Spectral and fluorescence lifetime (FLIO) characteristics of drusen in age-related macular degeneration

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Goal: Drusen, a hallmark of age-related macular degeneration (AMD), are known to show autofluorescence (AF) which is different from that of lipofuscin[1,2] (fig. 1). Here, for the first time, we combine spectral and lifetime data of drusen AF in vivo.

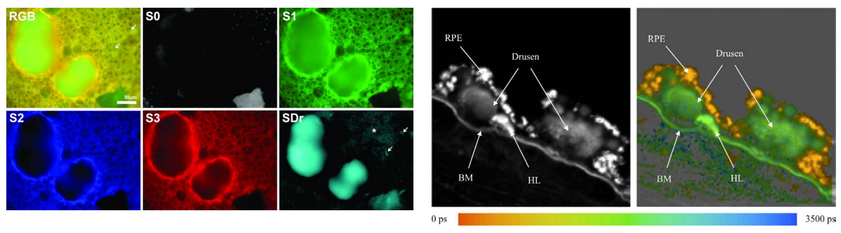
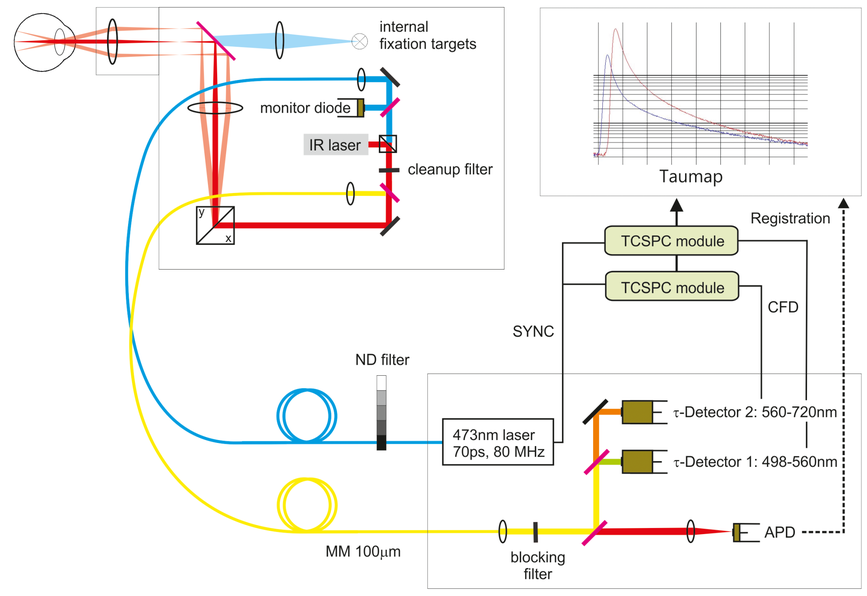


Fig. 1: hyperspectral[1] and lifetime[2] imaging of drusen AF in vitro

Methods: 12 patients with non-exudative AMD and no geographic atrophy were included. Fundus AF in a 30° retinal field was investigated with the Heidelberg Engeneering Spectralis® fluorescence lifetime imaging ophthalmoscope (FLIO), detecting the temporal decay of the fluorescence in a short (498-560 nm; Ch1) and a long (560-720; Ch2) wavelength channel upon excitation with <100 ps laser pulses at 473 nm. The amplitude weighted mean fluorescence lifetime □m was calculated from a three-exponential approximation of the decay. The spectral ratio sr of fluorescence emission in ch1 and ch2 was calculated. Drusen were identified from color fundus photographs and segmented in the AF images within an inner (IR) and outer (OR) ring centered at the macula according to the ETDRS-grid.

