FOURIER ANALYSIS FOR OPTIMISATION OF A STED IMAGING SYSTEM

David Merino, Jordi Andilla, David Artigas, Pablo Loza-Alvarez
ICFO - The Institute of Photonic Sciences, ICFO, Castelldefels, Spain
e-mail: pablo.loza@icfo.es

Stimulated emission depletion is a fluorescence-based microscopy technique in which diffraction limit is overcome by inhibiting fluorescence in the outer regions of the excitation point spread function by means of a depleting source [1,2]. Here, we present a method based on the Fourier Transform (FT) formalism that can provide information related to the performance of the STED system compared to a confocal microscope.

If we accept the image of a number of randomly organized beads can be described as follows:

\[ I(x, y) = \sum \delta(x - x_i, y - y_i) \ast b(x, y) \]

where \( b(x,y) \) is the image of a single bead, and \((x_i, y_i)\) are the positions of the image where a bead is found. According to this, the FT of this light intensity distribution can be obtained as:

\[ \{\text{FT}\{I(x, y)\}\} = \sum_i C e^{j\omega \delta} e^{-j\omega \cdot \delta} \{\text{FT}\{b(x, y)\}\} = \{\text{FT}\{b(x, y)\}\} \sum_i C e^{j\omega \delta} e^{-j\omega \cdot \delta} \]

Therefore, in an ideal situation, the FT of an image of randomly organized beads is proportional to the PSF of a single bead.

Under these premises, we have developed a model that describes the PSF of the system, and we have fitted it to the FT of the images of beads, as shown in fig 1. The fit provides information of the contribution of the high frequencies to the image. This information can be compared between the confocal and STED images obtained with the same imaging system, and the changes can be related to the performance of the STED system. Figure 2 shows this performance calculated using this method for different values of the power of the depleting source.

We suggest the use of this method as a fast way to characterise the performance of the system, and have applied to different configurations to find an objective parameter that can be used to determine the performance of the STED system based only on the images obtained.

---