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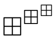

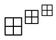

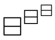

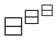

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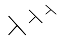

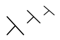

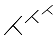

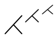

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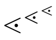

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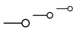

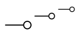

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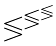

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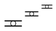

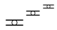

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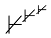

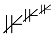

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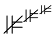

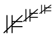

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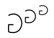

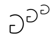

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NMF in LM

Abstract

We used non-negative matrix factorisation as a model for the

Introduction

Localisation microscopy (LM) is a conceptually simple and accessible technique providing super-resolution fluorescen images.[5,7,9,13]
The structure of the sample is reconstructed by localising individual fluorophores with precision surpassing[14]
resolution limit $\delta = \frac{\lambda}{2\mathrm{NA}}$

, where λ

is the wavelength of the emission light and NA is the numerical aperture of the objective lens. LM makes use of the fluorophore transition between bright (ON) and dark (OFF) states to discriminate individual sources separated by a distance

$d < \delta$

. The super-resolution image is achieved by a repetitive localisation of the different individual spatially separated subsets of fluorophores. The optimal number of ON sources in each acquisition frame must be experimentally estimated.[17]
A high density of the ON fluorophores results in overlapping sources and complicates localisation whereas a small density leads to a long acquisition time. Standard LM techniques (fPALM,[5]
STORM[16]
the density of the ON sources by photo-switching. However, some recent concepts suggest algorithms which can deal with of ON sources.[6]

Quantum dots (QD) are an order of magnitude brighter compared to the organic dyes used in conventional LM.[8,15]
Under continuous excitation the QDs exhibit a stochastic blinking between ON and OFF states.[18]
Excellent photo-stability, low cyto-toxicity and distinctive spectral properties make QDs very attractive for biological research.[15]
However, the stochastic blinking of QDs is impractical for standard LM as the rate of ON-OFF transition and hence the density of ON sources is difficult to control. QD labeled data typically consist of highly overlapping sources which canno localised with standard LM techniques.

Some concepts exploiting the blinking behaviour of the QDs have been proposed. Maximum a-posteriori (MAP) fittir of the positions and the intensities of known point spread functions (PSFs) to blinking QD data has been proposed.[4]
(ICA) of the QD data was suggested.[12]
A resolution improvement by analysis of the intensity fluctuation (SOFI) has been demonstrated.[3]

NMF

We used non-negative matrix factorisation (NMF)[10]
as a natural model for QD data. NMF decomposes a movie of the blinking QDs into spatial (time independent images of individual sources) and temporal parts (fluctuating intensities of each emitter). NMF imposes natural non-negativity con both the spatial (the images of the individual sources) and the temporal (intensities) components. Moreover, we used the algorithm[11]
which maximises the likelihood of the model for data corrupted with Poisson noise. This makes NMF preferable to ICA[12]
for QD data as ICA allows negative entries in the separated components and does not account for noise in the measured data (fig.A.2).

NMF does not put any additional constraints on the shape (point spread function PSF) or blinking behaviour of the individual sources, apart from being non-negative. Therefore NMF can separate overlapping sources of differing shapes a blinking characteristics. Variability in PSFs can, for example, arise in a 3D sample where fluorophores can be in different focal depths and therefore each exhibiting a different PSF.

Model comparison

The NMF algorithm requires prior knowledge about the number of sources
K

to be separated. Principal component analysis of the data (PCA) can be used as a simple method for dimensionality estimation. However, for noisy data the estimation of K

is difficult.
The results of NMF are the maximum-likelihood estimates and therefore a higher
K

leads to a higher likelihood (or a lower residual error) as more sources are better able to fit the data. However, overes
K

produces spurious sources. On the other hand, underestimation of K

results in incorrect source shapes as one component must fit several sources. Therefore a reliable estimation of K

is vital for a successful separation.
The posterior distribution $p(K|D)$

represents evidence for a particular value of K

given a data set D

. This quantity can be estimated by Bayesian treatment of the probabilistic NMF formulation. [1,2]
then choose the K

which maximises $\quad p(K|D)$

. However, a test on simulated data suggests that this approach systematically underestimates K

.

