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## Two-photon microscopy and fluorescence lifetime imaging of retinal tissues under lipid peroxidation

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Autofluorescence (AF) measurement with two-photon microscopy (TPM) and fluorescence lifetime imaging (FLIM) enables the discrimination of different fluorescence molecules located in a different depth of the tissue. We investigated the AF and fluorescence lifetime (FLT) of retinal cells focusing on retinal pigment epithelial (RPE) cells and photoreceptor outer segments (POS). Lipid peroxidation was induced in the RPE and POS of the porcine explants by FeSO<sub>4</sub>, and the tissues were investigated with TPM and FLIM. Furthermore, the RPE explants were examined with immunofluorescence study for 4-hydroxynonenal (4-HNE)-adducts, adipocyte differentiation related protein (ADFP), and rhodopsin. TPM-AF in RPE cells is mostly originated from the melanosomes under normal conditions, which have a very short fluorescence lifetime (FLT) (mean=117 ps). FeSO<sub>4</sub> exposure leads to the appearance of bright granular AF inside and around RPE cells, whose FLT is significantly longer (mean=1388 ps) than melanosome-AF. FeSO<sub>4</sub> exposure increases the fluorescence intensity of POS-AF and shortens their FLT. In the immunofluorescence studies, strong 4-HNE staining was observed intra- and pericellularly of RPE cells under lipid peroxidation, whose localization was partially consistent with rhodopsin and ADFP, respectively, which suggests that the FeSO<sub>4</sub>-induced bright AF granules inside/around RPE cells are suggested to be the oxidized POS-membranes and the retinoid-storing inclusions, respectively. From these results, 4-HNE-adducts may be one of the main fluorophores of these AF. Since the amount of 4-HNE-adducts is suggested to reflect cellular oxidative stress status, TPM with FLIM might be a useful tool to detect oxidative stress status of RPE cells and the POS.

### Biography

Yoko Miura completed her M.D. in 1997 and Ph.D. in 2002 from Osaka City University in Japan. Clinical training was completed in eye clinic of Osaka City University Hospital and since 2002 a specialist of ophthalmology. In 2005 started research work in University of Kiel in Germany and in 2009 moved to Institute of Biomedical Optics, University of Luebeck. Today engages in the research and clinical works in Institute of Biomedical Optics and Department of Ophthalmology, University of Luebeck. Leading some research projects related to retinal cell biology. Since 2010 adjunct lecturer of Osaka City University.

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