Resonant out-of-phase fluorescence microscopy and remote imaging overcome spectral limitations

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We present speed out-of-phase imaging after optical modulation (OPIOM), which exploits reversible photoswitchable fluorophores as fluorescent labels and combines optimized periodic illumination with phase-sensitive detection to specifically retrieve the label signal. Speed OPIOM can extract the fluorescence emission from a targeted label in the presence of spectrally interfering fluorophores and autofluorescence. Up to four fluorescent proteins exhibiting a similar green fluorescence have been distinguished in cells either sequentially or in parallel. Speed OPIOM is compatible with imaging biological processes in real time in live cells.

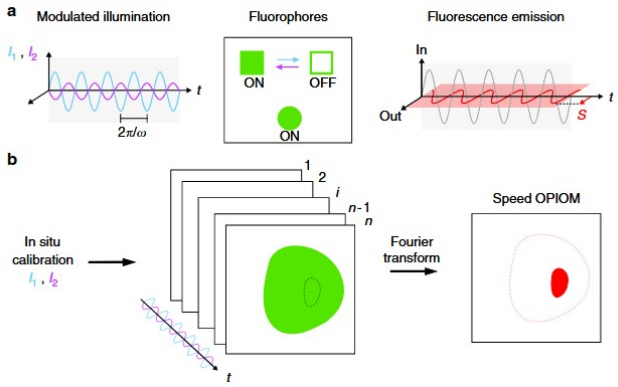

Fig. 1 Principle of speed OPIOM .a A sinusoidally modulated antiphase-synchronized dual illumination generates the quadrature-delayed component S (in red) of the fluorescence emission used for selective speed OPIOM imaging of RSFPs. b After in situ calibration, the fluorescence images are recorded under modulated illumination and processed to yield S after Fourier transform

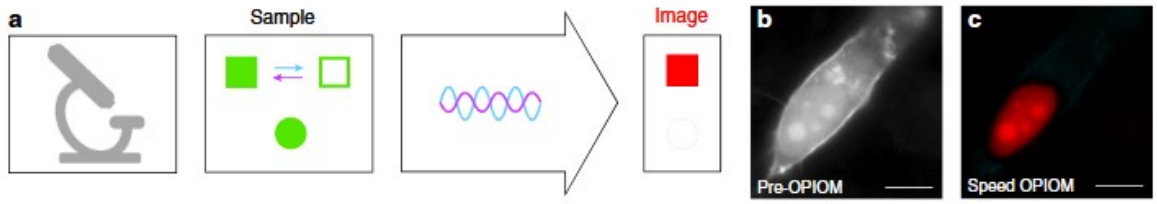

Fig.2 Speed OPIOM selectively retrieves RSFP signal in presence of spectral interference. In contrast to pre-OPIOM (b), speed OPIOM microscopy selectively unveils a RSFP target even in presence of spectrally interfering fluorophore and autofluorescence (c)

[1] J.Querard, Nature communication, 2017,8, 969