Title: Imaging protocols in clinical studies in advanced age-related macular degeneration

Subtitle: Recommendations from Classification of Atrophy (CAM) Consensus Meetings

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Abstract

Purpose: To summarize the results of a consensus meeting (Classification of Atrophy Meeting) on conventional and advanced imaging modalities used to detect and quantify atrophy due to late stage non-neovascular and neovascular age-related macular degeneration (AMD) and to provide recommendations on the use of these modalities in natural history studies and interventional clinical trials.

Design: Systematic debate on the relevance of distinct imaging modalities held in a sequence of meetings.

Participants: A panel of retina specialists.

Methods: During the Classification of Atrophy Meetings (CAM) a consortium of international experts evaluated the advantages and disadvantages of various imaging modalities based on the collective analysis of a large series of clinical cases. A systematic discussion on the role each modality in future studies in non-neovascular and neovascular AMD was held.

Main Outcome Measures: Advantages and disadvantages of current retinal imaging technologies and recommendations for their use in advanced AMD trials.

Results: Color fundus photography (CFP), confocal fundus autofluorescence (FAF), confocal near infrared reflectance and high resolution optical coherence tomography volume scans are valuable to detect, quantify and monitor progression of atrophy and are recommended at regular intervals throughout studies, aiming to detect, quantify and monitor progression of atrophy. In studies of non-neovascular AMD (without evident signs of either active or regressed neovascularization at baseline) CFP may be sufficient at baseline and end of study visit. Herein, fluorescein angiography (FA) may become necessary to evaluate for neovascularization at any visit during the study. Indocyanine-green angiography (ICG-A) may be considered at baseline under certain conditions. For studies in patients with neovascular AMD, increased need for visualization of the vasculature must be taken into account. Accordingly, these studies will benefit from FA (recommended at baseline and selected follow-up visits) and ICG-A under certain conditions.

Conclusions: A multimodal imaging approach is recommended in clinical trials for the optimal detection and measurement of atrophy and its associated features. Specific validation studies will be necessary to determine the best combination of imaging modalities. These recommendations may need to be adjusted to the particular focus in individual studies and will be updated as new imaging technologies become available in the future.

Introduction

In industrialized countries, late stage age-related macular degeneration (AMD) is the leading cause of legal blindness in the elderly. [1, 2] It either presents with neovascularization (NV) or geographic atrophy (GA). [3] Both manifestations are not mutually exclusive; atrophy develops in eyes with NV effectively treated with intravitreal anti-VEGF injections both, within as well as outside the area of NV. [4-7] In eyes developing atrophy without initial signs of NV, GA may be complicated by NV over time. The term "geographic atrophy" has been used with various definitions in the past as recently reviewed. [8] Herein, we use the term GA for complete retinal pigment epithelium (RPE) and outer retinal atrophy (complete RORA), excluding any region of presumptive NV. In this sense, GA can be present in eyes with no evident signs of active or regressed NV (*non-neovascular AMD*) as well in eyes with active or regressed NV outside the NV region (*neovascular AMD*).

The use of intravitreal vascular endothelial growth factor (VEGF) inhibitors has led to an unprecedented improvement in functional outcomes for patients with neovascular AMD, significantly reducing the incidence of blindness in the elderly. [9] However, visual outcomes of neovascular AMD treated with intravitreal anti-VEGF therapy are often limited by the occurrence of atrophy or fibrosis. [10] Many potential pathogenic factors are thought to cause the formation of atrophy in eyes with AMD and some pathways are being targeted with pharmacological agents in clinical development. [11] Currently no approved drugs are available to significantly slow atrophy progression and its associated vision loss.

Recent advances in retinal imaging technology, including spectral-domain and swept-source optical coherence tomography (SD-OCT and SS-OCT, respectively), scanning laser ophthalmoscopy (SLO) modalities, and wide-field imaging, have markedly improved detection of atrophy and the morphological biomarkers associated with disease progression. Anatomical endpoints have recently been validated and introduced as primary outcome parameters in interventional clinical trials for atrophic AMD with acceptance by regulatory authorities. [12-15] SLO-based fundus autofluorescence imaging together with semi-automated analysis tools and *en-face* OCT imaging are most commonly used for the delineation and quantitation of GA areas. [14, 16-18] Correlating the functional consequences of atrophy is highly relevant to clinical trials and clinical care of the condition. At this time, spatial resolution of these imaging modalities is far superior to currently available functional tests such as fundus-controlled perimetry (including so-called "microperimetry" with tracking of eye movements).

Selection of appropriate imaging modalities is a key factor for studies evaluating the efficacy of drugs in eyes with advanced AMD. Optimized selection of these modalities may be critical to detect an efficacy signal and the choice may impact duration and cost of such studies. A major challenge is the

fast pace of technological development in imaging devices as well as image analysis algorithms with a concurrent lack of timely validation. Each modality has its strengths and weaknesses. Moreover, the use of a single imaging modality may not be optimal, and multimodal imaging may be a sensible approach to obtain the most reliable detection and measurement of atrophy. [19]

The Classification of Atrophy Meetings (CAM) were organized to gather an international group of experts (see **appendix 1**), including representatives of established reading centers, to evaluate currently available imaging technologies and to propose consensus recommendations regarding the modalities to be used in interventional trials and in natural history studies of both non-neovascular and neovascular AMD, particularly taking into account the need for detection, quantification and monitoring of atrophy over time. Herein, the results of these consensus meetings are presented.

