Contents

1 Introduction

How does a virus reproduce itself? Which proteins are essential for neuro transmitters? How do proteins interact with each other? These questions and many more arise in biology or related sciences. Microscopy is the most powerfull tool to answer these questions. But light microscopy is limited in spatial resolution due to the diffraction limited, as described by Abbe (1873). Recently there were developed different methodes to increase the resolution of light microscopy beyond the diffraction limit, like photoactivated localization microscopy (PALM) Betzig et al. (2006) or stochastic optical reconstruction microscopy (STORM) Rust (2006). This techniques use many images with sparsely distributed signals, comming from flourophors and are blured by diffraction, to determine their center with a sub pixel accuracy, instead of one image with all signals together which would be blured and impossible to find the true position of the flourophors.
STORM can also be used to investigate the distribution of different proteins within a cell. Therefore each protein is labeled with different flourophores. Images can be aquired showing just signal from one kind of flourophore. But to be able to seperate the different signals from the flourophores the emission spectrum must be destinct. This leads to cromatic aberration which results in images that are distorted and thus can't be aligned easily. Many people have developed their own software to process STORM data sets. But most of these programs have a huge amount of parameters that must be set so that it is difficult for someone who is not familiar with image processing or does not know the parameters to use this software right from the beginning.

How to build a software that is easy to use even with no prior information about the data or knowledge of image processing?

SimpleStorm is a software that calibrates itself. It estimates the camera parameters and the width of the point spread function of the flourophores. In contrast to many other software applications no threshold is needed.
The results of two channels can be aligned automaticly, if the data contains enough beads.

2 Data processing

STORM data from different cells or structures show many different features, there can be clusters of flourophores with a high density of spots, low density, variable background in space and time, beads can be present or not. This section is about how the algorithm processes the data sets and how it is possible to find good settings that will work for all kind of input data.

2.1 Import and processing

The STORM data has usually a size of around 3 gigabyte. There are even larger datasets possible, so that it is important to work on smaller parts of the data, instead putting the whole dataset into memory. This is done using chunks of user defined size. The data is processed chunkwise, there is parallelisation for the frames of each chunk. This is possible because the signals in each frame are considered to be independent from each other.

2.2 Workflow

2.2.1 Chose parameters

At the begining the user has the option to set all important parameters, if no parameter is set the default ones are used and will give a good result because all crucial parameters are either determined from the data or set to reasonable values that work for every data set. The focus of the default parameters is to give a good result with no adjustment. This means parameters are chosen to produce almost 100 % precission. The loss of some points that are not detected using this conservative setting won't affact the final result as much as a lower precission will do.

2.2.2 Estimating camera gain and offset

First of all it is checked whether there exists a file containing settings for gain and offset from an earlier run. If this is not the case new parameters are estimated based on the first part of the data, usually 200 frames are sufficiant.
The method described by Hwang et al. (2012) is used to estimate the gain factor. For this methode a Skellam distribution is used. Each dataset is three-dimensional where time is the third dimension. Therefore mean μ and variance σ^2, in time, can be calculated

from the data for each pixel individually

$$\mu(i,j) = \frac{\Sigma_t(I_t(i,j) - I_{t+1}(i,j))}{n} \tag{2.1}$$

$$\sigma^2 = \frac{\Sigma_t(\mu - (I_t(i,j) - I_{t+1}(i,j)))^2}{n-1} \tag{2.2}$$

To determine the gain factor the Skellam parameter are plotted over the mean intensities. A straight line can be fitted and its slope gives the gain factor.

2.2.3 Recursively adjusting gain and offset

After the estimation of gain factor and offset, the transformations described in 6.3.1 and 6.3.2 are applied and the background subtracted.
Due to the Anscombe transformation the background pixels of the image should only vary around a mean intensity of zero with a variance of 1. Therefore a histogramm of the pixel intensities is created. The after background subtraction the background pixels should contribute only to the lower intensities in the histogramm. A gaussian function is fitted to the histogramms values. This is done under the assumption that there is much more background in the image than signal or the intensities coming from signals are distributed over a larger range, so the gaussian for the background intensity distribution can be fitted correctly.
If the estimated value for the variance is too far off 1 the originally estimated gain factor is corrected, applied and the fit is done again. This is done until the background variance converges or the maximal number of iterations is reached. In this case the initial gain factor will be used and a warning printed to the screen.

2.2.4 Estimating the width of the point spread function

For a certain number of frames the Fourier transform is calculated and averaged. The result is called mean power spectrum. It can be used to estimate the variance of the point spread function of the signal. A two dimensional gaussian functions Fourier transform is again a gaussian but with inverse variance. This relation is used to determine the variance of the point spread function in spatial domain, using the fit parameter for the variance in frequency domain.

2.2.5 Processing the data

Import Data

Storm data sets can consist of several thousand frames with resolutions up to one mega pixel per frame. This makes it necessary to break the data into smaller parts because otherwise it might be much larger as the RAM of an ordinary machine. Because of the background estimation it is not possible to process every frame completly independent as it was in the older version of this software Schleicher (2011). It is also faster with

some datatypes to load a larger consecutive part of the dataset into memory.
This algorithm uses chunks of user defined size. There are some limitations to the chuncksize that are discussed later. The data set is split into parts of equal size in x- and y-dimensions and independently also in t-dimension. If this partition does not fit at the edge of the data set, the last chunks will be smaller.
The data is transformed to be Poission distributed and after that the Anscombe transform is applied.

Background estimation

For each chunk the median is determined to get a robust estimate of the background value for this chunk. The Bspline interpolation implemented in vigra Köthe (2011) is used to get interpolated values for the full resolution of the current frames. For this interpolation three chunks in t-dimension have to be available. Therefore the maximal chunksize in t-dimension must not be larger than a third of the total stacksize.
This background is then subtracted from the transformed data to give finally background pixels with zero mean and a variance of one, both in xy- and in t-dimension.

Create mask for background suppression

With the given p-value from the settings a global threshold can be determined, because inhomogeneities of background intensities has been removed. The threshold value is that intensity that is explained from a gaussian distribution with mean zero and variance one with the given p probability. This is possible because the background intensity follows such a distribution after all the applied transformations.
The threshold is applied to the current frame and stored as a mask. Due to the probability that background pixels intensitis might exceed the threshold the connected components of the mask are calculated. Pixels that belong to connected components with too few members are discarded.

