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Retinal imaging with compact custom phase plates and two-photon imaging methods

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High-quality retinal imaging is vital in discovering pathogenesis and monitoring and diagnosing retinal disorders. This dissertation presents confocal retinal imaging with phase plates and two-photon retinal imaging methods to improve the retinal image quality for early detection and diagnosis of retinal diseases. It also addresses the feasibility of two-photon retinal imaging concerning laser safety in animal models.

Confocal retinal imaging with phase plates: Aberrations degrade image quality. A custom phase plate was designed and developed to compensate for the ocular aberrations and to improve the retinal image quality. A compact compensation unit with phase plates was implemented in the confocal scanning laser ophthalmoscope (cSLO) with a smaller field of view. Retinal microstructures and cone photoreceptors were resolved in human eyes. A significant improvement in image quality was observed with phase plates. Further, the cSLO retinal images with phase plates was compared to the adaptive optics scanning laser ophthalmoscope (AOSLO) retinal images.

Two-photon retinal imaging: Two-photon excited fluorescence (TPEF) imaging offers the theoretical potential for better anatomical resolution of retinal cells and better detection of autofluorescence pattern of the retina due to its greater penetration and superior resolution of microstructures. The near-infrared (NIR) fundus two-photon retinal imaging on animal models was demonstrated. A two-photon autofluorescence image of the retina including the retinal cells and their associated vasculatures was obtained by a real-time scan. Two-photon fluorescein (FA) and two-photon indocyanine green angiography (ICGA) were performed to record the retinal and choroidal vessels. 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 and single light source. The minimum laser power threshold required to elicit two-photon fluorescence was determined. The prototype can be adapted to image the retina of rodents and rabbits.

Retinal safety evaluation of two-photon imaging: Safe use of retinal imaging with two-photon excitation in human eyes is crucial, as the effects of ultrashort pulsed lasers on the retina are relatively unknown. In this study, rat retinas were evaluated at various laser exposure levels and with different laser parameters to determine the effects of laser-induced optical damage. The results were verified using confocal reflectance imaging, two-photon fluorescein angiography, immunohistochemistry, and correlated to the IEC 60825-1 laser safety standard.