rsFPs FOR NON INVASIVE SUPER-RESOLUTION MICROSCOPY

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Reversibly photo-switchable fluorescent proteins (rsFPs) allow to significantly lower the illumination intensities in super-resolution imaging techniques, such as reversible saturable optical fluorescence transition microscopy (RESOLFT) and nonlinear structured illumination microscopy (NL-SIM), defining them as the techniques of choice for non-invasive live-cell imaging at the nanoscale. Those techniques take advantage of the molecular transitions provided by rsFPs of different types and especially their positive and negative switching mechanism.

Several kinds of rsFPs have been developed during the last few years, often in strong relation with a specific imaging technique. Dronpa, GFP, YFP, Cherry, mMaple and mEos mutants have been used for RESOLFT, SOFI and NL-SIM imaging. However, each nanoscopy approach asks for specific measurement protocols, which make difficult to find a common framework for comparison. Here we provide a photo-physical and imaging toolbox to quantify and compare several rsFPs parameters to best perform RESOLFT imaging, both in the point-scanning and in the parallelized novel implementation. Brightness, switching efficiency and kinetics, fluorescence lifetime, contrast between ON (fluorescent) and OFF (not fluorescent) states and last but not least the switching fatigue, which results from the fine balance between photo-switching kinetics and photo-stability, are quantified and compared for different rsFP families and also for mutants belonging to the same family. We take advantage of the full characterization of the rsFPs behaviour to model and to provide the correct set of parameters for RESOLFT and NL-SIM super resolution imaging.