Wide-field fluorescence microscopy beyond the diffraction limit on resonant gratings

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Far-field optical microscopy is the most widespread imaging tool in biology for its non-invasive properties and its high sensitivity when combined to fluorescent labelling. However its diffraction-limited spatial resolution is a major drawback for modern bioimaging applications. In this contribution, we proposed an innovative technique based on structured illumination that allows improving spatial resolution well beyond the diffraction limit. Structured illumination consists in illuminating the sample with a periodic light grid that stems from interferences between coherent beams (standing-wave pattern). Images of the sample are recorded for different positions of the grid and the fluorescence density is numerically recovered using an inversion algorithm. This approach has the advantage to resort on neither saturation effects nor probe scanning; therefore it is compatible with wide-field imaging. It has notably been applied to total-internal-reflection fluorescence microscopy (TIRFM) to achieve highly sensitive surface imaging. Still the spatial resolution is defined by the spatial frequencies contained in the light grid, which are limited by the numerical aperture of the microscope objective. Here we report significant improvements in spatial resolution from the use of sample substrates that generate structured illumination patterns with much higher spatial frequencies. These substrates consist of glass slides supporting a thin wave-guide layer of high refractive index and low losses, and a periodic grating of nanoscopic period. This grating is designed for a plane wave to resonantly excite the guided mode at a given angle of incidence, i.e. using a setup much similar to that of TIRFM. We show by numerical simulations that the resolution of this imaging system is not limited by diffraction but only by the wave-guide index and the grating period. We report spatial resolution down to a tenth the excitation wavelength in the lateral direction.