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.

### I. Imaging system artifacts

![Diagram showing possible sources of imaging artifacts](image)

#### Monochromatic aberration

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

#### 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.

### 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.

### 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 localization.

#### Multicolor imaging of extended samples

The degree of the chromatic offset depends on the amount of defocus which play key role 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

Simulation arrays (with here 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 PSFs.
- Decreasing the exposure time, decreases the occurrence of the overlapping PSF spots. However, with shorter exposure times the worse localization precision begins to blurring and an increase in the apparent size of the vesicles.

#### Bridge artifact

![Diagram showing bridge artifact](image)

#### Edge artifact

![Diagram showing edge artifact](image)

### 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 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 suppression of these artifacts.

### References


### Acknowledgment

This research was partially funded by the Hungarian Brain Research Program (KIA_13,NAP-A14/14). AE acknowledges support from the Marie Curie Career Integration Grant (PIEJCI-2014-19718) and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. We acknowledge Lambda Research Corporation for providing the OSLO optical system design software for the simulations.

### Information

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