LS-10-O-2054 Investigating deposit-crowded cells by SIM microscopy to quantify the progress of age related granule-accumulation in human RPE cells.

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Age related macular degeneration (AMD) is closely connected to the non-reversible accumulation of degradation products in the inside of human retinal pigment epithelium (RPE) cells[1]. There they form granules with a typical volume of around (0.6 +/- 0.5)μm³. To better understand the mechanisms of AMD formation, granules are analyzed quantitatively, especially their size, number and composition. Conventional light-microscopy is usually unable to resolve single granules reliably. Also it is practically impossible to manually identify and characterize up to over 100 granules in a single cell for a statistically relevant number of cells. As solution we used Structured Illumination Microscopy (SIM)[2,3] to resolve the granules inside the cells and to distinguish between different deposit materials. Furthermore, we introduce an algorithm that separates individual granules even in cells with high granule density. Besides, the algorithm determines characteristic quantitative parameters of the granules.

We present these characteristic parameters gained by analyzing over 200 RPE cells in histological samples of human donors of different age. All work on human tissue was done according to the Declaration of Helsinki.

[1] V. L Bonilha; Age and disease-related structural changes in the retinal pigment epithelium, Clinical Ophthalmology 2008, 2:413-424

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Fig. 1: SIM-Image of human RPE. Age of donor was 53 years. Color-code is according to the excitation wavelengths 488nm, 568nm and 671nm.

Fig. 2: Left: 2D-Image of a single RPE-cell imaged by SIM. Right: Automatically separated granules of the left Image. The single granules are colored randomly.