Methods

The planning committee for the Consensus in Atrophy Meetings (CAM) consisted of three retinal experts who developed the format for the consensus proceedings. The composition of the consensus panel was determined on the basis of previous notable scientific contributions to the field. Individuals were considered to contribute notably to the field if they had previously mad substantial contribution in AMD and retinal imaging research such as in form of key publications and involvement in clinical trials. Participants are listed in **Appendix 1**. During two consensus meetings (CAM-1 and CAM-2 held in 2015), these experts discussed and evaluated the advantages and disadvantages of various imaging modalities proposing the optimal combination for use in future natural history and interventional clinical studies. The formats of both meetings included the collection and discussion of clinical cases by the participants. A pre-meeting exercise was additionally distributed in preparation of the meetings aiming to identify strengths and challenges for detection of atrophy in each imaging modality. Furthermore, a list of imaging modalities currently used in large scale clinical trials in AMD was prepared for future discussion. During CAM-2 in a first session, cases and study questions were discussed to provide a common basis for the consensus finding process. Next, a debate was held on each of the listed imaging modalities. Within the debate it was questioned which particular advantages and disadvantages existed for each modality and which acquisition systems and protocols were best used. Furthermore, there was systematic discussion to the role each modality should play in future studies in non-neovascular and neovascular AMD aiming to provide a guideline that can serve as a starting point when designing protocols for clinical trials. Consensus was achieved by discussion. A grading system was used to classify each modality as 1) R = recommended, 2) O = optional, 3) N = not recommended, or 4) D = at discretion of the sponsor. If no

consensus was achieved during the debate, then a decision was obtained by majority vote. Results of the debate were logged.

Results

Color fundus photography

Color fundus photography (CFP) has been the historical standard used for documenting funduscopic abnormalities and it is still used in current AMD trials. [12, 20, 21] CFP was the basis of studies performed prior to the advent of many newer forms of imaging. Therefore, the continued use of CFP in future studies is necessary to ensure comparability with data gathered from the newer modalities and to allow comparison with past studies. In addition, CFP with its broad spectrum of illumination is the closest imaging modality to correlate with clinical ophthalmoscopy. With CFP, it is possible to detect a wide array of phenotypic alterations associated with AMD including drusen, crystalline deposits, pigmentary changes, lipid, atrophy and fibrosis as well as neovascular findings such as hemorrhages, fluid and exudate (**Fig. 1 A, Fig. 2 A**). Yet, compared with other imaging modalities it has relatively low contrast, which makes identification and quantification of atrophic lesions and other AMD associated changes challenging. For instance, without previous processing CFP provided a sensitivity of only 33 – 42% for the detection of reticular pseudodrusen, an important risk factor for fast GA progression (**Fig. 1 A-D**). [22, 23] Moreover the use of high intensity and broad spectrum light makes CFP susceptible to media opacities and in most devices its quality is limited when there is poor mydriasis. In addition, the bright flash used during image acquisition is uncomfortable for patients. The sensitivity needed to detect topographical changes at the borders of atrophy regions can be improved with stereoscopic viewing of CFP. However, stereoscopic image capture requires experienced and specifically trained personnel as well as good patient cooperation, which limits its practical use in clinical studies.

CFP was used in the initial definitions of GA, requiring a circular or oval area of depigmentation with sharply demarcated borders and typically increased visibility of large choroidal vessels within the atrophic area [24, 25]. In 1995, the International Age-Related Maculopathy (ARM) Epidemiological Study Group agreed on the following criteria: sharp demarcation, hypo- or depigmented appearance, round or oval shape, increased visibility of the underlying choroidal vessels and a diameter of at least 175 µm measured using a 30° or 35° fundus camera. [26] CFP has also been used to quantify areas of GA and to monitor expansion over time. [27] However, CFP is limited for this purpose due to difficulties in precisely delineating lesion boundaries, particularly in eyes with relatively small and multifocal lesions. Recently, a new white light confocal instrument has become available which can potentially

increase the contrast of atrophy margins due to its confocality. However, no large studies of the device are currently available.

Recommendation: CFP remains a valuable option and is recommended to be used while other imaging modalities are being validated for detection of atrophy. In addition, CFP is still the modality of choice for detection of several AMD-associated phenotypic changes, particularly hemorrhages and focal pigmentary changes. For a comprehensive documentation of funduscopic findings non-stereoscopic CFP is recommended to be performed at baseline and at the end of the study in studies on non-neovascular and neovascular AMD.

