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Two-Photon Microscope with Spectral Resolution RUSSELL FUNG, MIKE MELNICHUK, ANURAG CHATURVEDI, DEVIN GILLMAN, VALERICA RAICU, Department of Physics and Department of Biological Sciences, University of Wisconsin, Milwaukee — Two-photon microscopy has many distinct advantages over other types of microscopy: it is faster, there is no out-of-plane photobleaching, and using near-infrared laser light (to produce visible fluorescence signal) allows deeper penetration into thick samples. We have built a two-photon microscope based on a novel design that uses a diffractive optic, a nondescanned detection scheme and an EM-CCD camera to produce spectrally resolved fluorescence images of samples after only one full scan of the sample and with relatively high speed. Our design is readily extended to incorporate control in the excitation channel through pulse shaping using spatial filtering in the frequency domain. This microscope, in conjunction with Fluorescence Resonance Energy Transfer (FRET) between fluorescent tags, has been used to detect interactions between proteins in various systems including yeast (Saccharomyces cerevisiae) cells. Also, its exquisite sensitivity makes it suitable to spectrally resolve signals from single quantum dots and single molecules.

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