

- Hassenstein et al. **Clinical use and research applications of Heidelberg retinal angiography and spectral-domain optical coherence tomography – a review.** Clin Experimental Ophthalmol 2009; 37:130-143

Fluorescein angiography (FA) was discovered by Nowotny and Alvis in the 1960s of the 20th century and has evolved to become the 'Gold standard' for macular diagnostics. Scanning laser imaging technology achieved enhancement of contrast and resolution. The combined Heidelberg retina angiograph (HRA2) adds novel innovative features to established fundus cameras. The principle of confocal scanning laser imaging provides a high resolution of retinal and choroidal vasculature with low light exposure providing comfort and safety for the patient. Enhanced contrast, details and image sharpness image are generated using confocality. For the visualization of the choroid an indocyanine green angiography (ICGA) is the most suitable application. The main indications for ICGA are age-related macular degeneration, choroidal polypoidal vasculopathy and choroidal haemangiomas. Simultaneous digital FA and ICGA images with three-dimensional resolution offer improved diagnosis of retinal and choroidal pathologies. High-speed ICGA dynamic imaging can identify feeder vessels and retinal choroidal anastomoses, ensuring safer treatment of choroidal neovascularization. Autofluorescence imaging and fundus reflectance imaging with blue and infrared light offer new follow-up parameters for retinal diseases. Finally, the real-time optical coherence tomography provides a new level of accuracy for assessment of the angiographic and morphological correlation. The combination of various macular diagnostic tools, such as infrared, blue reflectance, fundus autofluorescence, FA, ICGA and also spectral domain optical coherence tomography, lead to a better understanding and improved knowledge of macular diseases.

- Schmitz-Valckenberg et al. **Fundus Autofluorescence Imaging – Review and Perspectives.** Retina 2008; 28:385–409,

Fundus autofluorescence (FAF) imaging is a novel imaging method that allows topographic mapping of lipofuscin distribution in the retinal pigment epithelium cell monolayer as well as of other fluorophores that may occur with disease in the outer retina and the subneurosensory space. Excessive accumulation of lipofuscin granules in the lysosomal compartment of retinal pigment epithelium cells represents a common downstream pathogenetic pathway in various hereditary and complex retinal diseases, including age-related macular degeneration. FAF imaging has been shown to be useful with regard to understanding of pathophysiologic mechanisms, diagnostics, phenotype–genotype correlation, identification of predictive markers for disease progression, and monitoring of novel therapies. FAF imaging gives information above and beyond that obtained by conventional imaging methods, such as fundus photography, fluorescein angiography, and optical coherence tomography. Its clinical value coupled with its simple, efficient, and non-invasive nature is increasingly appreciated. This review summarizes basic principles and FAF findings in various retinal diseases.

- Schmitz-Valckenberg et al. **Fundus Autofluorescence and Progression of Age-related Macular Degeneration.** Surv Ophthalm 2009; 54: 96-117

Fundus autofluorescence imaging is an imaging method that provides additional information compared to conventional imaging techniques. It permits to topographically map lipofuscin distribution of the retinal pigment epithelial cell monolayer. Excessive accumulation of lipofuscin granules in the lysosomal compartment of retinal pigment epithelium cells represents a common downstream pathogenetic pathway in various hereditary and complex retinal diseases including age-related macular degeneration (AMD). This comprehensive review contains an introduction in fundus autofluorescence imaging, including basic considerations, the origin of the signal, different imaging methods, and a brief overview of fundus autofluorescence findings in normal subjects. Furthermore, it summarizes cross-sectional and longitudinal fundus autofluorescence findings in patients with AMD, addresses the pathophysiological significance of increased fundus autofluorescence, and characterizes different fundus autofluorescence phenotypes as well as fundus autofluorescence alterations with disease progression.