We achieved more reliable K

estimation by analysing the residuals (data-model). Models with low
K

gives rise to residual correlations as multiple individual emitters have to be represented with fewer components. Corr
with higher K

. We selected the smallest K

which reduces the residual correlations to a certain level.
The ability to estimate K

correctly becomes increasingly difficult for closely spaced sources. Simulations of the noisy data comprising two QDs show that NMF can reliably separate two closely spaces emitters ($d > \delta/10$)

when $\qquad K = K_{\text{true}}$

. However, the correct estimation of K

is possible only when the two sources are separated at least by $d > \delta/5$

, where $\delta = \lambda/2\mathrm{NA}$

is the classical resolution limit.

Out of focus PSF

A Supplementary materials

A movie of blinking quantum dots can be regarded as an $N \times T$

data matrix $\quad D$

where N

is the number of pixels and T

is the number of time frames in the movie. Each frame in the movie is transformed into a column of the matrix D

by concatenating columns of the 2D image into a $N \times 1$

vector. Non-negative matrix factorisation (NMF) makes an approximative decomposition

$$D \approx WH,$$

(A.1)

where the $N \times T$

matrix D

is expressed as a multiplication of the $N \times K$

matrix W

and $\quad K \times T$

matrix H

subject to non-negativity constraints on the entries $w_{nk} \geq 0$

and $\qquad h_{kt} \geq 0$

. Each column w_k

of the matrix W

($N \times 1$

vector) then represents an image of the k

th source and each row h_k

of the matrix H

($1 \times T$

vector) represents the time profile of the k

th source intensity.

The NMF algorithm [11]

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

$$\mathbb{E}\,D = WH,$$

(A.2)

where $\mathbb{E}$.

denotes expectation value of the noisy data with respect to the Poisson distribution.

The gamma-Poisson (GaP) model has been proposed as a probabilistic model for NMF. ^{2}

entries h_{kt}

of the intensity matrix H

are regarded as latent variables generated from a Gamma distribution with parameters α_k, β_k

and the data are modelled as a Poisson variable with mean WH

as in (A.2). Variables $\theta = \{w_k, \alpha_k, \beta_k, k = 1..K\}$

are then parameters of the GaP model. The variational approximation of the GaP model [1]

a lower bound $\mathcal{L}$

on the likelihood function $p(D|K, \theta)$

with latent variables h_k

integrated out. We can express the posterior distribution $p(K|D) \propto p(D|K)p(K)$

where $p(K)$

is a prior distribution over values of K

and $$p(D|K) = \int p(D|\theta, K)p(\theta|K)d\theta$$

is a marginal likelihood.
The number of components K_{true}

can also be estimated from the $N \times T$

residual matrix S

(entries s_{xt}

). After evaluating the model (A.2) for different values of
K

, we compute a standardised residual matrix with entries

$$s_{nt} = \frac{d_{nt} - \sum_{k=1}^{K} w_{nk} h_{kt}}{\sqrt{\sum_{k=1}^{K} w_{nk} h_{kt}}}.$$

The factor $1/\sqrt{\sum_{k=1}{}^{K} w_{nk} h_{kt}}$

is applied in order to standardise the residuals of Poisson distributed data. We can then compute the $N \times N$

$$C_S = SS^T,$$

correlation matrix

and the $N \times N$

matrix of the correlation coefficients R_S

with entries

$$r_{ij} = \frac{c_{ij}}{\sqrt{c_{ii}c_{jj}}}.$$

(A.3)

Underestimation of the number of sources ($K < K_{\text{true}}$

) will lead to correlations between some pixels as the model will try to explain multiple sources with one component.

