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Early detection of retinal disease and glaucoma with non-linear optical (NLO) imaging using the Two-Photon Ophthalmoscope

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In the present dissertation, we have presented a prototype, a novel two–photon ophthalmoscope based on a scanning laser unit, to perform high resolution *in vivo* imaging of the retina of rats and rabbits. It is a new technique that may potentially help with the diagnosis and treatment of glaucoma and retinal diseases. Two-photon fluoresceine angiography (TPFA) offers high resolution structural and functional imaging of the retina and reveals new possibilities for diagnosing retinal diseases. An *in vivo* study of TPFA on rat and rabbit retina is conducted for the first time in which the choroidal and vascular structures have been depicted with superior image quality than regular fluoresceine angiography (FA) and indocyannie green angiography (ICGA). The autofluorescence properties of the retina from rat and rabbit eyes are studied for their diagnostic values.

Other applications of the prototype is also explored on trabecular meshwork (TM). The intraocular pressure is thought to be one of the main causes of open angle glaucoma. The small focal spot size of the prototype and the nature of the non-invasive imaging method make it superior than commercial trabeculoplasty surgery device. The effect of the heat conduction due to the exposure of the ultra-short pulsed laser is assessed. Compared to confocal imaging, the image quality of the two-photon emitted fluorescence (TPEF) is better with higher resolution and higher signal-to-noise ratio.

A laser safety analysis and in vivo toxicity study in rat retina is performed to delineate the safety limit threshold for this prototype. There is no damage to retina under regular imaging operation of both two-photon autofluorescence (TPAF) and TPFA. Toxicity occurs only when the retina is exposed to a stationary laser beam focused on a given area at over 70mW for an exposure duration of 100s. A range of possible thermal damages for the ultra-short pulsed infrared laser scanning device are demonstrated by imaging and histology. It is shown that TPAF from the retina can be detected *in vivo,* and the abnormalities of the retina, especially the laser-induced damage on the retina ganglion cells (RGC) and outer nuclear cell (ONC) layers, emit higher autofluorescence than normal retinal tissue under TPAF imaging.

In summary, this emerging infrared ultra-short pulsed two-photon imaging prototype has demonstrated multiple advantages in ocular imaging over some of the currently available imaging modalities. The limitations on the image quality of the prototype are addressed, and potential future direction in applying adaptive optics (AO) and employing acoustic optical modulator (AOM) to eliminate the aberrations from the animal eyes is proposed.