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Multiphoton imaging of the living retina

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In vivo two-photon fluorescence imaging through the pupil of the living macaque eye allows us to visualize fluorescent molecules that cannot be excited in vivo with single photon fluorescence due to the transmission of the ocular optics. By using an adaptive optics scanning laser ophthalmoscope, the associated challenges of the low numerical aperture, the poor optical quality of the eye, the required high light levels, and eye motion were overcome. Based on the eye motion observed with simultaneously acquired high signal-to-noise ratio near-infrared reflectance images of the cone mosaic, 16,500 (12 minute acquisition) low signal-to-noise ratio two-photon images were registered and averaged to produce fluorescence images of the inner segments of the cone mosaic. The specific molecular origin of the fluorescence is as yet unknown, but the most probable candidate fluorophores are involved in the visual cycle (retinol) and metabolism (FAD or NADH). In vivo two-photon fluorescence has the potential to become a useful tool for functional imaging of the retina by recording the molecular dynamics occurring with photon absorption and pigment regeneration in normal and diseased retina. To this end, we observed an increase in the fluorescence response following light stimulation, which could provide a functional assay of the effects of light on photoreceptors.