$K \geq K_{\text{true}}$

the correlations are expected to drop to a base level and the residuals become uncorrelated.

Simulated data

We used simulated noisy data of two QDs separated by
d

to asses the performance of NMF when we assumed that the number of sources is known $K = K_{\text{true}}$

. Data were simulated with parameters shown in A.1 for 10 random geometrical configuration of two sources separate
by d

.

NMF updates

We used iterative NMF updates [11] to compute the facrorisation (A.2)

$$w_{nk} = \frac{w_{nk}}{\sum_{t=1}^{T} h_{kt}} \left((D./WH)H^{\top} \right)_{nk}$$

$$h_{kt} = \frac{h_{kt}}{\sum_{n=1}^{N} w_{nk}} \left(W^{\top}(D./WH) \right)_{kt}$$

where the ‘ ./

' operation refers to an element-wise division. The matrix elements
w_{nk}

and h_{kt}

were initialised at random from uniform distribution. The columns of the matrix W

were normalised such that $\sum_n w_{nk} = 1$

. One component was added as a homogeneous, static background: $w_{n(K+1)} = \frac{1}{N}$

, $h_{(K+1)t} = Nb$

, where the background value b

was estimated from the dark regions of the data averaged over time $\frac{1}{T}\sum_t d_{nt}$

. This component was not updated over the iterations. We run each NMF evaluation with five partial restarts - after ea
we restarted the values of h_{kt}

while keeping w_{xk}

. This procedure helps to reach a better local minimum.

Localisation error

Extracted components were localised as a maximum likelihood estimator of a gaussian centre
x_i

. We computed the mean localisation error per separation

$$\varepsilon = \frac{1}{d}\frac{e_1 + e_2}{2}$$

where $\qquad e_i = x_i - x_i^{\text{true}}$

corresponds to the localisation error of the i

th source located at x_i^{true}

and $\qquad d = {x_1}^{\mathrm{true}} - {x_2}^{\mathrm{true}}$

is the true separation of the emitters. For $d > 30$ nm the mean localisation error is less than 20%

of the separation d

(fig.A.1).

Correlations in residuals

We used the same data to evaluated the model for $K = K_{\text{true}} \pm 1$

and analysed the residual correlations A.3. The maximum correlation in residuals is shown in fig.A.1. For closely spa
sources ($d \leq 30$ nm)

the correlations are approximately on the same level for any K

. However, for $d \geq 40$ nm

there is a steep increase of the correlations for $K = 1$

compared to $K = 2$

. The model with $K = 3$

does not lead to any further improvement. For this noise level we can estimate the correct $K = 2$

for emitters with $d > 50$ nm

. This can be the actual limiting factor for the resolution of the method.

Real samples

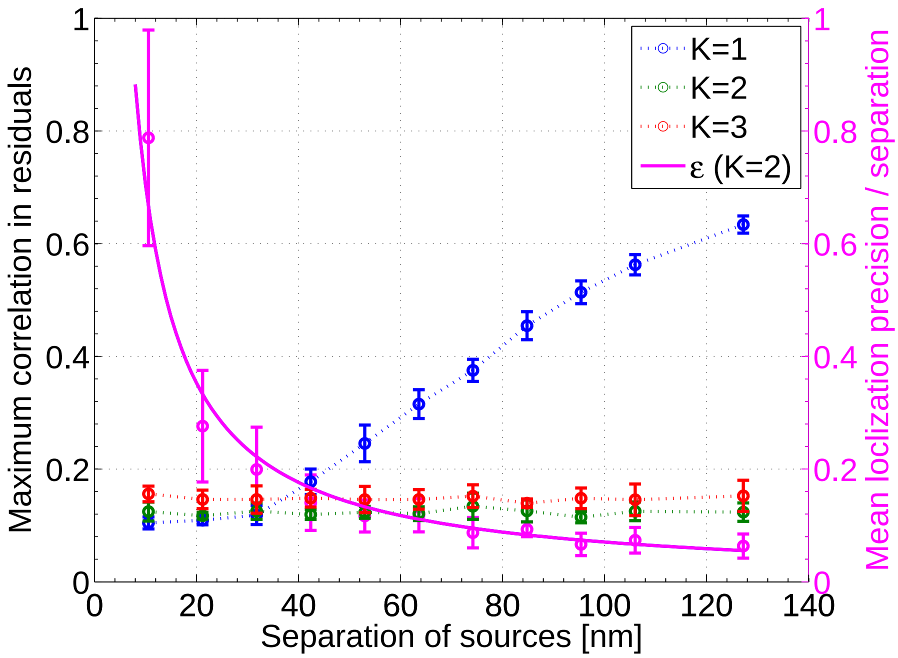
A QDs (Qdot 565, invitrogen) deposited on the coverslip were imaged with

