ARTIFACTS ANALYSIS IN LOCALIZATION BASED MICROSCOPY

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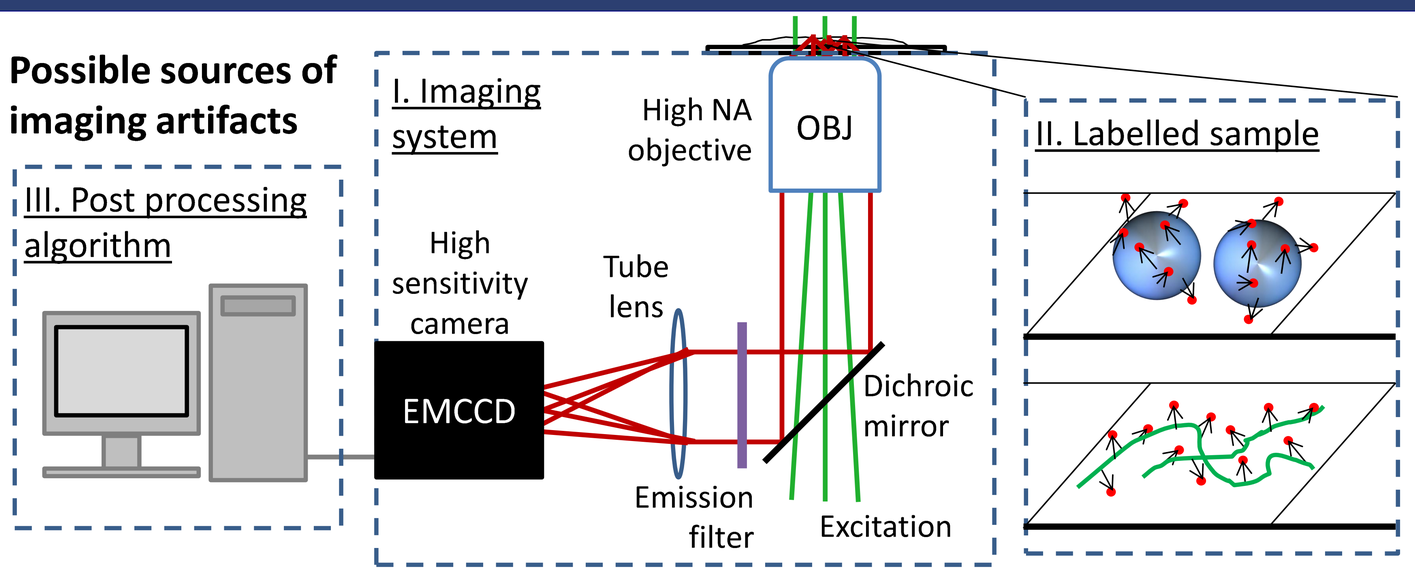
Introduction

Super-resolution systems are implemented into traditional microscope systems, optimized for diffraction limited imaging, so the possible errors need to be reconsidered. The post processing algorithm can also lead to less known artifacts. These random or systematic errors can lead to the misinterpretation of the final image. We present some errors and artifacts, and give methods for their minimization.

Possible sources of imaging artifacts



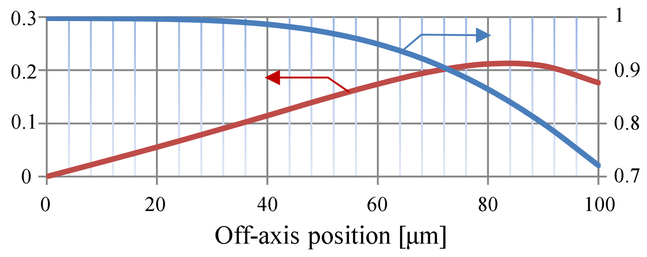
Such undesirable effects are ranked by their origin and classified as introduced by:

- the imaging system,
- the labeled sample,
- the post processing algorithm.

The simulations were made by means of OSLO, optical design program and TestSTORM [1], test sample generator program. We used rainSTORM [2] software for localization.