Fundus autofluorescence

Fundus autofluorescence (FAF) provides high contrast retinal images particularly valuable for the detection of atrophic areas. Within atrophic areas, RPE and lipofuscin (containing the major fluorophores responsible for fundus autofluorescence) are lost. Thus, FAF signal intensities are severely reduced at the site of atrophy (**Fig. 1 B**). Blue light excitation FAF is currently the most commonly used mode for FAF imaging. Absence of the signal is associated with loss of retinal sensitivity, [28, 29] providing a direct structure-function relationship. Furthermore, the contrast between intact retina and RPE usually allows for reproducible semi-automated quantification of atrophic areas. [30] Both these features have led to the acceptance of FAF as an anatomical outcome parameter for progression of GA in clinical trials [15] by international regulatory agencies. FAF signal measurements have been and are currently used in interventional clinical trials for GA including the SEATTLE-study (NCT01802866), GAP (NCT00599846) and GATE-studies (NCT00890097), MAHALO-Study (NCT01229215), Proxima A (NCT02479386) and B (NCT02399072) studies, the Zimura® trial (NCT02686658) and the CHROMA- (NCT02247479) and SPECTRI-studies (NCT02247531).

In eyes with neovascular AMD correlation between reduced FAF and presence of scotomas may be less pronounced. Yet, it could be shown that the area of confluent autofluorescence loss in the central macula is highly correlated to visual acuity, contrast sensitivity, and reading speed in eyes treated with anti-VEGF agents for neovascular AMD. [7, 31] In addition, FAF has proved useful in the differential diagnosis of disease entities that cause atrophy, and in classifying GA lesions according to distinct progression rates. [12, 32-34]

Two types of FAF imaging systems are currently available: Flash fundus camera-based systems and scanning laser ophthalmoscopy systems (SLO), usually equipped with a confocal aperture to filter out backscattering light from outside the focus plane (i.e. the retina). SLO-based systems have the advantage of higher contrast and spatial resolution but they cannot be integrated into conventional fundus cameras, making the acquisition of additional instrumentation necessary. [35] Nonetheless, SLO

devices are preferred for acquiring FAF images in most studies because of their excellent image contrast and resolution. Some technical skills are required by clinical sites to consistently obtain high quality images, and these include uniform illumination, good focus and central alignment of the camera with the eye to avoid obstruction by the iris.

Fundus autofluorescence imaging with SLO was originally introduced with blue light excitation. Later on, the use of longer excitation wavelengths (e.g. 532 nm) has become broadly available for SLO. Using fundus cameras, the use of blue light excitation for autofluorescence imaging is not practical due to technical limitations. Therefore fundus camera autofluorescence is based on excitation wavelengths in the green to orange range (510 – 610 nm). The quality of the blue FAF signal is influenced by several factors including nuclear lens opacities, vitreous floaters and pupil dilation. In addition, macular pigment blocks blue light leading to a relatively decreased signal intensity at the fovea. Therefore, when the atrophic border is close to the fovea, identification of the border of atrophy may be challenging (**Fig. 1 D**). [36] In addition, optimal contrast between intact and atrophic RPE can be lost in the presence of certain GA subtypes including the "diffuse-trickling" phenotype [37] or refractile drusen (also called "calcified drusen" or "crystalline deposits" in parallel) [38] at the margin and within areas of atrophy, which results in some limitation to FAF use alone. In such cases combined analysis of FAF and near infrared reflectance (NIR) images is typically helpful for adjudicating the precise measurement of the atrophic lesion. [19] In atrophy associated with concurrent NV distinction between atrophy and areas of fibrosis may be difficult with FAF alone [39]. Furthermore, hemorrhages or hard exudates may result in decreased FAF signals. Therefore, the additional use of fluorescein angiography (FA) has been suggested. [39] In addition, a disadvantage of bright blue excitation light is the discomfort it may cause to the patient. Within atrophic areas, the FAF signal may not be zero. This may be due to retained RPE cells or fragments and basal laminar deposits with a low level of 488-nm-excited autofluorescence. [40, 41]The combination of FAF and NIR may help improve demarcation of the atrophic areas confounded by the limitations of using FAF alone.

Recently, longer wavelength light sources have been integrated with confocal SLO devices (e.g., green). Longer wavelengths are less sensitive to optical media opacities and macular pigment. However, few validation studies (for 532 nm fundus cameras or 514 nm SLO systems) are currently available. [17, 42]

Recommendation: In view of its properties and recent validation in various clinical studies, confocal FAF images may provide valuable information when included in imaging protocols of interventional and non-interventional trials in non-neovascular AMD, and protocols involving neovascular AMD so that atrophy development and progression can be detected and monitored.