- Fleckenstein et al. **High-Resolution Spectral Domain-OCT Imaging in Geographic Atrophy Associated with Age-Related Macular Degeneration.** Invest Ophthalmol Vis Sci 2008; 49:4137-4144

PURPOSE. To describe morphologic variations in outer retinal layers in eyes with atrophic age-related macular degeneration (AMD) using high-resolution, spectral-domain optical coherence tomography (SD-OCT).

METHODS. SD-OCT scans were obtained with a combined confocal scanning laser ophthalmoscope (cSLO) and SD-OCT for simultaneous tomographic and topographic in vivo imaging. A total of 81 eyes of 56 patients (mean age, 77.8 ± 7.4 years) with geographic atrophy (GA) were examined. Morphologic alterations were analyzed and classified in the perilesional zone, at the junction between GA and nonatrophic retina, and in the atrophic area itself.

RESULTS. In the perilesional zone, distinct morphologic alterations included elevations of the outer retinal layers, thickening, and spikes of the outer hyperreflective band as well as clumps at different neurosensory retinal levels. At the junction, highly variable transitions of the outer retinal layers were present with different degrees of loss of the normal hyperreflective bands. Within the actual GA, hyperreflective clumps at different retinal levels, segmented plaques of the outer band and elevations with variable reflectivity were visualized.

CONCLUSIONS. SD-OCT imaging in eyes with GA revealed a wide spectrum of morphologic alterations, both in the surrounding retinal tissue and in the atrophic area. These alterations may reflect different disease stages or, alternatively, heterogeneity on a cellular and molecular level. Longitudinal studies using in vivo SD-OCT imaging may allow evaluation of the relevance of these phenotypic changes as potential predictive markers for the progression of disease (i.e., enlargement rates of GA over time) and may be used for monitoring of future therapeutic interventions.

- Holz et al. **Progression of Geographic Atrophy and Impact of Fundus Autofluorescence Patterns in Age-Related Macular Degeneration.** Am J Ophthalmol 2007; 143:463-472

PURPOSE: To test if fundus autofluorescence (FAF) patterns around geographic atrophy (GA) have an impact on GA progression rates over time in atrophic age-related macular degeneration (AMD).

DESIGN: Prospective longitudinal multicenter natural history study.

METHODS: Standardized digital FAF images were obtained from 195 eyes of 129 patients with GA using confocal scanning laser ophthalmoscopy (excitation 488 nm, emission >500 nm). Areas of GA were quantified and patterns of abnormal FAF in the junctional zone were classified. Repeated FAF images were obtained over a median follow-up period of 1.80 years (interquartile range [IQR], 1.28 to 3.34).

RESULTS: Areas of GA (median, 7.04 mm² at baseline; IQR, 3.12 to 10.0) showed a median enlargement of 1.52 mm²/year (IQR, 0.81 to 2.33). Progression rates in eyes with the banded (median 1.81 mm²/year) and the diffuse FAF pattern (1.77 mm²/year) were significantly higher compared to eyes without FAF abnormalities (0.38 mm²/year) and focal FAF patterns (0.81 mm²/year, $P < .0001$). Within the group of the diffuse pattern, eyes with a diffuse trickling pattern could be identified that exhibited an even higher spread rate (median 3.02 mm²/year) compared to the other diffuse types (1.67 mm²/year, $P = .001$).

CONCLUSIONS: The results indicate that distinct phenotypic FAF patterns have an impact on disease progression in eyes with atrophic AMD and may therefore serve

- Schmitz-Valckenberg et al. **Evaluation of Autofluorescence Imaging with the Scanning Laser Ophthalmoscope and the Fundus Camera in Age-related Geographic Atrophy.** Am J Ophthalmol 2008; 146:183-192

PURPOSE: To compare fundus autofluorescence images (FAF) between a modified fundus camera (mFC) and a confocal scanning laser ophthalmoscope (cSLO).

DESIGN: Evaluation of diagnostic technology.

