NAD(P)H in the rod inner segments and all-*trans*-retinol in the outer segments. They also obtained similar results with isolated mouse retina. Here, we have shown, *in vivo* and in an *ex vivo* preparation, an increase in two-photon fluorescence signal from cone inner segments in response to light exposures that would result in cone bleaching.

When enabled by a high-resolution adaptive optics scanning laser ophthalmoscope, two-photon imaging provides functional measurements at the cellular scale in the living eye. The nature of current functional measurements is still under investigation. The increased fluorescence may represent the creation of new fluorescent molecules or an increase in the concentration of existing fluorescent molecules either directly by the two-photon excitation light or by its stimulation of a visual response. The capability to image changes in cellular metabolism (if imaging FAD or NAD(P)H) or the influx and conversion of 11-*cis*-retinol in cone inner segments in response to visual stimuli is of interest, not only in young and aging healthy eyes, but also in eyes with retinal pathology. If the two-photon imaging signal emanates from NAD(P)H or FAD, mitochondrial dysfunction, such as that originating from Leber's hereditary optic neuropathy [30] or NARP (neurogenic muscle weakness, ataxia, retinitis pigmentosa) [31], the technique can display different fluorescence changes over time when compared to normal healthy eyes. Altered responses to light may also be observed with diseases of the visual cycle, including Leber's congenital amaurosis and Stargardt disease [2]. Two-photon fluorescence imaging capabilities could be highly useful for monitoring the efficacy of proposed therapies.

5. Conclusions

With the use of an adaptive optics scanning laser ophthalmoscope with dual imaging capabilities, the many challenges for *in vivo* two-photon fluorescence imaging were overcome to produce the first two-photon fluorescence images of the living primate retina. Images were obtained with light levels that did not produce observable retinal damage. Although the specific fluorophore is as yet unknown, a strong *in vivo* fluorescence signal originates from cone inner segments producing images of the cone mosaic and providing functional measurements of an early stage in the visual process. Future applications of two-photon fluorescence imaging in the primate eye may also include the use of extrinsic fluorophores.

Acknowledgments

For their assistance with this research, we thank Kamran Ahmad, John Moonan, Jennifer Norris, Lee Anne Schery and Robert Wolfe. *Ex vivo* two-photon imaging was performed at the Multiphoton Core Facility at the University of Rochester Medical Center with the assistance of Karl Kasischke, Gheorghe Salahura and Anita Sun. We appreciate financial support from the following sources: Polgenix, Inc.; National Institute for Health, Bethesda, Maryland Grants P30-EY001319, R01-EY004367, BRP-EY014375, T32-EY007125, R01-EY009339, R24-EY021126, R43-EY020715. This work made use of STC shared experimental facilities supported by the NSF under Agreement No. AST-9876783, Research to Prevent Blindness. Alfredo Dubra-Suarez, Ph.D., holds a Career Award at the Scientific Interface from the Burroughs Welcome Fund; Klaus Tschira Foundation Heidelberg, Germany; European Life Scientist Organization; TECH 09-004 State of Ohio Department of Development; Third Frontier Commission. DeMotion image registration software was developed by Alfredo Dubra and Zach Harvey with funding from Research to Prevent Blindness and the National Institute of Health, Bethesda, Maryland through the grants BRP-EY014375 and 5 K23 EY016700. Adaptive optics control software was developed by Alfredo Dubra and Kamran Ahmad.

#137817- $15.00USD Received 8 Nov 2010;revised 14 Dec 2010;accepted 14 Dec 2010;published 17 Dec 2010