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MUSICAL and super-resolution fluorescence microscopy: latest developments

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Extended Abstract

Fluorescence microscopy allows the study of biological structures in optical microscopy with high specificity and contrast by using special molecules attached to the structure of interest. Under proper illumination, such molecules behave as independent light sources whose photons are then captured in the camera of the system. However, due to diffraction of light, optical microscopes act as low-pass spatial filters. Therefore, small structures are no longer visible as high spatial frequencies are lost before reaching the camera. As small structures are fundamental for the study of processes at a subcellular level, surpassing this limit is of critical importance. As a result, several techniques coming different approaches have been developed. Among them, Multiple Signal Classification Algorithm (MUSICAL) [1] is a technique inspired in the use of MUSIC in electromagnetic inverse scattering problems.

MUSICAL is a computational algorithm that exploits the variation in the intensity of light produced by independent fluorophores in the sample without the need of special configurations or fluorophores. Similarly to MUSIC, the algorithm requires several snapshots of a particular sample in order to capture the photokinetics of the molecules. Then, through eigen-decomposition, signal and noise are separated into two different sets of images (eigenimages) and used to build an indicator function that enables detection of emitters at a subpixel level. As a result, since its publication MUSICAL has enabled super-resolution fluorescence microscopy on a variety of setups and samples [2]. In addition, the theory behind has been expanded to provide new insights to the algorithm [3], enabling novel processing techniques closely related to MUSICAL [4].

This presentation has two parts. On one side, it outlines the generalization of MUSICAL's indicator function. This allows its customization and to modify the way the eigenimages in the signal and noise sub-spaces are used to enable a soft separation of the subspaces, or to focus in contrast rather than super-resolution [3, 4]. On the other side, it presents examples of different platform where MUSICAL has been used, such as in chip-based and lattice illumination microscopy [2, 5].

References

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