Filter data and finding maxima

To imporve the accuracy of the spot detection the transformed signal is convolved with a two dimensional gaussian function with the previously determined or user set width. The convolved image will further be used to find the maxima. Each maxima found is tested to be covered by the mask or discared otherwise. A region of intrest around the remaining maxima is interpolated to a higher resolution. In the interpolated region it is searched for maxima for the last time. This maxima will be detected with super resolution.
To determin the signal-noise-ratio the unfilterd and uninterpolated pixelintensity is used.

Quality control for detections

Especially in data sets with a high density of spots it can happen, that two spots are near each other and the point spread functions overlap. It may happen that instead of

two maxima just one maximum will be detected right between the true ones. This leads to large errors in the localisation. To avoid this a threshold for the asymmetry of the spots can be set.

2.3 Comparison with older version of the storm algorithm

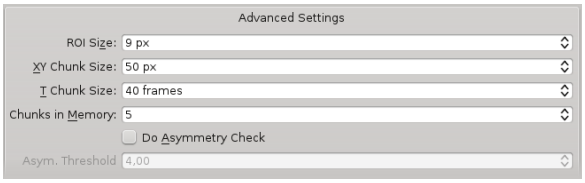
2.4 New graphical user Interface (GUI)

2.4.1 Input widget

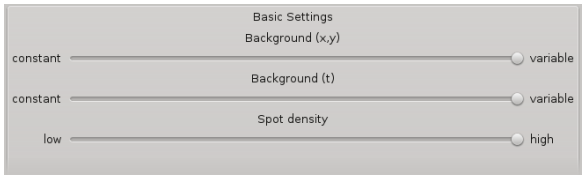
The new GUI for SimpleStorm was designed because where are many new features. Figure 2.2 shows the new design. The GUI was mainly designed by Ilia Kats. In principle there are three categories of parameters. The first category tells the program which upsampling factor shall be used or what the pixel width of the input data is given in nanometers. This are informations that can be chosen by any kind of user without knowing anything about the algorithms the SimpleStorm software uses. The most challenging parameter of this section is the alpha value that sets the sensitivity for false detections. If the user doesn't know what this means or which values gives the best results, he or her can stick to the default setting and alternate it after the run.
The next category of parameters is about region of interest (roi) widths for the estimation of the point spread function, and chunk sizes. There are two different ways to set this parameters (see figure 2.1). One way is to give values for all parameters. This is difficult without understanding the influence of this parameters on the algorithm. Therefore there is also a second way to set this parameters. The user should know some properties of the data that shall be processed, such as is the spot density high or low, is there variable background in time and space or not? Depending on the sliders position the best parameters are set automaticly. With this second option the user can process his or her data, treating variable background or dense data without deep insight or understanding of the used algorithms.
The last catagory of parameters describes which camera gain and offset was used, the width of the signals point spread function and a prefactor that can be used to alter the estimated gain.

2.4.2 Result widget

After the run button at the lower left edge of the input widget was pressed, a new tab opens. In this widget the reconstructed image is shown, also a progress bar and the stop/save button.

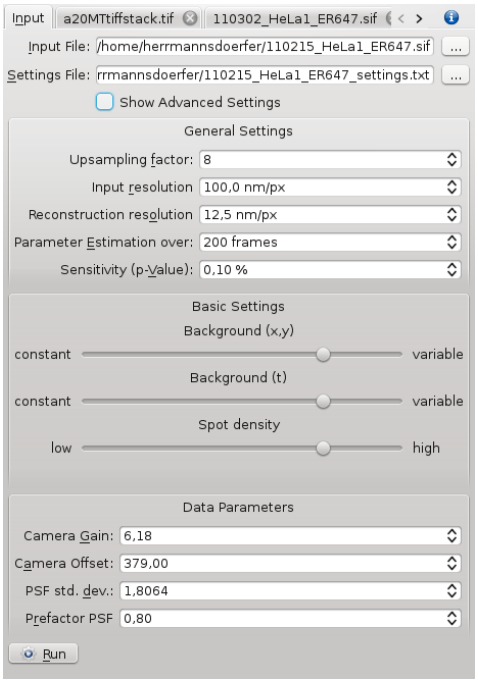


(a) Advanced setting widget

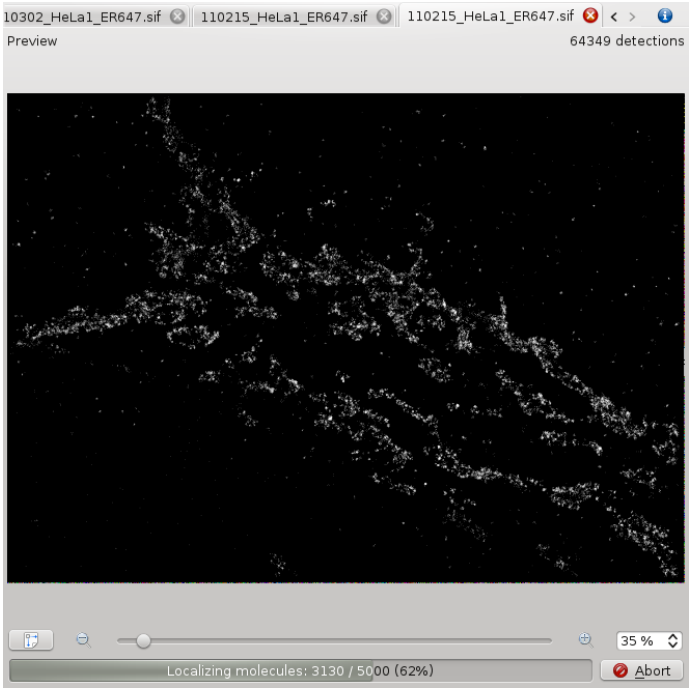


(b) Easy setting widget

Figure 2.1: There are two different ways to set the parameters important for the algorithm. In the picture on the left the standard way of setting parameters can be seen. On the right, there are sliders that can be adjusted between two extrems each. The program sets the value for the parameters in a way to produce the best results for the selected attributes of the data set.



(a) Input widget



(b) Result widget

Figure 2.2: The new gui design. On the left is the window for selecting input file and parameters show. On the right the result widget showing the procession of a data set in progress.

3 Multicolor registration

3.1 Background

In microscopy it is often desirable to label different structures in a cell with different colors. To do so our collaborators use different fluoroscent molecules that emit light at different and distinguishable wavelengths. Using different filters it is possible to capture pictures just containing light emited from one fluorophore. To get a mulit-channel picture the different channels must be aligned. Because different flourophores emit different wavelengths, cromatic aberration apears. This means that the light for the same spot but with different wavelenghts is not mapped to the same spot in the image. To align the different channels despite cromatic aberration, beads are used. Beads are flourophores added to the probe, that emit light in all wavelengths the different markers do and therefore are visible in all channels. The beads can be used as landmarks, because their position in the original image is at the same spot. The task is to find a transformation that maps corresponding beads on each other.

