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## Resolft Nanoscopy in Life Sciences: Unraveling Fine Details with Low Light Levels

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Lens-based fluorescence microscopy, which has long been limited in resolution to >200 nanometer by diffraction, is rapidly evolving into a nanoscale imaging technique. Here, we show that emergent RESOLFT fluorescence microscopy enables fast and continuous imaging of sensitive, nanosized features in living brain tissue. using low intensity illumination to switch photochromic fluorescent proteins reversibly between a fluorescent ON-state and a non-fluorescent OFF-state, we obtained more than a 3-fold increase in all three spatial dimensions over that of confocal microscopy. Dendritic spines located 10-50  $\mu m$  deep inside living organotypic hippocampal brain slices were recorded for hours without signs of degradation. using a fast-switching fluorescent protein increased the imaging speed 50-fold over reported RESOLFT schemes, which in turn enabled us to record spontaneous and stimulated changes of dendritic actin filaments and spine morphology occurring on time scales from seconds to hours.