

Reconstruction procedures in structured illumination fluorescent microscopy

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Recently, fluorescent microscopy with structured illumination has been proposed as an alternative to classical confocal microscopy [1,2]. In this technique, the sample is illuminated by a light-grid and the fluorescence is detected with a standard objective. The position of the grid is moved several times (in general 5 positions are necessary) in order to illuminate the object in an uniform way. For each position of the grid, an image of the fluorescence is recorded. Then, a numerical treatment, based on a fourier transform is performed on the data to reconstruct the image of the whole sample [3]. In a classic confocal microscopy, the sample is illuminated by a localised spot and the detection is performed in a localised volume. Moving in parallel both the illumination spot and the collection volume permits the building of a map of the sample. The advantage of structured illumination microscopy compared to classical confocal microscopy is that it requires less mechanical manipulations to reconstruct the whole sample, it presents a better signal to noise ratio and a better power of resolution. Its main drawbacks are the difficulty of controlling the structured incident field and the sensitivity of the reconstruction technique to the errors on the incident intensity.

In this work, we propose a numerical technique, based on the inversion algorithms that prevail in microwave imaging, which permits to reconstruct the image of the sample from the recorded data [4] in structured illumination microscopy. The advantages of our approach, as compared to the classic fourier transform method, is that it allows one to use complex structured incident fields. We study the robustness of our reconstruction procedure with respect to the signal to noise ratio and the errors on the grid positioning.

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