Quantitative fundus autofluorescence

Recently, a FAF method has been introduced which incorporates an internal fluorescent reference to account for variable laser power and detector sensitivity, enabling quantitative fundus autofluorescence (qAF). Intensity of autofluorescence is calculated against the calibrated reference standard in the optical pathway. To derive reliable qAF measurements, it is critically important to obtain high quality images. [43, 44] Key requirements for images suitable for qAF measurement include uniform signal intensity, fine-tuned focus, central alignment of the camera with the eye to avoid obstruction by the iris, and exposure within the linearity range of the detector. Lens and vitreous opacities may limit measurements, as absorption confounds measured FAF intensity levels originating from the retina and the RPE. Normative studies have shown that qAF levels exhibit a significant increase with age, that qAF values are higher in females, and that there may be ethnic differences. For example, as compared with Hispanics, qAF is significantly higher in Whites and lower in Blacks and Asians, [44] but these variations would have limited impact if looking at individual qAF change over time. Currently, this novel technology is increasingly employed in eyes with retinal dystrophies [45-47] as it appears to be of value for differential diagnosis. [46, 48] More recently, it has been used to image AMD. [49] Of potential interest is the magnitude of qAF signals outside atrophic areas as well as within atrophic zones, for instance to study the rather grayish than black atrophy observed in classical FAF images in some GA subtypes. [37]

Recommendation: Overall, this method may be considered to help validating current and generate novel disease-related hypotheses.

Near infrared reflectance

For near infrared reflectance (NIR) SLO imaging, wavelengths of light at the higher end of the visible spectrum are used (750 nm to 840 nm in current devices). This causes minimal discomfort to the patient. There is very little interference and absorption by media opacities, and, importantly, by the luteal pigment in the macula. Therefore, in contrast to blue-light FAF imaging, visualization of atrophic areas within the fovea and involvement of the fovea is facilitated (**Fig. 1 B**). [19] The available evidence suggests that NIR images allow for the detection of reticular pseudodrusen with the highest sensitivity. [22, 50-52] Systematic validation studies for the use of NIR alone in atrophic AMD are lacking and precise sensitivity and specificity need to be determined. NIR imaging originally served as a guidance modality for OCT, but the NIR image is now available as an independent output image for most OCT/SLO devices resulting in broad availability.

Recommendation: NIR images provide valuable information complementary to FAF. Their capture time is short and does not materially affect recording procedure. Therefore NIR images should be considered whenever FAF images are acquired.

Multicolor confocal scanning laser ophthalmoscopy

Confocal multicolor images consist of a composite image simultaneously obtained by confocal scanning lasers from three different wavelengths. [53] It comprises three reflectance images: blue reflectance (wavelength [ex.]: 488 nm), green reflectance (ex.: 518 nm) and near infrared reflectance (ex.: 820 nm). These different light wavelengths penetrate the retinal layers at different depths to reveal details of the respective layer. Blue reflectance highlights details of the inner retina and the vitreoretinal interface. Green reflectance facilitates visualization of deeper retinal layers, and near infrared reflectance highlights structures in the outer retina and choroid. [54] In contrast to CFP, multicolor images are "false-" or "pseudocolor" images comprised of information gathered from separate wavelengths, and not from the whole visible light spectrum. To-date, only a few studies using this novel technique are available. [23, 55, 56] In the context of GA, multicolor imaging can be used to visualize atrophic areas (**Fig. 2 C**). [54, 55] Due to their optical reflection properties, retinal hemorrhages and fibrotic changes may also be seen, yet, subtle hemorrhages and pigmentary lesions may not be distinguished from each other by multicolor imaging alone (**Fig. 2 C**). Whether the addition of the two other wavelengths, blue and green, increases or obscures the information contained in the NIR image, has not been systematically investigated. Furthermore, it may turn out that visualizing the individual wavelength component images in isolation may have advantages under certain conditions over a composite. It is possible to acquire multicolor images through non-dilated pupils covering a field of view of up to 55°. However, when the image is acquired through a non-dilated pupil, the signal quality may be adversely affected, in particular for wavelengths in the blue spectrum.

Recommendation: Due to the limits of current knowledge, the inclusion of multicolor imaging into study protocols for atrophic and neovascular AMD should be optional since its specific utility remains to be demonstrated.

Optical coherence tomography

In contrast to the other imaging modalities currently available in clinical routine, SD-OCT provides *in vivo cross-sectional* and *en-face* visualization of the retina and the RPE. [57] Using the enhanced depth imaging acquisition technique, additional details can be seen at the level of the choroid. [58] The anatomic correlates of the single bands and layers identified in an SD-OCT line-scan are now largely established. [59] In addition, anatomical tracking functions that are implemented in some devices, allow the alignment of follow-up with baseline images. While a single line-scan (= B-scan) may be obtained quickly, a dense raster pattern of line-scans ("volume scan" or C-scan) is necessary to extract "*en-face*" images that represent macula topography (**Fig. 1 E, F and Fig. 2 J, K**). The spacing between scans must be sufficiently small so that pathologic features of interest such as drusen,

reticular pseudodrusen (**Fig. 1 E**), and pigment migration into the inner retina, are not missed. However, it is recognized that the denser the scan spacing, the longer the acquisition time. Hence, often a compromise is required depending on the particular study questions. For instance, reliable detection of reticular pseudodrusen, which are significantly associated with fast atrophy progression, and volume-rendering of outer retinal tubulations would require scans spaced as thin as 11 µm. [60] Also volume-rendering of pathologic structures, like outer retinal tabulation, will require equally dense scan spacing. [61] However, within the context of longitudinal large scale clinical studies, a less dense scan (e.g., spacing of 120 µm) is usually preferred. [22] The near infrared laser light used to acquire SD-OCT images is well tolerated, yet discomfort due to fatigue and tear film desiccation during prolonged acquisition times is reported by some patients.