Tables

sty-LR11C		Parameter
sty-LR11C	T	
sty-LR11C	K_{true}	
sty-LR11C	b	
sty-LR11C	I_{max}	
sty-LR11C		blinking
sty-LR11C	λ_{em}	
sty-LR11C	NA	
sty-LR11C		pixel-size
sty-LR11C	δ	
sty-LR11C		noise
sty-LR11C		

TablexA.1: Parameters of the simulation

Figures



FigurexA.1: The maximum value of correlations in residuals for $K = 1$

(blue), 2

(green) and 3

(red). Mean localisation error per separation ε

for $\quad K = 2$

(magenta) with fitted curve $\propto 1/d$

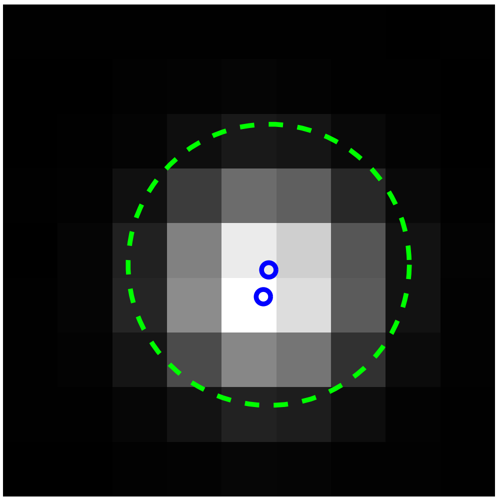
. Simulated data of two sources ($K_{\text{true}} = 2$

). The difference in residual correlations for $K = 1$

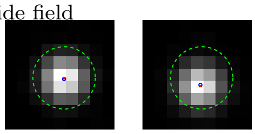
and $\quad K = 2$

are apparent only when the two sources are separated by at least 50 nm

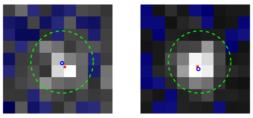
. The classical resolution limit $\delta = 273$ nm



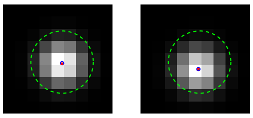
(a) wide field



(b) NMF (noise)



(c) ICA (noise)

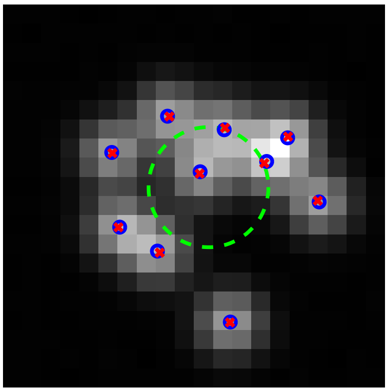


(d) ICA (noise free)