3.2 Features of the colercomposer application

3.3 Bead detection

The input for the colorcomposer application is a text file created by the storm algorithm that contains information about the position, intensity, symmetry, framenumber and signal-to-noise ratio of each detection. The beads should ideally be visible in most of the images, this means one must search for detections that appear in almost every frame at the same position. Therefore it is plausible to take every detection of the first 50 frames as initial candidates for beads. After that candidates that are closer than a threshold are merged to get a list of all location where beads might be. Given that list every other detection is tested to belong to one of the bead candidates. If a candidate gets too few members it is no longer considered to be a bead and removed from the list.

3.4 Align Beads

After the beads for each channel are found the next task is to find the same bead in each channel. It can happen that some beads occure in just one channel, if this is the case there will be no corresponding bead in the other channels.
To do so, the minimal number of beads, three to four, that are neccesary to calculate

the transformation are chosen randomly from the first channel. After that, based on a probabilistic approach and a distance matrix containing information about the distances between all beads of the two channels, three to four beads from the second channel are chosen.
Using this pairs of beads linear transformation is found like described by Schleicher (2011). Using this transformation it is tested how many beads match in total. It is assumed that the correct transformation will match other bead pairs that were not chosen to calculate this transformation. After that the whole procedure is done multiple times. In the end the best transformation is chosen.
In principle shearing should also be allowed for this transformation, but tests indicate that shearing does not occure. There is a problem if there are just three beads in each channel, then every time a perfect transformation is found, but with the constraint of forbidden shearing, the right solution can be identified.

3.5 Accuracy of Registration

3.6 Colocalisation

3.6.1 Global colocalisation

3.6.2 Local colocalisation

3.6.3 Validation of colocalisation approaches

"Image set CBS001RGM-CBS010RGM from the Colocalization Benchmark Source (www.colocaliza
benchmark.com) was used to validate colocalization."

4 Futur work

4.1 3d Storm

4.2 Improved methode to detect maxima

4.3 Colorcomposer implemented in Storm-Gui

4.4 Interactive parameter selection

An unprocessed image is shown to the user. The user marks areas with spots, where he thinks that it is signal or dark spots he wants to have detected. Then the program sets the parameters in a way that at the user labeled areas will contain signal in the end.

Alternatively one could set the parameters and get a direct feedback which points would be selected in the shown frame.

5 CCD camera

5.1 Image acquisition

5.1.1 Photon sources with shot noise

The emission of photons is a random process that occures at unpredictible times. Therefore the number of photons passing through a plane is never constant but varies around some average value. The phenomena, that one can never determine exactly how many photons should hit the sensor chip of a CCD camera for example, is called shot noise. It playes a major role if the total number of photons is low, as from dark sources or with short exposure times of the camera.

5.1.2 Quantum efficiency

Quantum efficiency describes the fraction of photons that create a detectable electron in a sensor chip. The quantum efficiency is dependent of the wavelenght of the incoming photon. Photons with energies below the band gap can't produce a free electron that can be detected. The quantum efficiency has a maximum basically caused by two effects. The higher the photons energy the higher the kinetic energy of the freed electron, but it is absorbed earlier and can therefore recombinate with a electron hole more likely.

5.1.3 Gain

There are two different gain factors involved in the capturing process of a camera. First the electric signal for each pixel might be amplified. And there is also a gain factor that describes the proportionality between collected electrons and the digital number it is associated with.

5.1.4 Readout noise

The origin of readout noise is the amplifier. The aplification is never perfect, this means the exact number of electrons at the end of the amplification has some variation around the expected linearly increased value. There might also be some random signals of the electronics that add to the "true" signal. The readout noise is independent of the exposure time.

5.1.5 Dark current noise

Dark current noise is generated by the thermal movement of the atoms in the sensor chip. The movement of molecules and atoms is dependent of the temperature of the material, because of that dark current noise depends strongly on the temperature of the chip and can be reduced by cooling. Dark current noise generates electrons in the bins of each pixel even with closed shutter it is constantly increasing with time and follows Poisson statistics.

5.1.6 Quantisation

The signal must fit into the output color depth. It has to be rounded or truncated to fit in. This process introduces errors that can be seen as additional noise that is dependent on the intensity of the signal. High intensities are disturbed less relative to low intensies.

6 Theoretical background

6.1 Distributions

6.1.1 Poisson distribution

One very important probability distribution in physics is the Poisson distribution. It describes the results of "counting experiments" and is therefore very important for image processing as the pictures taken with a camera are in principle counts of photons reaching the camera. Photon counting noise is one important example.
Poisson distributions are just defined for integer values and the variance is the same as the mean value of the distribution. Another important attribute is the skewnes which is the inverse of the squarerot of the mean or variance and describes the assymetry.
The probability mass function is:

$$p(n, \mu) = \frac{\mu^n}{n!} \exp(-\mu) \tag{6.1}$$

6.1.2 Skellam distribution

The probability mass function of a Skellam distribution is a function of the difference between two Poisson random variables

$$p(k; \mu_1, \mu_2) = \exp(-(\mu_1 + \mu_2)) \left(\frac{\mu_1}{\mu_2}\right)^{k/2} I_{|k|}\left(2\sqrt{\mu_1 \mu_2}\right) \tag{6.2}$$

where n_1, n_2 are the Poisson random variables and $k = n_1 - n_2$. $I_{|k|}$ means the modified Bessel function of the first kind.
Mean μ and variance σ of the Skellam distribution are given by

$$\mu = \mu_1 - \mu_2, \qquad \sigma^2 = \mu_1 + \mu_2 \tag{6.3}$$

$$\Rightarrow \qquad \mu_1 = \frac{\mu + \sigma^2}{2}, \qquad \mu_2 = \frac{-\mu + \sigma^2}{2} \tag{6.4}$$

6.1.3 Approach using skewness of poisson distribution

For every pixel there is a set of multiple values in the set. This allows to calculate the different parameters individually for each pixel. One can calculate mean and variance of the measured intensities $I_{\text{meas}}(i, j)$ and gets

$$\text{mean}(I_{\text{meas}}(i, j)) = g \cdot \text{mean}(I_{\text{true}}(i, j)) + o \tag{6.5}$$

$$\text{var}(I_{\text{meas}}(i, j)) = g^2 \cdot \text{var}(I_{\text{true}}(i, j)) \tag{6.6}$$

