Super-resolution capacity of variance-based stochastic fluorescence microscopy.

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Abstract

The challenge of super-resolution microscopy is to recover sample spatial frequencies above the diffraction limit of 2/λ from images the frequency of which are necessarily under 2/λ. The solution (common to all techniques) is to record several images of the same sample under different non-uniform excitations of the fluorescence and to process the data, either numerically or analogically, to form the super-resolved map of the sample. One can distinguish two main approaches in super-resolution microscopy [1]: those using a known deterministic excitation as in Structured Illumination Microscopy or point-scanning Microscopy and those using an unknown stochastic excitation, either caused by speckled illuminations, as in the recent Random Illumination Microscopy (RIM) or based on fluorescence blinking as in Fluctuation Imaging (SOFI) and Single Molecule Localization method (SMLM). The theoretical (noiseless) resolution gain is well established when the excitation is known (as in SIM or point-scanning microscopy). Generally, the sample frequency can be reconstructed up to the sum of the illumination and observation cut-off frequencies. On the other hand, determining the resolution of methods using stochastic unknown excitation like SMLM or SOFI is more difficult because it depends on assumptions concerning the sample fluorescence density (such as binarity or sparsity). A notable exception concerns Random Illumination Microscopy where the excitation is due to unknown speckled illuminations [2] . In this case, it has been demonstrated mathematically that the sample Fourier amplitude could be recovered from the covariance operator of the speckled images up to 4/λ [3]. However, the reconstruction algorithm of RIM is not based on the covariance operator (which is not tractable numerically) but uses simply the variance of the images [4]. In this work, we study the information that is contained in the variance of images recorded under stochastic excitations. We analyse SOFI and RIM and question whether they permit to recover unambiguously the sample spatial frequencies on a Fourier support beyond 2/λ.

References

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