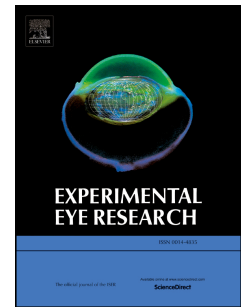


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# In vivo two-photon imaging of retina in rabbits and rats

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## ABSTRACT

The purpose of this study was to evaluate the retina using near-infrared (NIR) two-photon scanning laser ophthalmoscopy. New Zealand white rabbits, albino rats, and brown Norway rats were used in this study. An autofluorescence image of the retina, including the retinal cells and its associated vasculatures was obtained by a real-time scan using the ophthalmoscope. Furthermore, the retinal vessels, nerve fiber layers and the non-pigmented retina were recorded with two-photon fluorescein angiography (FA); and the choroidal vasculatures were recorded using two-photon indocyanine green angiography (ICGA). Two-photon ICGA was achieved by exciting a second singlet state at ~398 nm. Simultaneous two-photon FA and two-photon ICGA were performed to characterize the retinal and choroidal vessels with a single injection. The minimum laser power threshold required to elicit two-photon fluorescence was determined. The two-photon ophthalmoscope could serve as a promising tool to detect and monitor the disease progression in animal models. Moreover, these high-resolution images of retinal and choroidal vessels can be acquired in a real-time scan with a single light source, requiring no additional filters for FA or ICGA. The combination of FA and ICGA using the two-photon ophthalmoscope will help researchers to characterize the retinal diseases in animal models, and also to classify the types (classic, occult or mixed) of choroidal neovascularization (CNV) in macular degeneration. Furthermore, the prototype can be adapted to image the retina of rodents and rabbits.

**Keywords:** nonlinear optics; two-photon ophthalmoscope; retinal imaging; fluorescein angiography; indocyanine green angiography; femtosecond laser; animal models

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