METHODS: Thirty-two eyes of 16 patients with age related geographic atrophy (GA) treated in an institutional setting were included. FAF images were obtained with both the cSLO (excitation, 488 nm; emission, >500 nm) and the mFC (excitation, approximately 500 to 610 nm; emission, approximately 675 to 715 nm). Using established algorithms, images were graded by two independent observers and agreements were evaluated. The main outcome measures were image quality, quantification of total atrophy, and classification of FAF patterns.

RESULTS: In two eyes with advanced cataract (lens grade 7 according to the Age-Related Eye Disease Study classification), FAF image quality with both systems was not sufficient for any meaningful analysis. In the remaining 30 eyes, the mean differences of the interobserver agreements for atrophy quantification were 0.16 mm² (95% confidence interval [CI], 0.07 to 0.38) for mFC and 0.15 mm² (95% CI, 0.04 to 0.33) for cSLO images. Because of inferior signal-to-noise ratios, FAF pattern classification was possible in a lower number of mFC images (69%) compared with cSLO images (88%).

CONCLUSIONS: This study suggests that the agreements for atrophy quantification are similar with both devices. The lesser visualization of FAF patterns with the mFC and thus inferior determination of disease markers may be the result of the nonconfocality and the use of single instead of mean images compared with the cSLO. These findings may be important for the design of interventional trials as well as the routine use of FAF imaging in age-related geographic atrophy.

- Schmitz-Valckenberg et al. **In-vivo imaging of foveal sparing in geographic atrophy secondary to age-related macular degeneration.** Invest Ophthalmol Vis Sci 2009; 50:3915-3921

PURPOSE. To investigate morphologic alterations in geographic atrophy caused by age-related macular degeneration (AMD) in the presence of foveal sparing using high-resolution in vivo imaging.

METHODS. Simultaneous spectral domain optical coherence tomography (SD-OCT, 870 nm, 40,000 A-scans/s) and confocal scanning laser ophthalmoscopy (cSLO; fundus autofluorescence; excitation, 488 nm; emission, 500–700 nm) were performed in 18 eyes with geographic atrophy and foveal sparing using a combined instrument. Anatomic layers were evaluated, and retinal thickness in the fovea and the peripheral macula were measured and compared with those in controls of similar age.

RESULTS. Fundus autofluorescence imaging showed an inhomogeneously reduced signal at the residual foveal island. SD-OCT scans disclosed mitigation of the foveal pit in the absence of extracellular fluid accumulation and an increased mean central retinal thickness of $248 \pm 28 \mu\text{m}$ compared with $225 \pm 12 \mu\text{m}$ in control eyes ($P = 0.005$). No difference in retinal thickness in the peripheral macula was observed ($245 \pm 16 \mu\text{m}$ vs. $253 \pm 11 \mu\text{m}$; $P = 0.6$). Subanalysis revealed marked appearance of swelling and widening of visible structures at the central outer nuclear layer ($153 \pm 22 \mu\text{m}$ vs. $127 \pm 12 \mu\text{m}$; $P = 0.003$). Below the external limiting membrane, a broad band of irregular high reflectivity was detected instead of the normal three separate reflective bands.

CONCLUSIONS. Thickening at the foveal site may reflect a preapoptotic stage of neuronal cellular elements indicating imminent atrophy. Limited structure-function correlation found in our study suggests that future therapeutic intervention may be beneficial in only a subset of AMD patients with foveal sparing.

- Wolf-Schnurrbusch et al. **Morphologic Changes in Patients with Geographic Atrophy Assessed with a Novel Spectral OCT-SLO Combination.** Invest Ophthalmol Vis Sci 2008; 49:3095-3099

PURPOSE. To investigate the appearance of geographic atrophy in high-resolution optical coherence tomography (OCT) images, the fundus autofluorescence (FAF) pattern, and infrared images simultaneously recorded with a novel combined OCT scanning laser ophthalmology (SLO) system.