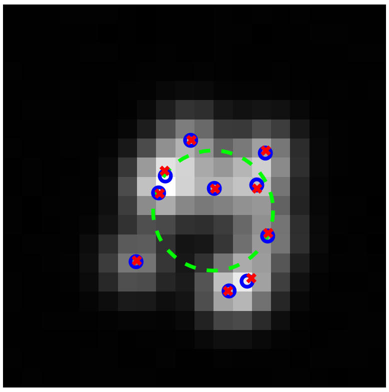
FigurexA.2: Comparison of the components separated with NMF (b) and ICA (c) for noisy data of two blinking QDs sep
by $d = 50$ nm

(mean value shown in (a)). ICA for noise free data shown in (d). The true and the estimated positions are shown as blue circle and red crosses, respectively. The radius of the green circle is the resolution limit
δ

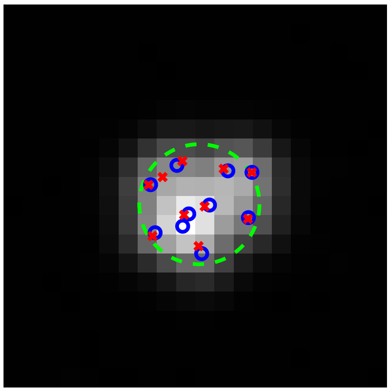
. Blue pixels contain negative values.