Results: The lifetimes of drusen were significantly longer than that of RPE (Ch1: 345 vs. 273ps, p=0.002 (IR) and 334 vs. 291ps, p=0.026 (OR), Ch 2: 382 vs. 324 ps, p=0.002 (IR) and 371 vs. 335 ps, p=0.014 (OR), example: fig. 2, statistics: fig. 3). Furthermore, drusen fluorescence was green-shifted compared to that of RPE (sr=0.64 vs. 0.57, p=0.017 (IR) and 0.60 vs. 0.53, p=0.003 (OR)). Overall, there was a correlation between fluorescence lifetime and sr (Ch1: p=0.001, Ch2: p=0.011) in drusen. In RPE we found a correlation only in Ch1 (p=0.002).

FLIO basics



- Modified Spectralis (Hedelberg Engineering GmbH)
- Fluorescence excitation by a fiber-coupled pico-second diode laser (473 nm, 89 ps pulse width, repetition rate: 80 MHz)
- Laser class 1, energy of a single pulse 10^{-4} of accessible exposure limit according to ANSI Z136.1-2007
- Fluorescence detection by time-correlated single photon counting (TCSPC) in two spectral channels
- Approximation of the fluorescence decay by a series of three exponential functions:

$$\frac{I(t)}{I_0} = IRF * \sum_i (\alpha_i \cdot e^{-\frac{t}{\tau i}})$$

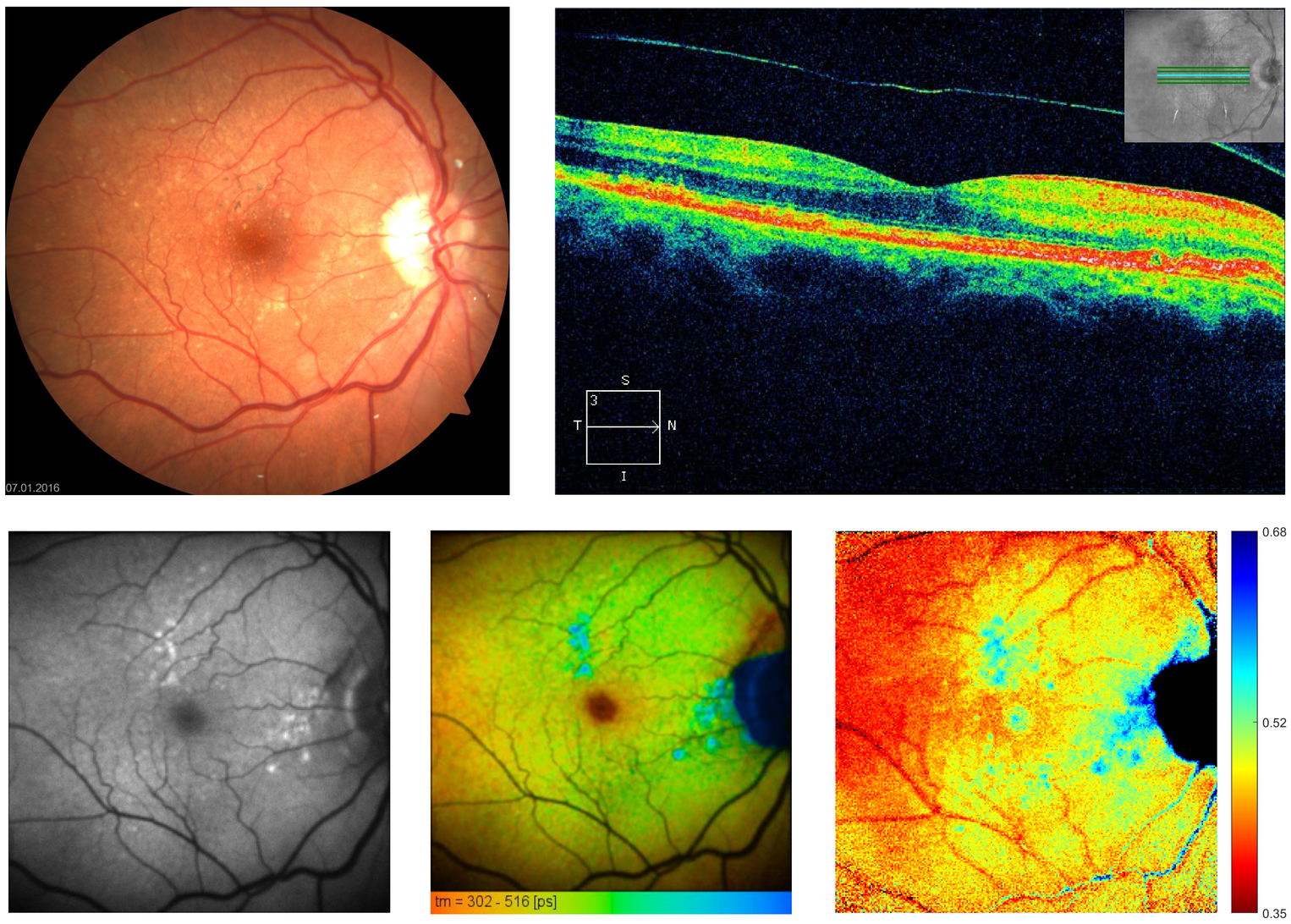