METHODS. Patients aged over 50 years with geographic atrophy secondary to dry age-related macular degeneration (ARMD) were assessed in a prospective cross-sectional study by means of simultaneous spectral OCT-SLO (Spectralis HRA OCT; Heidelberg Engineering, Heidelberg, Germany). The integrity of the retinal layers was analyzed in the apparently normal areas, the junctional zone between the normal retina and the geographic atrophy, and the atrophic area. The presence and integrity of the external limiting membrane, the photoreceptor inner segments, the outer segments, and the retinal pigment epithelium were assessed.

RESULTS. Fifty-two eyes of 52 patients (28 women, 24 men) aged 51 to 92 years were examined. Retinal layer alterations were documented, not only in atrophic zones, but also in junctional zones surrounding the geographic atrophy. Disintegration of the retinal layers began in the RPE and adjacent retinal layers, such as the photoreceptor inner and outer segments and external limiting membrane.

CONCLUSIONS. Novel imaging modalities will provide further valuable insight into ARMD pathogenesis. The key to understanding the morphologic change lies in in vivo depiction of retinal layers by spectral OCT technology in combination with other imaging modalities such as FAF.

- Dandekar et al. **Autofluorescence Imaging of Choroidal Neovascularization Due to Age-Related Macular Degeneration.** Arch Ophthalmol 2005

Objective: To describe the autofluorescence (AF) characteristics of choroidal neovascularization (CNV) in patients with age-related macular degeneration.

Methods: Autofluorescence images of 65 consecutive eyes with CNV at various stages of evolution were analyzed. Twenty images were of recent-onset CNV (group 1), 8 were of eyes 1 to 6 months after CNV diagnosis (group 2), and 37 were late-stage CNV (group 3). Autofluorescence images from groups 1 and 2 were compared with fundus fluorescein angiographic images.

Results: Group 1 showed areas of hyperfluorescence on fundus fluorescein angiography corresponding to areas of normal AF in 16 of 20 cases, with adjacent areas of increased AF in 13 cases. The main areas of abnormal AF were larger than the main areas of abnormal fluorescence on fundus fluorescein angiography in 18 of the 20 cases. Groups 2 and 3 showed areas of decreased AF corresponding to areas of previous leakage on fundus fluorescein angiography (in group 2) or atrophy.

Conclusions: Preserved AF in group 1 indicates viable retinal pigment epithelium initially, which has implications for visual prognosis. Decreased AF in groups 2 and 3 indicates loss of retinal pigment epithelium and photoreceptors. Autofluorescence imaging may increase our understanding of CNV in age-related macular degeneration.

- Heimes et al. **Foveal RPE Autofluorescence as a Prognostic Factor for Anti-VEGF Therapy in Exudative AMD.** Graefes Arch Clin Exp Ophthalmol 2008; 1229-1234

Autofluorescence (AF) of the retinal pigment epithelium (RPE) are thought to reflect metabolic activity of the RPE cells, which in turn is largely driven by photoreceptor outer segment renewal. In exudative AMD, choroidal new vessels (CNV) may be confined to Bruch's membrane, or transgress the RPE, with consequence loss of photoreceptor cells. It has been suggested that they may be distinguished with autofluorescence imaging. The aim of our study was to analyze the prognostic value of RPE autofluorescence in relationship to the therapeutic outcome of anti-VEGF (vascular endothelial growth factor) therapy in exudative AMD.

AF images (Heidelberg Retina Angiograph) were obtained from 95 eyes (95 patients, mean age 77.64 years, 39 male and 56 female) with exudative macular lesions and associated drusen before therapy with intravitreal Bevacizumab (Avastin®). Increased, normal, or decreased AF of a central area with diameters of 500 and 1,000 µm around the foveola were distinguished, and compared with the outcome of central vision. As a measure of data reproducibility (inter- and intraobserver variability), the kappa statistics ($K > 0.6$ "good", $K > 0.8$ "excellent") and exact agreement in % were calculated.