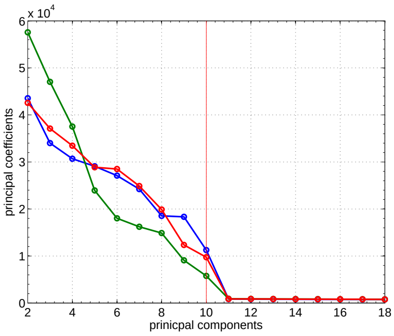
(a)
2δ



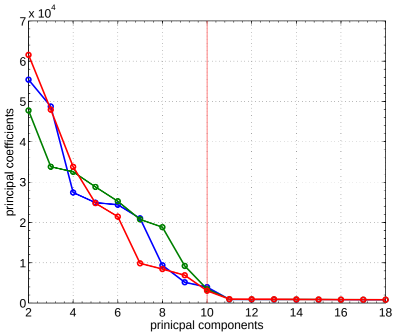
(b)
1.5δ



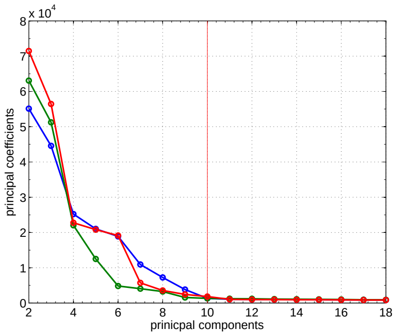
(c)
δ



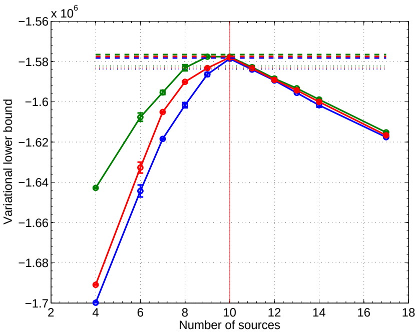
(d)
2δ
PCA



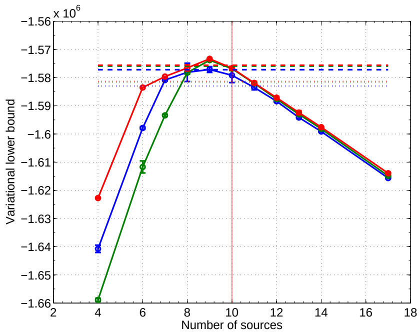
(e)
1.5δ
PCA



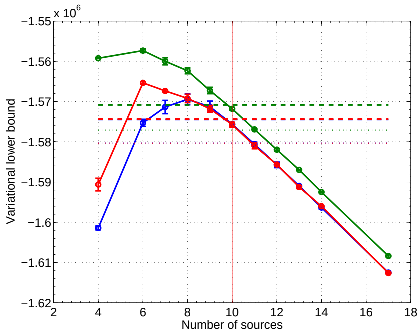
(f)
δ ,
PCA



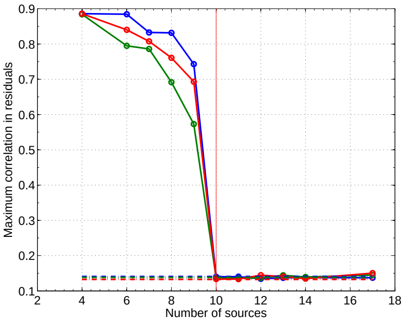
(g)
2δ
lower bound



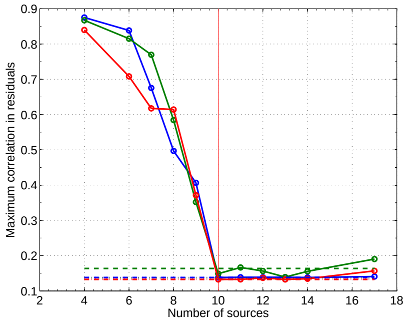
(h)
1.5δ
lower bound



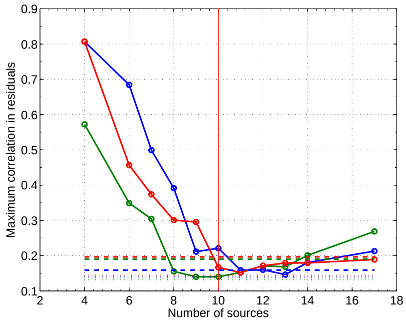
(i)
δ ,
lower bound



(j)
