BALANCED SUPER-RESOLUTION OPTICAL FLUCTUATION IMAGING

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1. INTRODUCTION

Super-resolution optical fluctuation imaging (SOFI) achieves 3D super-resolution by computing higher-order temporal cumulants, or spatio-temporal cross-cumulants of stochastically blinking fluorophores [1]. In contrast to localization microscopy, SOFI is compatible with very weakly emitting fluorophores and a wide range of blinking conditions [2]. The main drawback of SOFI is the nonlinear response to brightness and blinking heterogeneities in the sample, which limits the use of higher cumulant orders. Here, we propose an extension to the SOFI algorithm, which allows mapping molecular parameters with super-resolution and cancelling the nonlinearities without compromising resolution.

2. EXPERIMENTS

We used a widefield fluorescence microscope to acquire image sequences of Alexa647-labeled microtubules in fixed HeLa cells (Figure 1.1). We then computed different orders of cross-cumulants (Figure 1.2). Assuming a simple 2-state blinking model, at least three cumulant orders are needed to extract the spatial distribution of the on-time ratio, brightness and density (Figure 1.3). This information is used to generate balanced cumulants, which provide a balanced image contrast and a resolution improvement that scales linearly with the order.

3. CONCLUSION

bSOFI is a fast and simple yet effective imaging concept that cancels the nonlinear response to brightness and blinking heterogeneities in SOFI and adds a functional feature to super-resolution microscopy based on stochastic switching.