Analysis of AF showed a significant difference in outcome of visual acuity in eyes with changes in AF of the central 500 und 1000 µm (Mann-Whitney test, $p_{500 \mu m} < 0.001$, $p_{1000 \mu m} = 0.02$). Comparison of eyes with increased AF to the other eyes also resulted a significant difference in visual acuity at follow-up ($p_{incr} < 0.001$); those with decreased AF had no significant difference to the eyes with normal or increased AF ($p_{decr} = 0.1733$).

The RPE-AF of exudative AMD lesions varies greatly. The AF differences probably represent different kinds of metabolism disorders in the RPE. Furthermore, they apparently have a great influence on the chances of antivasculare endothelial growth factor (VEGF) therapy success; in particular the development of visual acuity is less favorable in eyes with initially increased central AF.

- Fleckenstein et. al. **Discrete arcs of increased fundus autofluorescence in retinal dystrophies and functional correlate in microperimetry.** Eye 2008: 1-9

Purpose To describe the occurrence of discrete arcs of increased fundus autofluorescence (FAF) associated with various retinal dystrophies and to assess their functional significance by fundus-controlled microperimetry.

Methods Seven patients, three with pigmented paravenous retinochoroidal atrophy (PPRCA), one with sector retinitis pigmentosa (RP), one with typical RP, and two with macular dystrophy were assessed by retinal imaging including FAF imaging. Serial images were obtained within a review period of 6 and 10 years in a patient with PPRCA and macular dystrophy, respectively. Fundus controlled microperimetry was performed in eight eyes of five patients to determine light increment sensitivity.

Results A discrete arched line of increased FAF was observed without obvious correlate on fundus biomicroscopy. The orientation of this line differed from ring shape in RP and macular dystrophy, a semi-circle structure in sector RP to crescent shape with tiplike extensions towards branching retinal veins in PPRCA. Longitudinal investigation revealed slight migration of the arc in PPRCA and peripheral extension of the ring diameter in macular dystrophy. Microperimetry revealed that the arc of increased FAF sharply delineated areas of severely impaired retinal sensitivity.

Conclusions The findings indicate that arcs of increased FAF in PPRCA and other retinal dystrophies demarcate areas of impaired retinal function and may migrate over time. FAF imaging may therefore reveal the exact extent of neurosensory dysfunction that may exceed the dimensions anticipated by conventional examinations.

- Gomes et al. **A Comparison of Fundus Autofluorescence and Retinal Structure in Patients with Stargardt Disease**. Invest Ophthalmol Vis Sci 2009; 50: 3953-3959.

PURPOSE. To improve the understanding of Stargardt disease by comparing structural changes seen on spectral domain optical coherence tomography (SD-OCT) to those visible on fundus autofluorescence (FAF).

METHODS. FAF and SD-OCT were performed on 22 eyes of 11 patients with Stargardt disease. SD-OCT images were obtained at the fovea and at the eccentric preferred retinal locus (PRL). The diameters of absent (hypoautofluorescence) and abnormal FAF areas were measured. The extent of the transverse defect of the junction between the inner and outer segments of the photoreceptors (IS-OS) was measured in the foveal area. The PRL was evaluated with fundus photography and microperimetry.

RESULTS. Twenty-one of 22 eyes showed defective FAF. In 17 eyes, FAF was absent in the fovea and in four eyes, FAF was abnormal. All eyes showed disorganization and/or loss of the IS-OS junction in the foveal area on SD-OCT. The diameter of the absent FAF area was smaller than the measurement of the IS-OS junction loss; the latter was closer to the diameter of the abnormal FAF area. Seventeen eyes had an eccentric PRL associated with a retinal area with no defects on FAF.

CONCLUSIONS. In the majority of eyes, changes shown by SDOCT correlated well with changes in FAF. However, in three patients, photoreceptor abnormalities were seen in the fovea on SD-OCT without an equivalent abnormality on FAF. This result suggests that in these patients, the structural integrity of the photoreceptors may be affected earlier than changes in the RPE at least as detected by FAF.

