

### Investigation of the two photon-excited bright autofluorescence granules in the RPE cells

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Two-photon microscopy is one of the great innovations of the 20th Century. For the ophthalmologist, so far, it is clinically not yet available, although its use for the ocular surface seems to be in the near future. Clinical frustration is that we observe fundus autofluorescences (FAF) but without knowing what they actually are, although lipofuscin is well known as a main fluorophore. Recently, the possibility of using different wavelengths has been realized in FAF cameras or ophthalmoscopes, and therewith more questions have arisen, because there is still very little basic knowledge about FAF. I introduce some experimental results with two-photon-excited autofluorescence (AF) of retinal pigment epithelial (RPE) cells and the change of AF under experimentally induced pathological conditions with porcine RPE cells *in vitro* and mouse RPE cells *in vivo*. Thermal damage was induced by laser irradiation. We found an appearance of very bright AF granules in the RPE cells around the laser spots, and analyzed these granules, including spectral characteristics and fluorescence lifetime measurement (FLIM). Characteristic of this bright AF granule is not consistent with the one of lipofuscin, but is theoretically quite consistent with the fluorescence characteristics with the one of the end-product of lipid peroxidation. We deduce that this AF granule is an oxidative stress-suffered phagocytized photoreceptor outer segment including many unsaturated fatty acids. This AF granule is probably related to the FAF in the clinical practice that seems to be non-lipofuscin. Two-photon microscopy and FLIM analysis provide significantly new information about AF molecules in the RPE cell.

### Biography

Dr. Yoko Miura has completed her Ph.D in Osaka City University School of Medicine and clinical training in Osaka City University Hospital. From 2003 she served as an assistant professor in the department of Ophthalmology of Osaka City University. She is currently a lecturer-researcher in Institute of Biomedical Optics, University of Lubeck, as well as a guest ophthalmologist in University Eye Clinic Lubeck, Germany. She is serving as an editorial board member of Journal of Experimental and Clinical Ophthalmology.