Fig. 2: 83 year old patient with hard drusen: top left: fundus photograph, top right: OCT, bottom left: AF intensity, bottom middle: AF lifetime, bottom right: AF spectral ratio

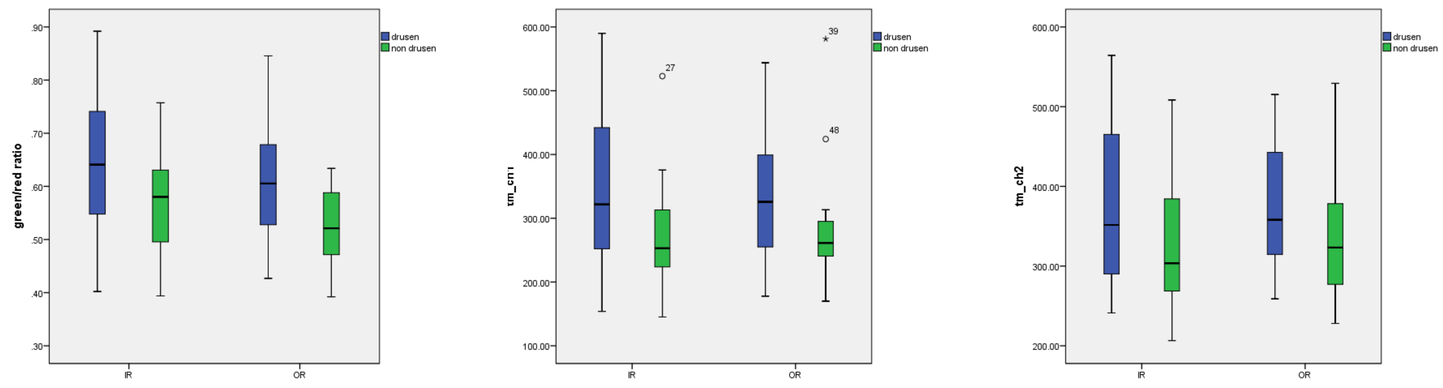


Fig. 3: Box plots of AF spectral ratio (left) and lifetimes at short (Ch1, middle) and long (Ch2, right) wavelength

Conclusions: Drusen contain fluorophores different from that of RPE lipofuscin. As these can be investigated by FLIO in vivo, this might help to understand the nature of sub-RPE drusenoid deposits and might give additional diagnostic information on their role in AMD progression.

Reference: 1.Tong Y, Ben Ami T, Hong S, et al. Hyperspectral Autofluorescence Imaging of Drusen and Retinal Pigment Epithelium in Donor Eyes with Age-Related Macular Degeneration. Retina 2016. **2.Schweitzer D, Gaillard ER, Dillon J, et al.** Time-resolved autofluorescence imaging of human donor retina tissue from donors with significant extramacular drusen. Invest Ophthalmol Vis Sci 2012;53:3376-3386.

Conflict of interests: Hammer: none, Kreilkamp: none, Sauer: none, Augsten: none, Meller: none