SIGNAL, NOISE AND (ULTRA-HIGH) RESOLUTION IN NONLINEAR STRUCTURED-ILLUMINATION MICROSCOPY

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Abbe’s classic diffraction limit to microscope resolution defines the highest spatial frequency $k_0$ that is observable by a wide-field light microscope. Periodically structured illumination light can extend the resolution beyond this limit by an amount equal to the spatial frequency $k_1$ of the illumination structure [1]. Unfortunately 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 $k_0$. The maximum resolution extension is therefore normally about a factor of two.

Much more dramatic levels of resolution extension are 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 contains harmonics at multiples of $k_1$, with correspondingly multiplied resolution-enhancing ability [2,3].

Such nonlinear structured illumination microscopy does not face any hard resolution limit at all; instead the maximum practically achievable resolution will be determined by the signal-to-noise ratio in the data, which in turn will be limited by photobleaching of the sample.

Nonlinear structured-illumination microscopy can be implemented in different ways; for example there is a choice between either by shifting a single 2D illumination pattern through a 2D raster of phases, or by shifting a 1D parallel-line pattern one-dimensionally and repeating that 1D shift sequence for a number of different orientations of the 1D pattern. There are also several kinds of nonlinear phenomena that can be exploited, including saturation of the first excited state [2,3] and switching of reversibly photo-activatable molecules [4]. The noise properties depend on the properties and photo-degradation modes of the nonlinearity and the sample.

It is not intuitively obvious what is the optimal choice of implementation, or what resolution could be supported by a given sample. Here we therefore analyze the noise properties and resolution potential of various implementations of nonlinear structured-illumination microscopy.