2δ
maximum correlation



(k)
1.5δ
maximum correlation



(l)
δ
maximum correlation

,

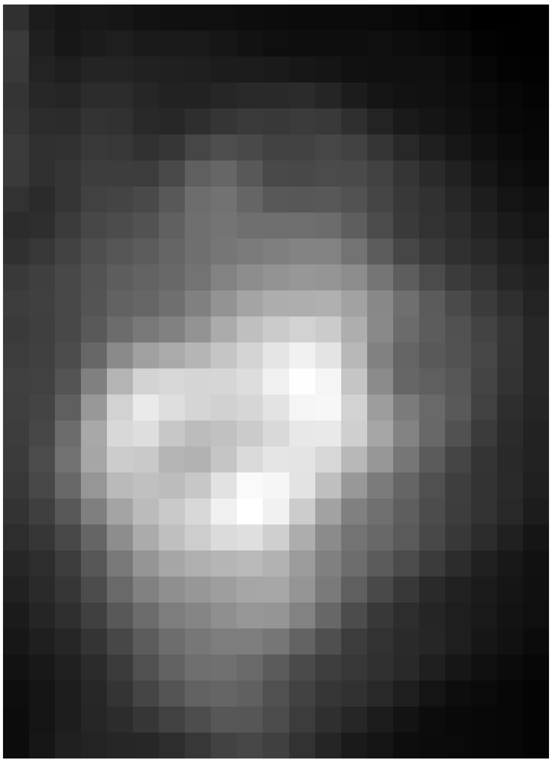
FigurexA.3: K

estimation for sources contained in a circular area with radius 2δ

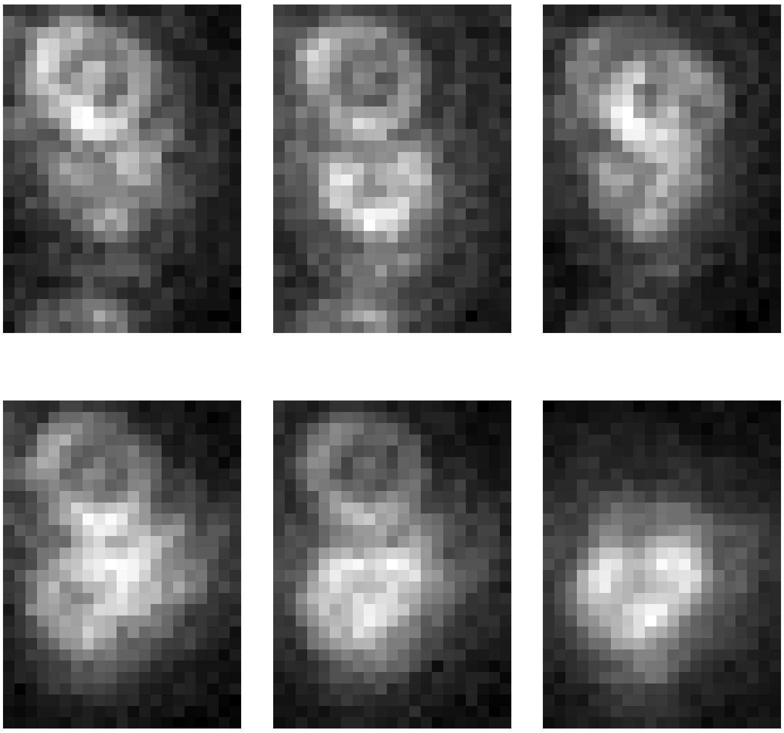
x(left column), 1.5δ

x(middle column) and δ

x(right column).

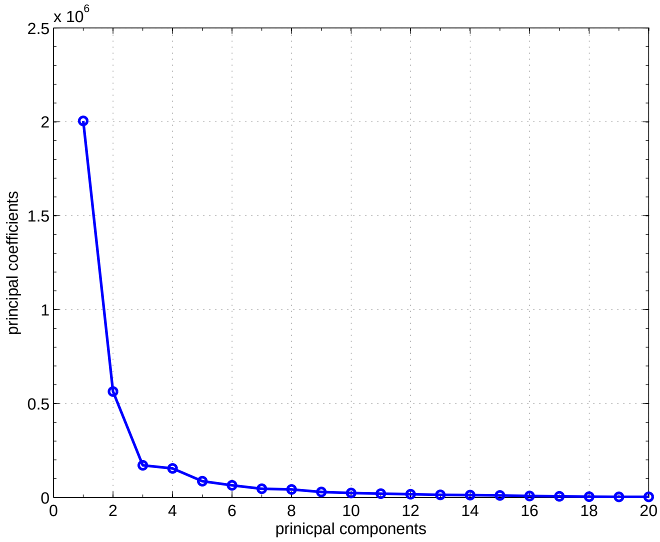


(a) Mean intensity image

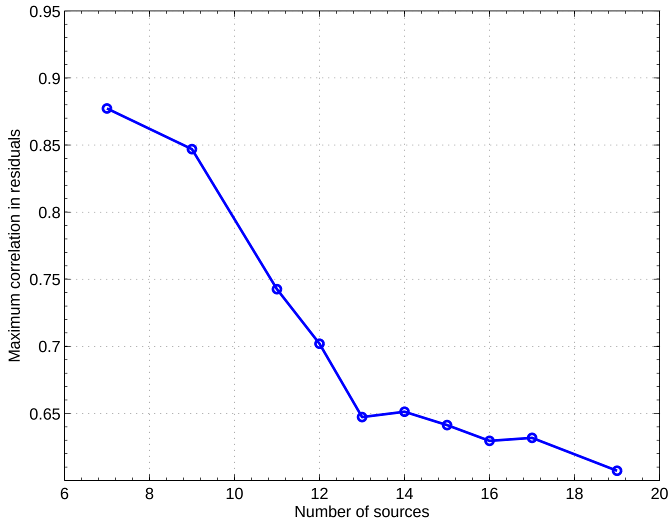


(c) Movie of the blinking sources (six frames out of 10^3)

FigurexA.4: Real QD data.