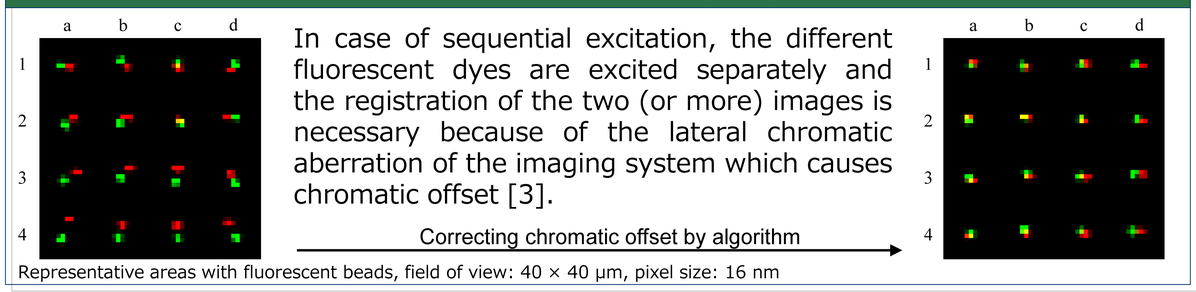
I. Imaging system artifacts

Monochromatic aberration



Monochromatic aberrations can distort the PSF, especially when the source is farther from the optical axis, and this cause the displacement of the center position of the fitted curve. Compensation: e. g. by means of calibration with post processing algorithm.

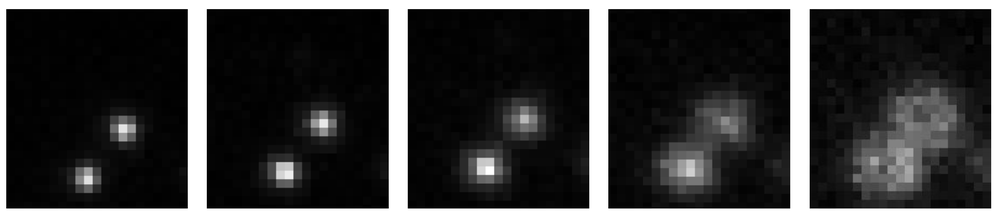
Multicolor imaging



In case of sequential excitation, the different fluorescent dyes are excited separately and the registration of the two (or more) images is necessary because of the lateral chromatic aberration of the imaging system which causes chromatic offset [3].

Representative areas with fluorescent beads, field of view: 40 × 40 μm, pixel size: 16 nm

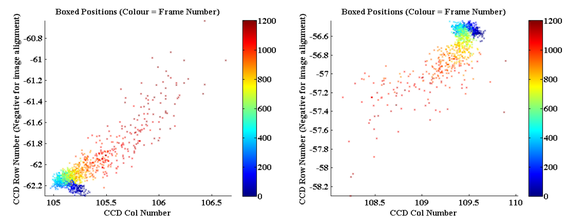
Three dimensional drift (thermal and mechanical)



The 3D drifts should be kept smaller than the localization precision (~10 nm) throughout the entire data acquisition time to avoid blur caused by the drifts.

Compensation:

- usage of autofocus system
- mechanically and thermally stable arrangement

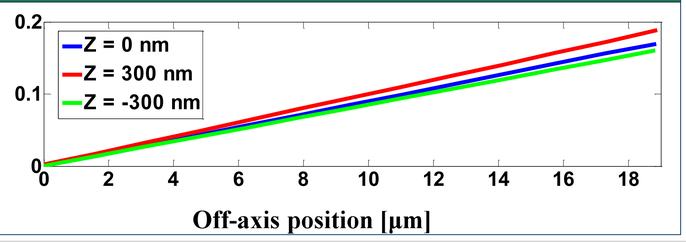


II. Labeled sample artifacts

The region located between the focal plane and the surface of the cover plate can affect the imaging in both the single and multicolor imaging. This type of error highly depends on the sample structure too.