Today, SD-OCT is widely available and has been validated to assess and quantify atrophy. [14] It is also an important tool to grade and monitor NV in AMD, [62, 63] and serves an integral role in several large-scale AMD trials. [64] *En-face* rendering of the structural OCT may provide an additional mode to analyze and quantitate areas of atrophy (**Fig. 1 F**).

Recent studies indicate that distinct morphologic findings identified with SD-OCT scans like splitting of band 4 (generally accepted to correspond to RPE/Bruch's membrane complex), presence of outer retinal tubulations, disruption of the surrounding outer retinal anatomy, [65] or local minimum SD-OCT signal intensity may predict atrophy progression. [37, 66-68] Additional capabilities of OCT include quantitation of GA areas and identification of putative precursor lesions. [69-72] While providing depth encoded images, compared to SLO imaging, the lateral resolution of SD-OCT devices is lower (up to 5 µm in current confocal SLO and 15 µm in current SD-OCT devices).

To accurately grade a volumetric SD-OCT dataset, it is necessary to inspect all individual line-scans within the volume scan, or to assess several different *en-face* slabs. Generally, it is more time consuming and labor intensive to interpret an SD-OCT dataset than to interpret any of the imaging modalities that produce single *en-face* images. In addition, if SD-OCT slabs are to be assessed, sophisticated software solutions are needed to automatically segment the images, a function that is complicated by the AMD-associated pathology distorting the reflective bands and that needs to be further addressed by ongoing software improvements. Though substantial advances have been made in this field errors in automated segmentation occurring in the presence of pathology may require time-consuming manual adjustment; [73-75] however, for simple viewing of GA, this is rarely necessary.

Currently, various SD-OCT devices are commercially available; however, industry standards concerning data compatibility (e.g.: Digital Imaging and Communication in Medicine [DICOM]) and acquisition settings are not generally supported by the manufacturers. This lack of compliance affects

comparability between examinations obtained with different devices, leads to fragmentation of imaging databases and limits the possibility of pooling data from different devices. Wherever possible, similar SD-OCT devices should be used at all study sites.

New developments in OCT imaging include the availability of swept-source OCT (SS-OCT), which features higher scanning rates and wider scan areas. In current commercially available devices a more than 40% faster acquisition time can be achieved. Moreover, SS-OCT devices use a longer wavelength light source, which has better tissue penetration in the axial direction, and produces more detailed images of the choroid. [76] Thus faster acquisition of large scan volumes is possible. The addition of eye-tracking functions further facilitates image acquisition in eyes with poorer fixation stability. Further evaluation of this imaging technology is needed, yet SS-OCT imaging seems at least as good as SD-OCT with the promise of easier imaging of larger scan areas measuring 12 mm x 12 mm (40° x 40°) on the retina. Thus, commercially available SS-OCT devices may be considered as an alternative for future clinical studies.

Recommendation: OCT imaging may provide valuable information at each visit in trials of non-neovascular and neovascular AMD. Evidence supports the acquisition of volume scans (SD-OCT or SS-OCT) covering an area of at least 6 x 6 mm (larger or overlapping scans should be performed depending on the expected size of GA at the end of the study) and a distance not greater than 120 µm between single line-scans in order to visualize AMD-related pathologies. In devices that allow for averaging of multiple images, averaging at least 10 line-for noise reduction is recommended. Variation of scan density and averaging of multiple scans may be considered depending on the study purpose.

Fluorescein angiography

Fluorescein angiography (FA) has been the gold standard to detect and grade NV. [77, 78] Flash fundus camera-based and SLO-systems are widely available and have been used in large trials for non-neovascular and neovascular AMD (to rule out NV). [4, 79] Exact lesion boundary demarcation may be obscured by progressive dye leakage. [62] Yet, validation analyses have shown that reliable quantification of lesion area is possible in SLO [21, 80] and fundus camera based devices. [81] To complement FA in this regard, SD-OCT can also be used to track neovascular activity over time (**Fig. 2 D-F**).

There are disadvantages to FA. It is an invasive method with relatively long examination times to acquire the late phase FA images. Injection of the dye and the use of bright light may be uncomfortable for the patient. There is also a risk of allergic reactions to the intravenously injected dye. [35]

Recommendation: FA is informative at baseline in trials of non-neovascular AMD to exclude the presence of concurrent NV and should be performed when incident NV is suspected. In neovascular AMD trials FA should be considered at baseline, selected follow-up visits (approximately every 6 months) and at the end of the study. Imaging should cover the central 30° and a 10 minute late phase should be included.

