

NONLINEAR STRUCTURED-ILLUMINATION MICROSCOPY USING PHOTO-SWITCHABLE LABELS

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Periodically structured illumination light can extend the resolution beyond the classical limit by an amount equal to the spatial frequency k_1 of the illumination structure [1]. The set of frequencies that can be physically generated in a light intensity field is limited by the wavelength and the laws of physics in the same way as the set of frequencies that can be observed, which implies that k_1 cannot be much greater than the classic resolution limit itself, and the resolution extension therefore cannot exceed a factor of approximately two.

Dramatically greater resolution extension is possible, however, if a nonlinearity can be introduced between the incoming illumination intensity and the outgoing emission rate, because such a nonlinearity can cause the effective excitation to contain harmonics at multiples of k_1 , with correspondingly multiplied resolution-enhancing ability [2,3].

Photo-switching of fluorophores [4] constitutes one promising form of such nonlinearity. This phenomenon has produced modest resolution improvements when applied on one dimension and analyzed with real-space methods [5]. We are using it to develop high-resolution microscopy of 2D samples based on our frequency-space-based approach to nonlinear structured-illumination microscopy. Here we describe recent progress toward multidimensional super-resolution of biological samples using this technique.

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