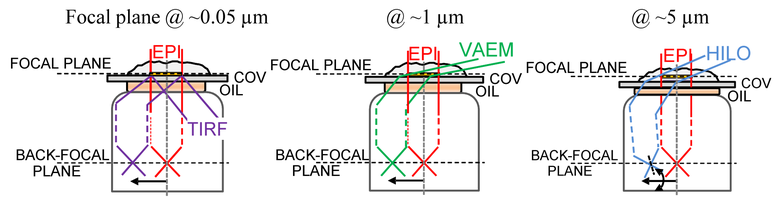
Multicolor imaging of extended samples

The degree of the chromatic offset depends on the amount of defocus which play key rule in extended sample imaging. 3D correction can be applied.



Fluorescent background

- The precision of the localization highly depends on the background level.
- Possible way of background reduction: applying inclined illuminations.



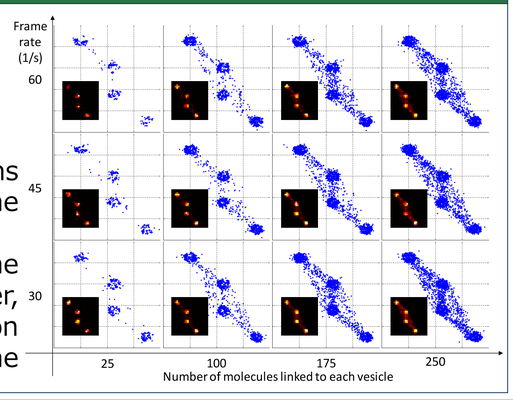
III. Post processing algorithm artifacts

One key requirement for successful localization microscopy is that fluorophore positions are determined precisely, but even more important is that false positive localizations are excluded. When using a single molecule fitting algorithm, it is desired to avoid situations where two or more molecules produce overlapping point-spread functions which can lead to a single false localization. Experimental optimization methods are expensive and time consuming, but simulations with software such as TestSTORM is a valuable method for initially estimating the best exposure time and other parameters for a measurement.

Bridge artifact

Simulation arrays (here with vesicles) are useful for optimizing experimental parameters. Ranking the results according to the labeling density and frame rate is an effective way to find the process window.

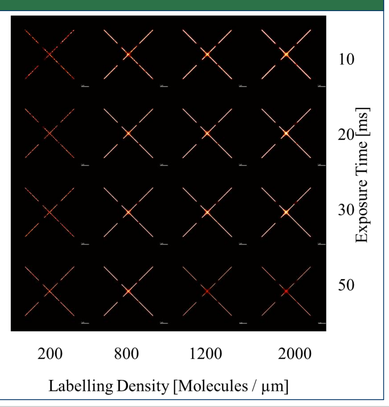
- Increasing the labeling density, mislocalizations appear between the vesicles because of the overlapping PSF.
- Decreasing the exposure time, decreases the occurrence of the overlapping PSF spots. However, with shorter exposure times the worse localization precision leads to blurring and an increase in the apparent size of the vesicles.



Edge artifact

Image sequences were simulated for crossing-lines samples and three different artifacts could be observed:

- The number of good localizations decreases near the crossing of lines, while the number of bad localizations increases.
- With high labeling density, the gap between the line parts is filled with false localizations especially in shorter gaps. Lower labeling density can help, but if it fails to satisfy the Nyquist sampling theorem, spurious extra gaps are implied.
- The line part with homogeneous labeling has decreased brightness in the central part.



Conclusions

Localization-based super-resolution microscopy methods can improve the spatial resolution to a few tens of nm. However, such high resolution requires at least the same degree of precision. Therefore, minor effects typically neglected in traditional microscopy start to play important role, and the implementation of the final high resolution images is sometimes challenging. Imaging artifacts were categorized by their origin and methods were given for the elimination or reduction of these effects.

References

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[2] E. J. Rees et al, “Blind assessment of localization microscopy image resolution,” Opt. Nanoscopy 1(**1**), 12 (2012)

[3] Miklos Erdelyi et al, "Correcting chromatic offset in multicolor super-resolution localization microscopy," Opt. Express **21**, 10978-10988 (2013)

Information

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