Indocyanine green angiography

Indocyanine green angiography (ICG-A) provides additional information regarding the choroidal vasculature and blood flow. [82] Indocyanine green has much greater binding affinity to plasma proteins than fluorescein and is associated with minimal leakage from the fenestrated choriocapillaris allowing more detailed visualization of the choroidal vessels (**Fig. 2 G-I**). [83] This additional information obtained with ICG is helpful to detect retinal-choroidal anastomoses and polypoidal choroidal vasculopathy (PCV). [84, 85] ICG-A has also been suggested for differential diagnosis of atrophy. For example, “dark atrophy” where no dye accumulation is found beneath the zone of atrophy is seen in Stargardt disease, but not in AMD in which late staining occurs. [86]

Choroidal imaging may be helpful for studying pathological processes involved in AMD and for differential diagnosis in atrophic and neovascular AMD. [86-88] However, ICG-A is an invasive method [35] and for atrophic AMD, alternative, non-invasive methods are available that are sufficient for differential diagnosis. Furthermore, acquisition also of late phase images makes ICG-A a time-consuming procedure.

Recommendation: The consensus committee suggests ICG-A to be included into the study protocol for clinical studies of neovascular AMD only if deemed necessary by the investigators at the baseline visit. Decision on whether to include ICG-A or not should particularly take into account regional qualifications like distinct PCV frequencies. In atrophic AMD, ICG-A may be optionally performed if doubts remain on the etiology of the atrophy (i.e. differential diagnosis).

Optical coherence tomography angiography

Optical coherence tomography angiography (OCT-A) allows blood flow detection based on motion contrast. The motion contrast can be derived from phase/Doppler shift and/or a variation in signal amplitude over time within a voxel due to movement of blood cells. [89-93] A significant advantage of OCT-A over conventional dye-based angiography is that it is depth-resolved and non-invasive. [94] Isolated analysis of flow in the various choroidal layers has been studied with OCT-A. [95] In the context of AMD, several investigators have demonstrated that OCT-A can be used for detailed characterization of NV in AMD (**Fig. 2 L**). [96-100] Other investigators have noted that a non-exudative type 1 neovascularization can be detected with OCT-A. [101, 102] OCT-A also enables analysis of different

choroidal layers inside and at the border of atrophic lesions. [103] Due to the ability to visualize the choroid and choriocapillaryis, it may be possible to use OCT-A instead ICGA to differentiate the different types of macular atrophy. [104] As only flow is visualized, no information on vascular wall integrity comparable to leakage in FA can be obtained. However, structural information is still maintained in the OCT-A and can show collections of fluid, suggesting leakage is occurring. Also, the availability of corresponding enface structural OCT images may be useful for revealing con-existing non-patent vascular structures. [102] To obtain useful OCT-A datasets, it is necessary to acquire dense, high quality SD-OCT or SS-OCT scans. Therefore, like for conventional OCT devices, OCT-A limitations include acquisition time and field; however, faster acquisition times with wider fields of view and deeper choroidal imaging can be performed using SS-OCTA instruments. Motion and retinal vascular projections are additional limitations of this technology, but these limitations could be resolved in the future. [105] The full clinical value and optimal application of OCT-A are still being defined, but the growing information to-date appears promising.

Recommendation: OCT-A may be optionally included in studies on non-neovascular and neovascular AMD for exploratory purposes.

Wide-field imaging

Wide-field imaging allows much larger areas of the retina to be visualized, when compared with conventional imaging modalities. The field of view extends to > 100° (values for the angle of view depending on point of reference and the optics of the individual eye). Commercially available wide-field imaging devices are based on SLO-technology, which, in general, is suitable for acquisition of FAF, reflectance images as well as both FA and ICG-A. Color images are obtained by using two or more discrete laser sources for illumination, thus providing a false-color image that may have regions of artifactually abnormal-looking color depending on the registration of the light sources. Due to the special optics required to cover such a wide field of view, separate devices can be used. Alternatively, fields of view of up to 150° can be achieved using a SLO device equipped with a contact lens for FA and ICG-A. [106]

The greater field of view is obtained at the expense of spatial resolution and contrast. The presence of eye lashes may limit the field of view inferiorly and superiorly, although additional steered images may be obtained to minimize this problem. Despite these developments, the full extent of the peripheral retina may not be visible in all subjects.

Previous studies have shown that when the features of the macular region from wide field images are graded, the results can be similar to those obtained by review of traditional CFP. [107] One important advantage of this novel technique is the opportunity to observe peripheral abnormalities

associated with AMD and to monitor these changes over time. [108] This is of particular relevance as peripheral changes in AMD are to-date incompletely understood. Data on their natural history, impact on disease progression, and functional implications need to be investigated. [109-112]

Recommendation: The use of wide-field imaging is likely to provide a more complete understanding of the fundus involvement in AMD, and its use should be considered for trials regarding non-neovascular and neovascular AMD for exploratory purposes.

Conclusions

The CAM group carefully evaluated the burgeoning number of imaging modalities and assessed their potential contributions to clinical studies and interventional trials. The advantages and disadvantages of each imaging modality are summarized in **Table 1**. Taking into account the particular needs when imaging eyes with AMD, the consensus committee provides recommendations which technologies may be appropriate in studies of non-neovascular and neovascular AMD. These recommendations should be considered as a starting point when designing imaging protocols for clinical studies but may require adjustments depending on the exact study purpose.

