TWO-PHOTON-LIKE MICROSCOPY WITH ORDERS-OF-MAGNITUDE LOWER ILLUMINATION INTENSITY

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ABSTRACT:
We describe “two-step” fluorescence microscopy, a new approach to nonlinear imaging based on positive reversible photoswitchable fluorescent probes. The protein Padron [1] approximates ideal two-step fluorescent behaviour: it equilibrates to an inactive state, converts to an active state under blue light, and blue light also excites this active state to fluoresce. Both activation and excitation are linear processes, but the total fluorescent signal is quadratic, proportional to the square of the illumination dose. We use Padron’s quadratic nonlinearity to demonstrate the principle of two-step microscopy, similar in principle to two-photon microscopy but with orders-of-magnitude better cross-section. As with two-photon, quadratic nonlinearity from two-step fluorescence improves resolution and reduces unwanted out-of-focus excitation. We also show two-step and two-photon imaging combined give quartic nonlinearity, further improving imaging in challenging samples.


Figure 1: One-step vs. two-step imaging of an artificial sample with substantial out-of-focus fluorophores. Fixed U2OS cells expressing F-tractin-Padron immersed in Padron solution, imaged with (a) one-step and (b) two-step fluorescence. Scale bars: 5 μm.