Assuming a Poisson distribution as the true intensity, mean and variance would be the same. Unfortunately the mean true intensities are unknown and it is not possible to determin g and o so far. For large mean Intensities μ the Poisson distribution becomes more and more similar to a Gauss distribution with the same mean. However, for small means, the Poisson distribution is not symmetric. The skewness s_p of a Poission distribution is the inverse of the square root of the mean $(\mu)^{-.5}$. It can also be directly calculated from data

$$s_p = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{x_i - \bar{x}}{\sigma}\right)^3 \tag{6.7}$$

The skewness is invariant to shift and multiplication with a constant. This means that the transformation caused by the camera gain and the dark current does not affect the skewness. This gives a third equation to solve for g and o.
This approach has very strict limitation at least for background pixels not to be too bright. If the mean of the true Poisson distributin is higher than roughly 30 the skewness gives due to noise no stabel results and it is impossible to determin the mean intensity in this way.

6.2 the data

The concept of direct stochastic optical reconstruction microscopy (dSTORM) Heilemann et al. (2008) is, to label interesting structures with fluorophors that can be exited using a laser with the appropriate wavelength. After a short time the flourophors emit a photon and go back to the unexited state or lose the ability to get exited, they bleach out. The datasets for dSTORM microscopy that we recieve from our collaborators from Bioquant are big datasets of several gigabyte in the Andor .sif format. Each file conains a stack of pictures, normaly between 1000 and 10000, taken consecutively with exposure times between 20 and 200 milliseconds.

Picture /refrawStorm shows a typical frame of raw data. In each frame there might be multiple flourophores visible at the same time. Due to the large magnification, beyond the diffraction limit, the almost pointlike flourophores appear as gaussian shaped signals, their point spread functions. The flourophores are either attached to the biological structures that are of interest or they form a cluster, called a bead.

Beads are larger and brighter than spots form only one flourophore and are used to align multiple channels in the postprocessing step. Beads are designt to show up in every frame of the sequence at the same position. They are composed of flourophores of different color to be visible in every channel.

The other spots, bound to some proteins for example, are just lighting up for a very short time. This is the key aspect of dSTORM. Instead of one frame that shows all flourophores at the same time, thousands of frames are captured containing just a point

spread functions per frame. This gives the possibility to determine the center of each point spread function with sub-pixel precision, and in the end when all points are displayed together in one picture, to an image with a resolution beyond the diffraction limit.

6.3 Transformations

6.3.1 Transformation to Poisson distributed signal

The images aquired from the camera show not the real intensities I_{true}, which result from the photon emission of the probe, but transformed ones I_{meas}. I consider two main reasons why the taken image differs from the true image, besides noise.
There is dark current which means that even a picture taken with closed shutter would get some intensity, even without any light hitting the sensor chip of the camera. This is a result of thermal movement of the atoms off the sensor chip and can be reduced by cooling. The dark current noise adds an almost constant value o to the output signal. Incoming photons create electrons via inner photoelectric effect. This electrons are collected for each pixel and might be amplified to get the final result. Assuming a linear relation between the number of incoming photons and the number of electrons created and a linear amplifier results in a factor g. This factor is multiplied with the number of photons captured during exposure time for each pixel.
If the gain factor g and the offset o are known the true intensity, the number of photons detected is:

$$I_{\text{true}} = \frac{I_{\text{meas}} - o}{g}. \tag{6.8}$$

6.3.2 Anscombe transformation

The Anscombe transform is used to transform a random variable with a Poisson distribution into one with an approximatly constant standard deviation. The transformation is defined as:

$$A(x) = 2\sqrt{x + \frac{3}{8}}. \tag{6.9}$$

As one can see in figure 6.2 the Anscombe transformations result has for mean intensities greater than 4 a intensity independent standard deviation of one.

6.4 Estimation of camera gain

Given a sample prepared for STORM microscopy. A sequence of images captured from this sample will show some active flourophores, beads and also illuminated background that comes from flourophores that lie not in the focus plane. Assuming an inhomogenous