For research trials in non-neovascular AMD (**Table 2**), imaging modalities should include CFP, confocal FAF (blue light excitation) and NIR (three field, 30°) and dense SD-OCT or SS-OCT volume scans (at least 6 x 6 mm larger depending on area of GA with maximum distance between scans not greater than 120 µm) at baseline and at the final outcome point. For follow-up visits, CFP may be considered and may be necessary if evaluating specific phenotypic features that are present and that are readily captured by this method (e.g. hemorrhages, pigmentary changes). Novel imaging technologies, namely multicolor imaging, OCT-A, qAF and wide-field imaging are likely to further contribute to our understanding of disease processes related to AMD, but data are still forthcoming. Thus, their value is currently of an exploratory nature and inclusion into trial protocols is optional. Nonetheless, the consensus committee encourages their inclusion in clinical studies to better determine their usefulness. FA may be required at any visit to detect incident NV.

Recommendations for imaging protocols regarding trials on neovascular AMD (**Table 3**) consider the need to identify and assess the vasculature and the more dynamic nature of the underlying pathological processes. In studies of neovascular AMD, FA and FAF are recommended at baseline, selected follow-up visits (approx. every 6 months) and at the final outcome point. NIR and CFP imaging is recommended at visits when FA or FAF is performed to enhance interpretation. ICG-A is optional at baseline. Decision on whether to include it into the trial protocol also needs to reflect regional peculiarities in the abundance of single NV subtypes. Whether ICG-A examinations are to be

carried out at interval visits depends on the type of lesion under observation and may be reasonable in eyes with retinal-choroidal anastomosis or PCV. SD-OCT or SS-OCT volume scans (6 x 6 mm or larger with distances between scans not greater than 120 µm) should be performed at every visit.

These recommendations reflect the status of our current knowledge and available imaging technologies as well as practical considerations. They will need to be updated as new information becomes available and imaging technology (for instance OCT-A) advances.

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References

1. Congdon N, O'Colmain B, Klaver CC, et al. Causes and prevalence of visual impairment among adults in the United States. Arch Ophthalmol 2004;122:477-85.

2. Resnikoff S, Pascolini D, Etya'ale D, et al. Global data on visual impairment in the year 2002. Bull World Health Organ 2004;82:844-51.

3. Lim LS, Mitchell P, Seddon JM, et al. Age-related macular degeneration. Lancet 2012;379:1728-38.

4. Chakravarthy U, Harding SP, Rogers CA, et al. Alternative treatments to inhibit VEGF in age-related choroidal neovascularisation: 2-year findings of the IVAN randomised controlled trial. Lancet 2013;382:1258-67.

5. Grunwald JE, Pistilli M, Ying GS, et al. Growth of geographic atrophy in the comparison of age-related macular degeneration treatments trials. Ophthalmology 2015;122:809-16.

6. Grunwald JE, Daniel E, Huang J, et al. Risk of geographic atrophy in the comparison of age-related macular degeneration treatments trials. Ophthalmology 2014;121:150-61.

7. Kumar N, Mrejen S, Fung AT, et al. Retinal pigment epithelial cell loss assessed by fundus autofluorescence imaging in neovascular age-related macular degeneration. Ophthalmology 2013;120:334-41.

8. Schmitz-Valckenberg S, Sadda S, Staurenghi G, et al. Geographic atrophy: semantic considerations and literature review. Retina 2016 [Epub ahead of print].

9. Campbell JP, Bressler SB, Bressler NM. Impact of availability of anti-vascular endothelial growth factor therapy on visual impairment and blindness due to neovascular age-related macular degeneration. Arch Ophthalmol 2012;130:794-5.

10. Bhisitkul RB, Mendes TS, Rofagha S, et al. Macular atrophy progression and 7-year vision outcomes in subjects from the ANCHOR, MARINA, and HORIZON studies: the SEVEN-UP study. Am J Ophthalmol 2015;159:915-24.

11. Holz FG, Strauss EC, Schmitz-Valckenberg S, van Lookeren Campagne M. Geographic atrophy: clinical features and potential therapeutic approaches. Ophthalmology 2014;121:1079-91.

12. Schmitz-Valckenberg S, Sahel JA, Danis R, et al. Natural History of Geographic Atrophy Progression Secondary to Age-Related Macular Degeneration (Geographic Atrophy Progression Study). Ophthalmology 2016;123:361-8.

13. Schmitz-Valckenberg S, Nadal J, Fimmers R, et al. Modeling Visual Acuity in Geographic Atrophy Secondary to Age-Related Macular Degeneration. Ophthalmologica 2016;235:215-24.

14. Yehoshua Z, Rosenfeld PJ, Gregori G, et al. Progression of geographic atrophy in age-related macular degeneration imaged with spectral domain optical coherence tomography. Ophthalmology 2011;118:679-86.