- Kellner et al. **Lipofuscin- and Melanin-related Fundus Autofluorescence in Patients with ABCA4-associated Retinal Dystrophies**. Am J Ophthalmol 2009; 147:895-902

PURPOSE: To compare melanin-related near-infrared fundus autofluorescence (NIA; excitation 787 nm, emission > 800 nm) to lipofuscin-related fundus autofluorescence (FAF; excitation 488 nm, emission > 500 nm) in patients with retinal dystrophies associated with ABCA4 gene mutations (ABCA4-RD).

DESIGN: Observational case series.

METHODS: Sixteen consecutive patients with ABCA4-RD diagnosed in one institution were included. FAF and NIA imaging were performed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2; Heidelberg Engineering, Heidelberg, Germany). The pattern and size of retinal pigment epithelial (RPE) alterations detected with FAF and NIA were evaluated.

RESULTS: FAF and NIA alterations were detected in all patients. In 7 of 16 patients, the alterations progressed beyond the vascular arcades, and in 9 of 16, they were confined to the macula. Spots of increased NIA (4/16) were less frequent compared with spots of increased FAF (15/16). Confluent patches of reduced NIA were frequent (12/16), and severely reduced NIA was observed in 3 cases. Areas with reduced NIA corresponded to either increased or reduced FAF. Preservation of subfoveal FAF or NIA corresponded to visual acuity > 0.4. Abnormalities detected with NIA were more extensive or more severe compared to FAF in 15 of 16 patients.

CONCLUSION: Patterns of FAF and NIA indicate different involvement of lipofuscin and melanin and their derivatives in the pathophysiologic process of ABCA4-RD. NIA imaging provides a noninvasive in vivo visualization of RPE abnormalities that may precede FAF alterations during the degenerative process. Combined FAF and NIA imaging will provide further insight in the development of ABCA4-RD and could help to monitor future therapeutic interventions.

- McBain et al. **Fundus autofluorescence in the diagnosis of cystoid macular oedema.** Br J Ophthalmol 2008; 92;946-949

Aim: To determine the sensitivity and specificity of the non-invasive imaging technique, fundus autofluorescence (AF), in the diagnosis of cystoid macular oedema (CMO), using fluorescein angiography as the reference standard.

Design: Retrospective, consecutive, observational case series.

Methods: Ninety-six consecutive patients with CMO suspected clinically were selected from the AF database of the Retina Unit, Ophthalmology Department, Grampian University Hospitals—NHS Trust, between August 2004 and June 2006. Only patients in whom CMO was secondary to (1) cataract extraction, (2) inherited retinopathies, (3) inflammatory eye disease or (4) idiopathic cases were included in this study. Only patients in whom AF images had been performed within 2 weeks of FFA and, when obtained following FFA, there was a minimum gap of 4 days (“washing out” period), were considered eligible for this study. A total of 34 eyes from 34 patients were eligible and were included in this study. FFA was used as the reference test to confirm the presence of CMO, and, based on fluorescein angiography (FFA), CMO was graded as either mild or florid. AF images were examined in a masked fashion for the presence or absence of CMO. The sensitivity and specificity of AF in detecting CMO were then calculated.

Results: CMO was seen on AF imaging as round or oval areas at the fovea with an AF signal similar to that of background levels. At this site (fovea), the AF signal is usually reduced compared with background, due to the blockage caused by luteal pigment. The diagnosis of CMO based on AF imaging had 81% sensitivity and 69% specificity when compared with the reference standard FFA. Based on the FFA, there were 12 cases of florid CMO and eight of mild CMO. Of the former, CMO was detected with AF imaging in 100% (12/12 eyes), and of the latter, in 50% (4/8 eyes).

Conclusions: AF imaging can be used as a rapid, non-invasive technique in the diagnosis of CMO