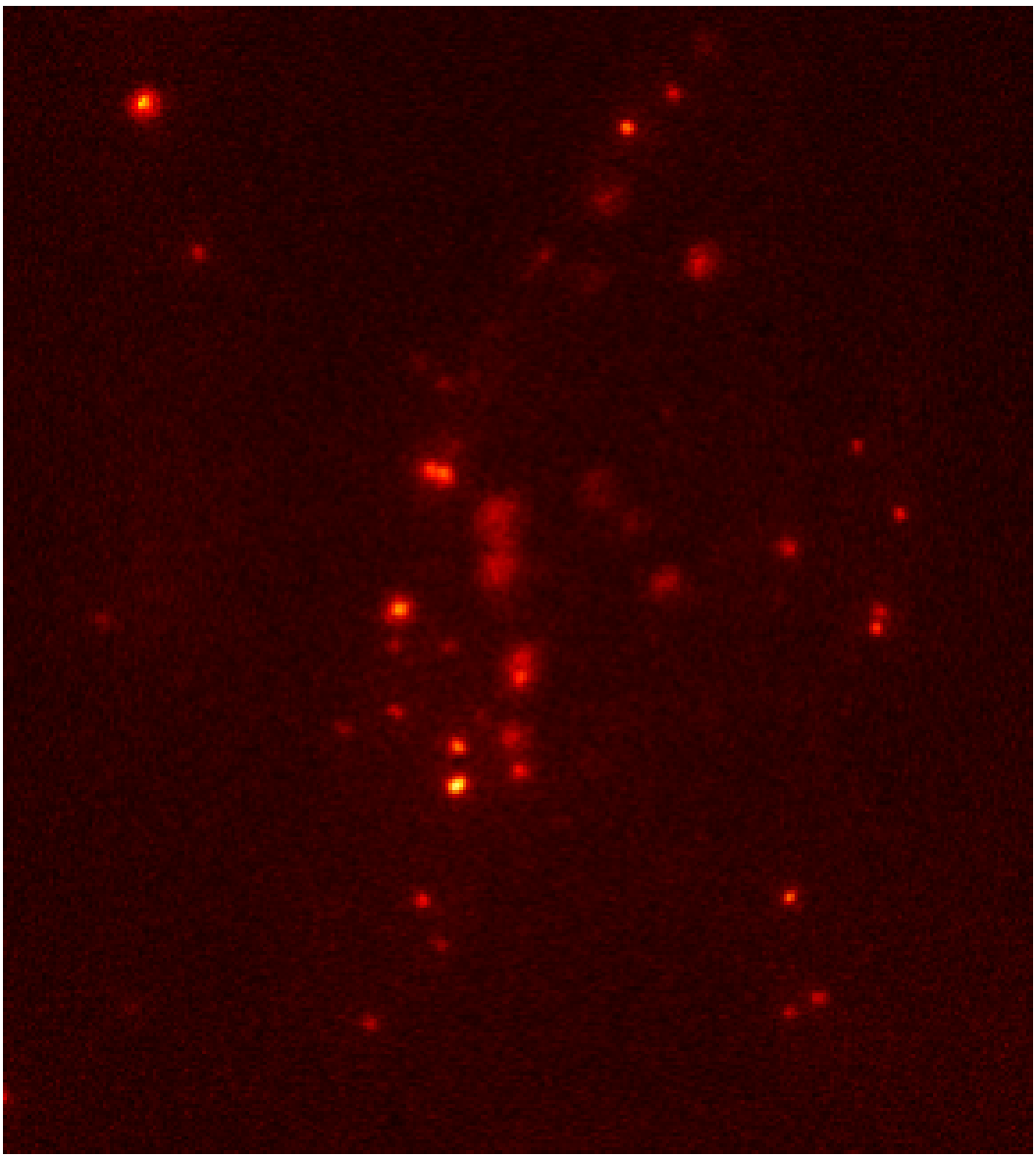


Figure 6.1: Raw image for dSTORM processing

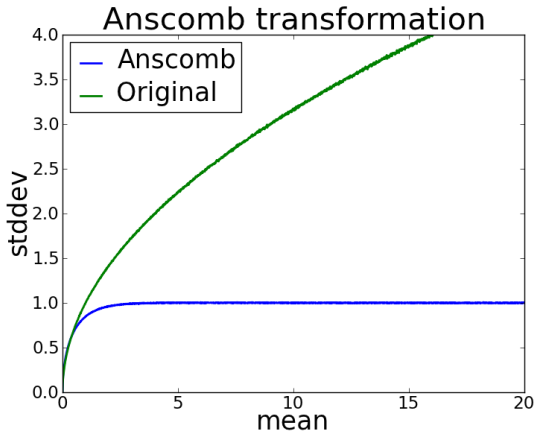


Figure 6.2: Standard deviation over mean intensities of different Poisson distributions

background signal that follows a Poisson distribution, or even better almost homogenous background and beads both with a Poisson distribution in time with different mean values. If this Signal I_{true} is transformed in the following way:

$$I_{\text{meas}} = g \cdot I_{\text{true}} + o \tag{6.10}$$

This is the inverse transformation of equatione 6.8.
Considering two pixels with different Poisson distributions P_1 and P_2 with mean value and variances of this distributions λ_1 and λ_2. If this distributions are transfomed as given in equation 6.10 their mean and variance change like shown in equation 6.5 and 6.6. This gives the oportunity to determine the gain and offset using two or more pixels.

$$\text{var}(I_{\text{meas1}}) = g^2 \cdot \text{var}(I_{\text{true1}}) \tag{6.11}$$
$$\text{var}(I_{\text{meas2}}) = g^2 \cdot \text{var}(I_{\text{true2}}) \tag{6.12}$$
$$\text{mean}(I_{\text{meas1}}) = g \cdot \text{mean}(I_{\text{true1}}) + o \tag{6.13}$$
$$\text{mean}(I_{\text{meas2}}) = g \cdot \text{mean}(I_{\text{true2}}) + o \tag{6.14}$$

The values for $\text{var}(I_{\text{meas1/2}})$ and $\text{mean}(I_{\text{true1/2}})$ can be calculated from the data and can be used to get the gain as follows:

$$\frac{\text{var}(I_{\text{meas1}}) - \text{var}(I_{\text{meas2}})}{\text{mean}(\text{meas1}) - \text{mean}(I_{\text{meas2}})} = \frac{g^2 \cdot \lambda_1 - g^2 \cdot \lambda_2}{g \cdot \lambda_1 + o - (g \cdot \lambda_2 + o)} \tag{6.15}$$
$$= \frac{g^2 \cdot (\lambda_1 - \lambda_2)}{g \cdot (\lambda_1 - \lambda_2)} \tag{6.16}$$
$$= g \tag{6.17}$$

In the same manner the offset o can be calculated.

$$\text{mean}(I_{\text{meas1}}) - \frac{\text{var}(I_{\text{meas1}})}{g} = g \cdot \lambda_1 + o - \frac{g^2 \cdot \lambda_1}{g} \tag{6.18}$$
$$= o \tag{6.19}$$

7 ISBI Challenge 2013

7.1 Introduction

The goal of the ISBI Challenge, as announced on their website (Biomedical Imaging Group (2013)), is to give an overview and understanding of availible algorithms for single particle localization microscopy. The focus was on 2d localisation, to give information about the depth of a localisated spot was optional. To benchmark results one needs groundtruth. Therefore the organisers created synthetic datasets of biologically relevant structures such as tubulins. To match realistic conditions the data was transformed to introduce different kinds of noise and background to it.
The participants were given training data sets and the corresponding groundtruth and one month before the deadline of the challenge the test sets. There were two different kind of datasets in principle. One with very dense spots and shorter sequences, the other with longer sequences and fewer spots per frame.
All paricipants were asked to submit their results and also the time it took to run the algorithm and the hardware configuration of the used system.

7.2 Terminology

To be able to compare different algorithms there must be a way to determine the correctly detected spots. To do so for each estimated positon of a flourophor, the nearest correct position of the molecule in the groundtruth data was searched within a lateral tolerance disc. Once a match was found this two spots were taken out of consideration for the matching.
One important parameter for this evaluation is the radius of the lateral tolerance disk, because it has big influence on the number of detections considered to be true positives (TP).
Detections with no associated spot in the groundtruth are called false positives (FP), spots in the groundtruth with no matching detection are called false negatives (FN).
This matching is done frame by frame, it is not possible to match a point from different frames even if the x and y coordinates match perfectly but the frame differs.
The precision (p) of a classification task is defined as the ration between the number of true positives and the sum of true positives and false positives:

$$\text{precision: } p = \frac{\text{TP}}{\text{TP} + \text{FP}} \tag{7.1}$$

It is a number between 0 at worst and 1 at best, telling how reliable the result is, how likely it is that a labeld sample really belongs to the predicted class. In this context it means how certain a detected spot has its origin in a fluorophore attached to the investigated structure and it's origin is not wrongly detected background noise.
An other important value is the recall r that is defined as the ratio of true positives and the sum of true positives and false negatives:

$$\text{recall: } r = \frac{\text{TP}}{\text{TP} + \text{FN}} \tag{7.2}$$

The recall lies also in a range from 0 to 1 and gives an impression on how many relevant spots were found.

7.3 Measures

For the evaluation three different measures were used. The f-score index f, the Jaccard index J and the rot-mean square distance RSME.

$$\text{f-score: } f \quad = \frac{2 \cdot p \cdot r}{p + r} \tag{7.3}$$

7.3.1 Jaccard index

Let A be the set of points of the groundtruth and B be the set of detected points. The Jaccard index J is defined as:

$$\text{Jaccard: } J = \frac{|A \cap B|}{|A \cup B|} \tag{7.4}$$

The intersection is done frame by frame. This means two spots from the groundtruth and the detection set just match if they occure in the same frame.

7.3.2 RSME

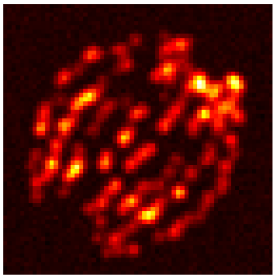
The root-mean square distance gives an impression how big the squared distance between a spot in the groundtruth and an associated detection was in average. It can be calculated like this:

$$\text{RSME} = \frac{1}{|A \cap B|} \sum_{i=1}^{|A \cap B|} \left(p_a(x, y) - p_b(x, y)\right)^2 \tag{7.5}$$

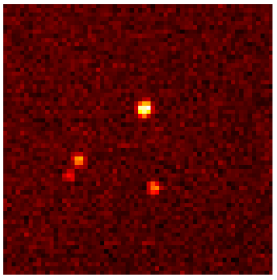
7.4 Trainingsdata

7.4.1 Bundled tubes datasets

There was two kinds of bundled tubes data sets, both created from the same underlying structure, one set with a high spot density and a short sequence of 360 frames, the other



(a) High spot density



(b) Low spot density

Figure 7.1: One frame from bundled tubes training data set

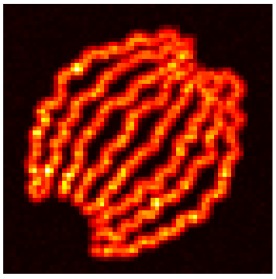


Figure 7.2: Maximum projection of bundled tubes data set

with fewer spots per frame but 12000 frames in total. Picture 7.1 shows one frame of each data set.

The original images very small, just 64 pixels in each dimension. Both sequences had spatial and temporal constant background. Picture 7.2 shows the maximum projection of the bundled tubes data set. The maximum projection is used to reduce the dimensionality of a data set. In this case for each pixel in x- and y-dimension, in the three dimensional dataset, the brightes value from all frames is taken.

7.4.2 Tubulin data sets

The other training data sets models 7 microtubules, a structure that is a long filament up several micrometers long and a diameter of about 25 nanometers. The spot density lies somewhere between the high density and the low density of the bundled tubes data sets. This data sets show strong inhomogeneity in spatial dimensions and moderate inhomogeneity in temporal dimension, see figure 7.3. This is the reason why in the lower left corner of the maximum projection 7.4 a brighter area can be seen.

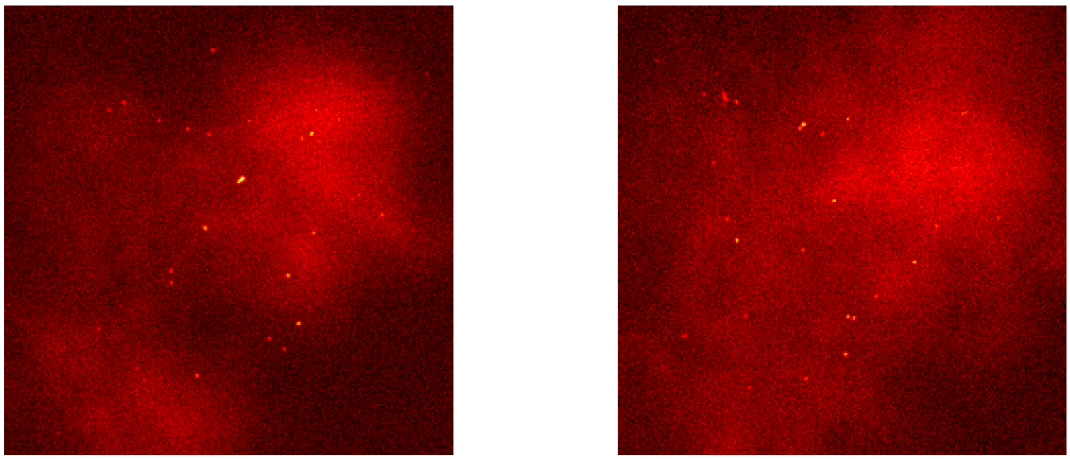
7.5 Submissions

7.5.1 High precision

7.5.2 High score

7.5.3 Highest score via postprocessing

7.6 Results



(a) Tubulin2 frame 10

(b) Tubulin2 frame 1010

Figure 7.3: This pictures show the variability of the background in the spatial and temporal dimensions

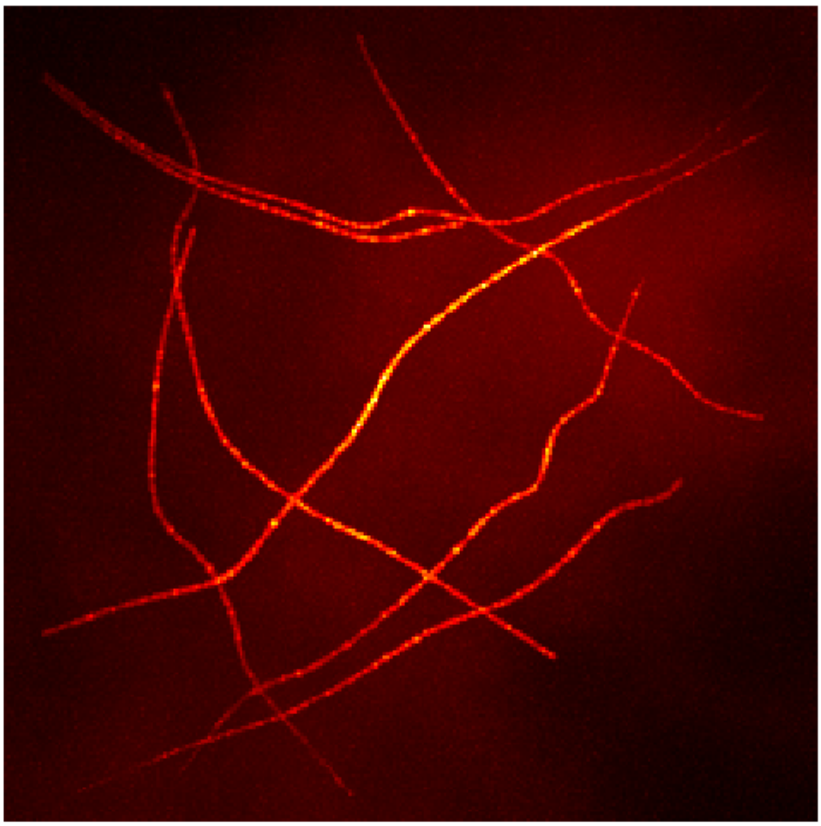


Figure 7.4: Maximum projection of tubulin data set

8 Check of the assumptions

8.1 Calibration measurement

As described by Newberry (1998) the gain can be determined using the mean and variance using data with different mean intensities. Therefore a calibration dataset was aquired. The temperature and the gain factor were set to be like the settings usuall used. A sample with cells was prepared for the Storm measurement. First eleven time series were taken, with opend shutter and with increasing exposure time from 0 milliseconds up to 1000 milliseconds. Afterwards the same procedure were repeated with the shutter kept closed. Figure 8.1 shows the results for the first 6 exposure time. All the points lie on a straight line. The gain factor determined from the calibration measurement was 3.9, the offset 315. This offset is too small. An other methode was used to determine the offset. There were also an other series of images taken with closed shutter. This series shows the response of the camera without any light around. The mean value of the shortest exposure time were taken as offset. The value is 380.

8.2 Correction to Poisson distributions

8.3 Result Anscombe transformation

8.4 Accuracy of detection

Unfortunately the position of the flourescent molecules can't be detected perfectly. There are three main contribution to the error in detection.
On the one hand, there is the problem of finding the maximum in a noisy signal. Due to noise the pixel next to the true maximum might gain some intensity and be therefore brighter.
On the other hand, the position is deteted by upscaling the pixel grid and interpolation. After that the maximums position of the upscaled grid is taken as the resulting position. This gives an error from roughly a 12th pixelwidth. This error becomes less important with higher upscaling factors.

Because there is no groundtruth for the real data, one has to produce test data and groundtruth. This was done simular to the method described by Grüll et al. (2011). The difference was, that instead of generating just one spot per frame a different number of spots were simulated for each frame. For different signal to noise ratios, a dataset with

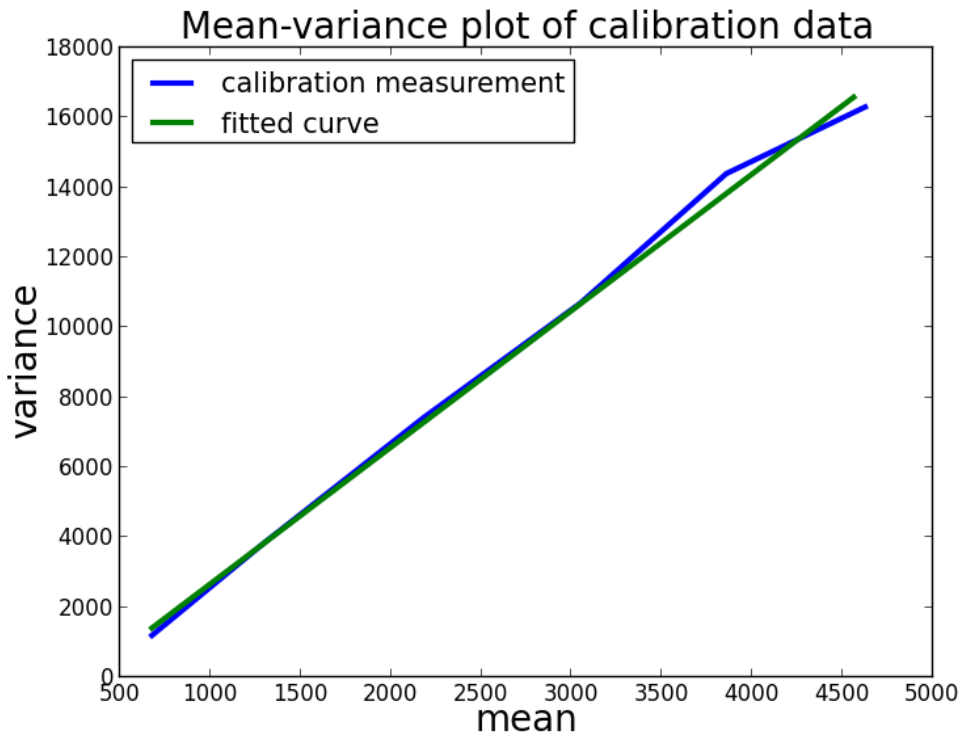
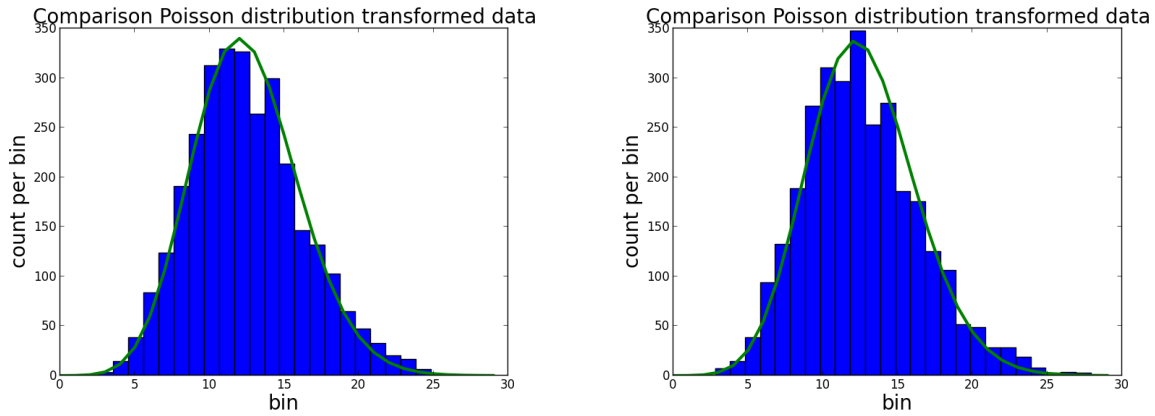


Figure 8.1: Result of the calibration measurements. The gain determined from this variance-mean plot is 3.9.



(a) Tubulin2 frame 10

(b) Tubulin2 frame 1010

Figure 8.2: This pictures show how well the histograms of background pixels taken over the first 3000 frames, follow a poisson distribution. For the gain the parameter from the calibration measurement of $g = 3.9$ were used. The offset was estimated from the minimal intensities of the original image.

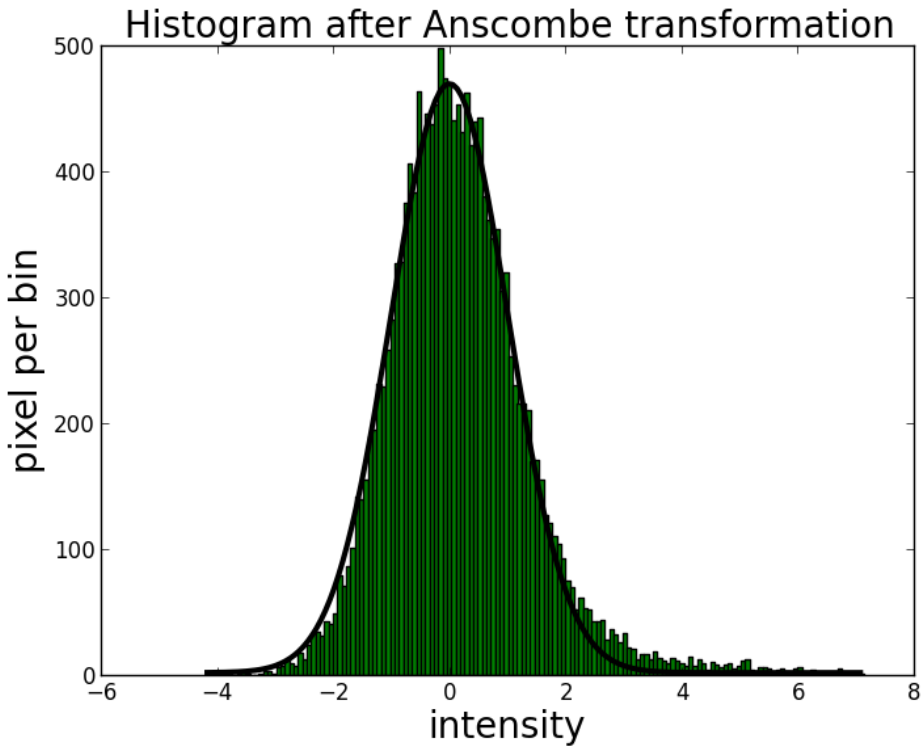


Figure 8.3: After the Anscombe transformation the background pixel intensities should be distributed with mean zero and variance one. This figure shows the histogram of the pixel intensities and a fitted gaussian with variance one and mean zero.

40 times 40 pixels and 1000 frames was created. Each frame containing one, three or five point spread functions. The position of the spots was determined beforehand, to use the same spots for each signal to noise ratio.

To determine the accuracy the standard deviation between the true position of the signal and its detection were used. For data sets with more than one spot per frame, it was searched for the best match within a certain distance around the true position. If a pair of true spot and detection was found, both were removed for further matching of the remaining signals. In the end the averaged standard deviation of the detection relative to the true positions were calculated as follows:

$$\text{std. dev.} = \sum_i \sqrt{(\mathbf{p}_{\text{true}^i} - \mathbf{p}_{\text{detec}^i})^2} \tag{8.1}$$

i runs over all found pairs of groundtruth and detections, $\mathbf{p}$ describes the two dimensional spatial vector of the groundtruth or detection respectivly.

The results can be seen in 8.4. It can be seen, that the more spots are present per frame the harder it is to detect them properly. This is a result of the fact that spots that lay near each other might be detected as just one spot, what gives rise to higher errors in the detection accuracy.

8.5 Bleaching signal

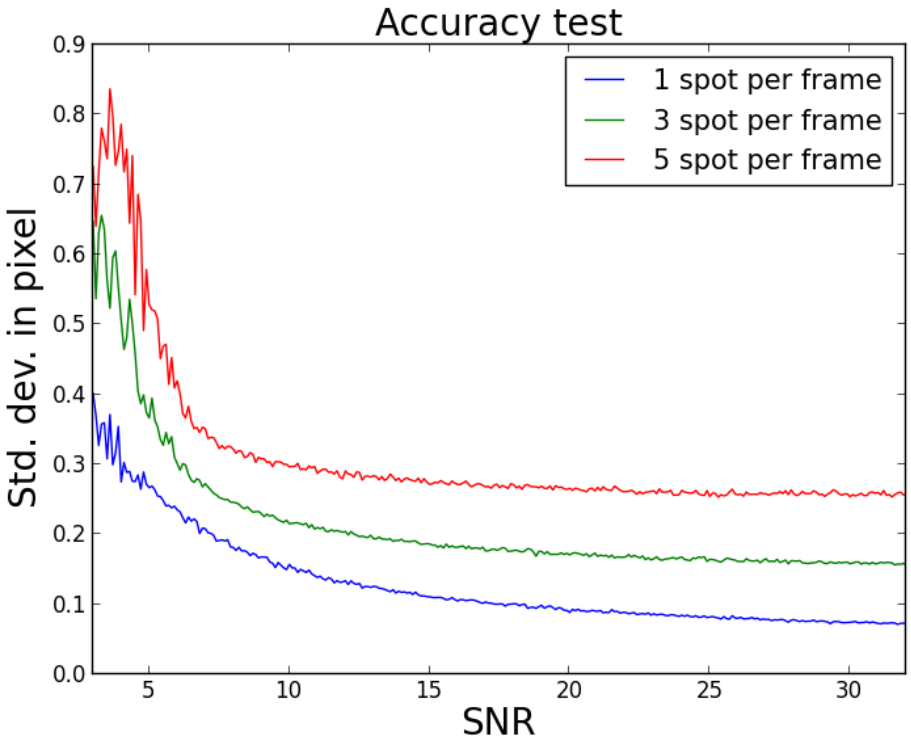


Figure 8.4: Result of the accuracy test. For datasets with one, three or five point spread functions per frame, evaluated for different signal to noise levels. The more dense the spots are the less accurate the detections are.

Erklärung:

Hiermit versichere ich, dass ich diese Master Arbeit selbstständig verfasst habe und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Heidelberg, den 11. Juni 2013 .....

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