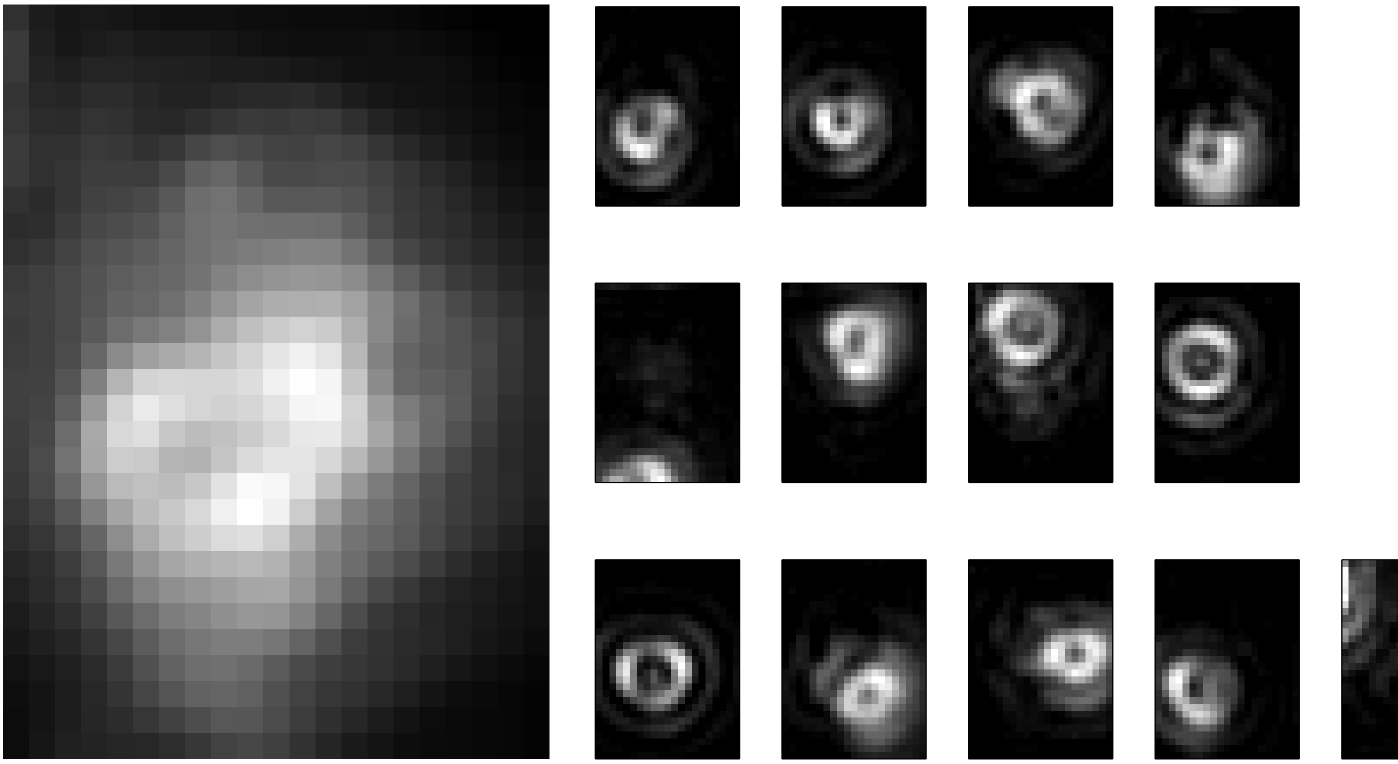


(a) PCA



(b) Correlations

FigurexA.5: Estimation of K



(a) Mean intensity image **(b)** Separated sources

FigurexA.6: Left: mean intensity image correspond to a standard wide-field image. Right: separated individual sources fo $K = 13$

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