

## RESOLFT IMAGING AND WRITING WITH A PHOTOCHROMIC GFP

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Lens-based optical microscopy failed to discern fluorescent features closer than 200nm for decades, but the recent breaking of the diffraction resolution barrier by sequentially switching the fluorescence capability of adjacent features on and off is making nanoscale imaging routine. We demonstrate an optical nanoscopy that records raw data images from living cells and tissues with low levels of light. The generation of the reversibly switchable enhanced green fluorescent protein (rsEGFP) facilitated this advance. The reversible switching also enabled all-optical writing and reading of features with subdiffraction size and spacings, which can be used for data storage.

### Experiments

We started from enhanced green fluorescent protein (EGFP) [1], expressed numerous EGFP variants in *Escherichia coli* and screened for colonies expressing a reversibly switching fluorescent protein with an automated microscope. After analysing ~30'000 clones, we identified rsEGFP, an EGFP variant that can be reversibly switched on at 405nm wavelength and off at 491nm wavelength more than a thousand times [2]. We compared rsEGFP with the fluorescent protein Dronpa [3], which could be switched less than ten times under the same conditions. We recorded distributions of functional rsEGFP-fusion proteins in living bacteria and mammalian cells at better than 40nm resolution. Dendritic spines in living brain slices were super-resolved with about a million times lower light intensities than before. Last but not least, we show all-optical rewritable short-term data storage and super-resolved write-once permanent data storage using rsEGFP embedded in a thin polyacrylamid (PAA) layer.

### Conclusions

We performed spatially super-resolved imaging and all-optical writing using a reversible photo-switchable fluorescent protein rsEGFP, which we found well suited for reversible saturable optical (fluorescence) transition (RESOLFT) nanoscopy.

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