

## ImageJ Tools for STED Performance Analysis

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### Abstract

Stimulated emission depletion (STED) microscopy is a super-resolution fluorescence-based technique where the diffraction limit is overcome. A ring shaped beam is used to inhibit fluorescence in the outer regions of the excitation point. The size of the resulting emitting point can be reduced, virtually, without any limitation. As the performance of STED is based on superimposing the STED beam to the excitation beam, the system is very sensitive to misalignments. In addition, other factors such as image exposure time and dwell time (travel's duration of the scanning laser on the area corresponding to a pixel in the final image), as well as temperature changes or small vibrations can produce important effects on the alignment of the setup and that compromise the resolution efficiency. Therefore, it is not easy to determine when the performances of the system are at its best. In this work we present an ImageJ plugin which implements an analysis of the image quality, based in the Fourier transform (FT) formalism. This quantifies the performance of our STED microscopy in order to determine the actual imaging conditions. In order to determine the increase of resolution, we use the build-in functions of ImageJ to calculate the dispersion of the FT of the image of nano-sized fluorescent beads. To be able to compare single values, we perform the average in the angular direction of the FT. We can, then, make use of the one-dimensional dispersion obtained, which is directly related to the resolution of the system.

### Keywords

STED microscopy, Fourier transform analysis, super resolution

HeLa cells' vimentin labeled with Horizon V500. a. Confocal Image b. STED image c. Fourier analysis