15. FDA Cellular, Tissue, and Gene Therapies Advisory Committee. CTGTAC Meeting #52, Cellular and Gene Therapies for Retinal Disorders. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/UCM259087.pdf; 2011 Accessed 02.06.2016

16. Csaky KG, Richman EA, Ferris FL, 3rd. Report from the NEI/FDA Ophthalmic Clinical Trial Design and Endpoints Symposium. Invest Ophthalmol Vis Sci 2008;49:479-89.

17. Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, et al. Comparison of Geographic Atrophy Growth Rates Using Different Imaging Modalities in the COMPLETE Study. Ophthalmic Surg Lasers Imaging Retina 2015;46:413-22.

18. Yehoshua Z, Garcia Filho CA, Penha FM, et al. Comparison of geographic atrophy measurements from the OCT fundus image and the sub-RPE slab image. Ophthalmic Surg Lasers Imaging Retina 2013;44:127-32.

19. Lindner M, Boker A, Mauschitz MM, et al. Directional Kinetics of Geographic Atrophy Progression in Age-Related Macular Degeneration with Foveal Sparing. Ophthalmology 2015;122:1356-65.

20. Chew EY, Clemons T, SanGiovanni JP, et al. The Age-Related Eye Disease Study 2 (AREDS2): study design and baseline characteristics (AREDS2 report number 1). Ophthalmology 2012;119:2282-9.

21. Grunwald JE, Daniel E, Ying GS, et al. Photographic assessment of baseline fundus morphologic features in the Comparison of Age-Related Macular Degeneration Treatments Trials. Ophthalmology 2012;119:1634-41.

22. Wu Z, Ayton LN, Luu CD, et al. Reticular Pseudodrusen in Intermediate Age-Related Macular Degeneration: Prevalence, Detection, Clinical, Environmental, and Genetic Associations. Invest Ophthalmol Vis Sci 2016;57:1310-6.

23. De Bats F, Mathis T, Mauget-Faysse M, et al. Prevalence of Reticular Pseudodrusen in Age-Related Macular Degeneration Using Multimodal Imaging. Retina 2016;36:46-52.

24. Klein R, Davis MD, Magli YL, et al. The Wisconsin age-related maculopathy grading system. Ophthalmology 1991;98:1128-34.

25. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. Eye (Lond) 1988;2:552-77.

26. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv Ophthalmol 1995;39:367-74.

27. Sunness JS, Gonzalez-Baron J, Applegate CA, et al. Enlargement of atrophy and visual acuity loss in the geographic atrophy form of age-related macular degeneration. Ophthalmology 1999;106:1768-79.

28. Panorgias A, Zawadzki RJ, Capps AG, et al. Multimodal assessment of microscopic morphology and retinal function in patients with geographic atrophy. Invest Ophthalmol Vis Sci 2013;54:4372-84.

29. Pilotto E, Guidolin F, Convento E, et al. Fundus autofluorescence and microperimetry in progressing geographic atrophy secondary to age-related macular degeneration. Br J Ophthalmol 2013;97:622-6.

30. Schmitz-Valckenberg S, Brinkmann CK, Alten F, et al. Semiautomated image processing method for identification and quantification of geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 2011;52:7640-6.

31. Sato T, Suzuki M, Ooto S, Spaide RF. Multimodal Imaging Findings and Multimodal Vision Testing in Neovascular Age-Related Macular Degeneration. Retina 2015;35:1292-302.

32. Holz FG, Bindewald-Wittich A, Fleckenstein M, et al. Progression of geographic atrophy and impact of fundus autofluorescence patterns in age-related macular degeneration. Am J Ophthalmol 2007;143:463-72.

33. Saksens NT, Fleckenstein M, Schmitz-Valckenberg S, et al. Macular dystrophies mimicking age-related macular degeneration. Prog Retin Eye Res 2014;39:23-57.

34. Spaide RF. Outer retinal atrophy after regression of subretinal drusenoid deposits as a newly recognized form of late age-related macular degeneration. Retina 2013;33:1800-8.

35. Holz FG, Pauleikhoff D, Spaide RF, Bird AC, eds. Age-related Macular Degeneration, 2nd ed. Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg, 2013.

36. Forte R, Querques G, Querques L, et al. Multimodal evaluation of foveal sparing in patients with geographicatrophy due to age-related macular degeneration. Retina 2013;33:482-9.

716 37. Fleckenstein M, Schmitz-Valckenberg S, Martens C, et al. Fundus autofluorescence and
717 spectral-domain optical coherence tomography characteristics in a rapidly progressing form of
718 geographic atrophy. Invest Ophthalmol Vis Sci 2011;52:3761-6.

719
720 38. Suzuki M, Curcio CA, Mullins RF, Spaide RF. REFRACTILE DRUSEN: Clinical Imaging and
721 Candidate Histology. Retina 2015;35:859-65.

722
723 39. Kuehlewein L, Dustin L, Sagong M, et al. Predictors of Macular Atrophy Detected by Fundus
724 Autofluorescence in Patients With Neovascular Age-Related Macular Degeneration After Long-Term
725 Ranibizumab Treatment. Ophthalmic Surg Lasers Imaging Retina 2016;47:224-31.

726
727 40. