^{K} w_{xk} h_{kt} - \sum_{k=1}^{K} w_{xk} h_{kt} \right) + const$$

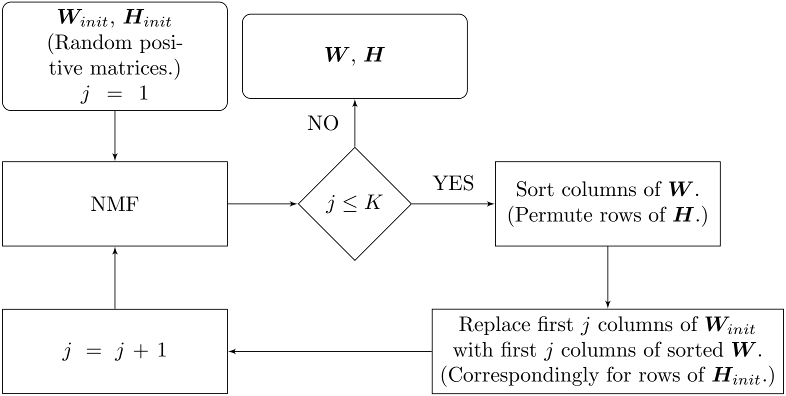
- Corresponds to Kullback-Leibler divergence used by Lee and Seung (2001)
- Multiplicative updates

$$w_{xk} = \frac{w_{xk}}{\sum_{t=1}^{T} h_{kt}} \left[(\boldsymbol{D} \oslash \boldsymbol{WH}) \boldsymbol{H}^\top \right]_{xk}$$
$$h_{kt} = \frac{h_{kt}}{\sum_{x=1}^{N} w_{xk}} \left[\boldsymbol{W}^\top (\boldsymbol{D} \oslash \boldsymbol{WH}) \right]_{kt} .$$

where $\oslash$ denotes the element-wise division of matrices

Iterative NMF (iNMF)

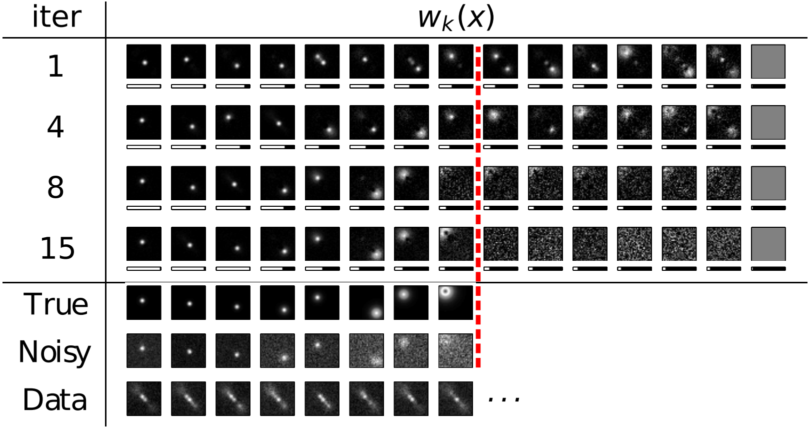
- Multiplicative updates are convex wrt W and H separately, but non-convex jointly
- Multiple restarts can be used, but we did not find good solutions with this method
- We exploit prior knowledge that $\mathbf{w}_k$s (PSFs) are likely to have compact structure
- Rank columns $\mathbf{w}_k$ of W according to their L_2 norm
- Larger L_2 scores tend to have sparser structure
- Hoyer (2004) used target L_2 sparseness, rather than as a ranking


$\boldsymbol{W}_{init}$, $\boldsymbol{H}_{init}$ (Random positive matrices.) $j = 1$
$\boldsymbol{W}$, $\boldsymbol{H}$
NO
NMF
$j \leq K$
YES
Sort columns of $\boldsymbol{W}$. (Permute rows of $\boldsymbol{H}$.)
$j = j + 1$
Replace first j columns of $\boldsymbol{W}_{init}$ with first j columns of sorted $\boldsymbol{W}$. (Correspondingly for rows of $\boldsymbol{H}_{init}$.)

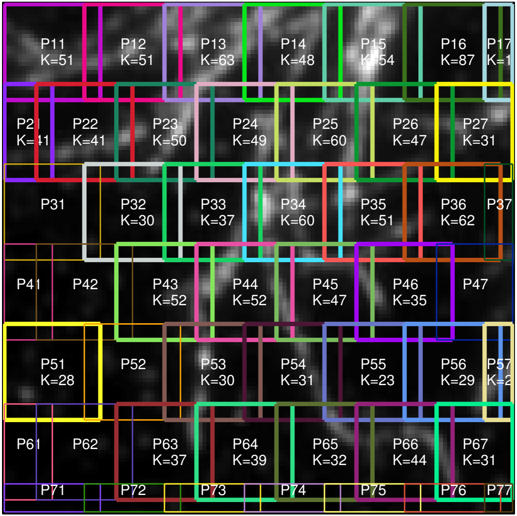
Choosing K

- We use a over-estimate based on PCA
- We demonstrate that iNMF recovers the optimal number of emitters if K is over-estimated

iNMF in Action



Handling many sources



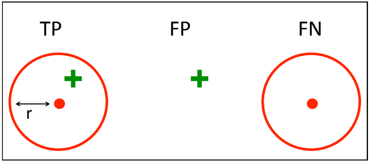
- iNMF applied to each patch, then the results are stitiched back together

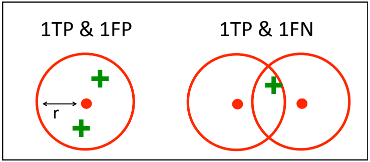
Competitor Methods

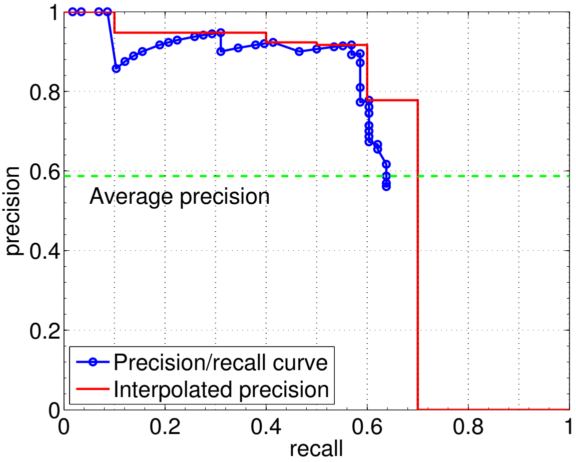
- **CSSTORM**: (Zhu et al, 2012). Acts on each frame separately, uses ideas from compressed sensing re spatial sparsity of sources
- **3B** (Bayesian Blinking and Bleaching, Cox et al, 2011). Fits a hidden Markov chain for each source. Expensive MCMC approximations over location, blur, and brightness of each source, and jump moves over number of sources
- **bSOFI** balanced Super-resolution Optical Fluctuation Imaging (Geissbuehler et al, 2012). Does not localize emitters but analyses higher order statistics of intensity fluctuation

Simulations: Quantitative Evaluation

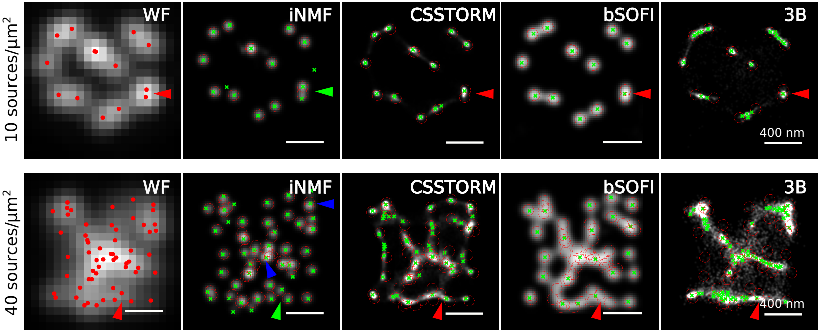
- Scatter sources randomly at a given density, time series generated by down-sampling a telegraph process
- For each method measure localization precision and ability to recover individual sources
- Use Precision-Recall curve and calculate the Average Precision (AP)
- Use methodology from PASCAL VOC competition to define TPs, FPs, FNs

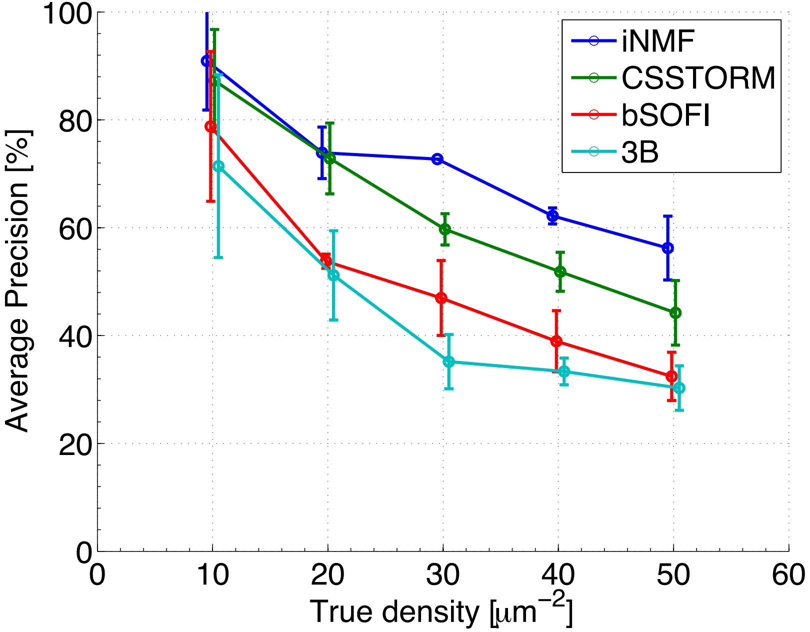




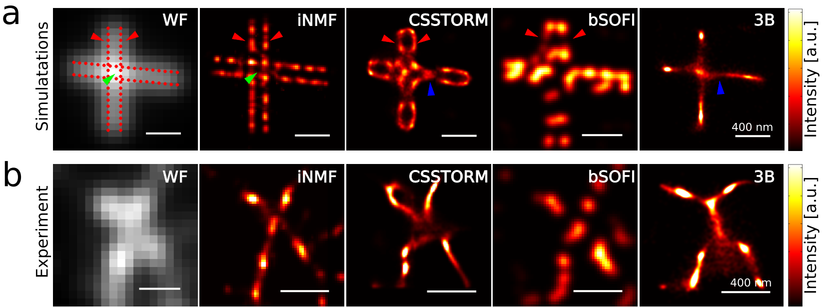


- Ranking of sources according to mean intensity $\mathrm{mean}_t(h_{kt})$


10 sources/µm²
WF
iNMF
CSSTORM
bSOFI
3B
400 nm
40 sources/µm²
WF
iNMF
CSSTORM
bSOFI
3B
400 nm

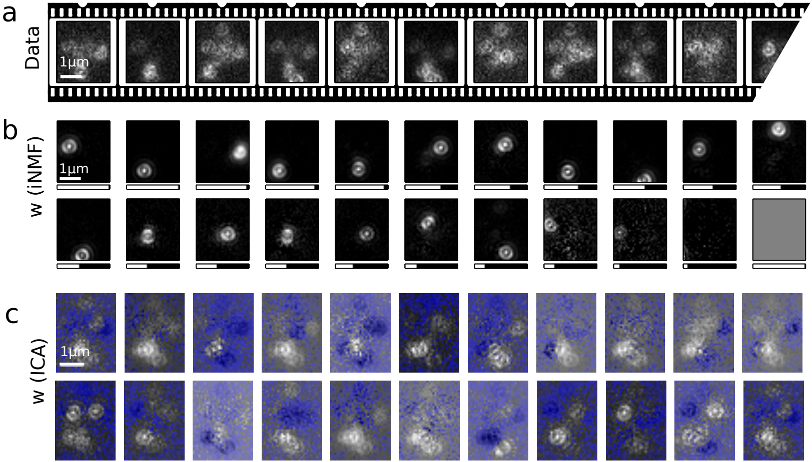

iNMF
CSSTORM
bSOFI
3B
Average Precision [%]
True density [μm^{-2}]
0
20
40
60
80
100
0
10
20
30
40
50
60

Comparisons on Simulated and Real Data

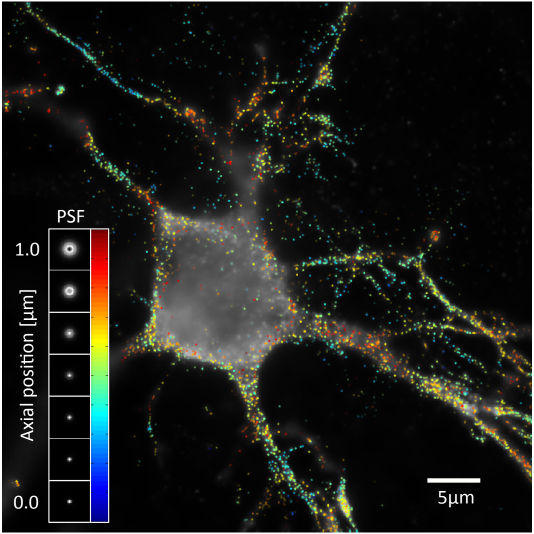


(b) is tubulin fibres of a HEp-2 cell immuno-labelled with QDs

iNMF vs ICA



Localization in Depth



A neurone with neurotransmitter receptor subunits labeled with QD605. Data kindly supplied by Anja Huss

Conclusions

- NMF is a natural formulation for localization microscopy with QDs
- Local optima problems in fitting led to the iNMF algorithm
- Outperforms competitors on localization and detection task (assessed on synthetic data)
- Promising results on real data
- Access to shape of each PSF allows localization in 3D
- Code at `https://github.com/aludnam/inmf`

Acknowledgments

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  - Stefan Geissbuehler and Marcel Leutenegger for providing the bSOFI algorithm and help with bSOFI evaluation
  - David Baddeley and Anja Huss for providing us with data of three dimensional samples
  - Susan Cox, Martin Kielhorn and Kai Wicker for interesting discussions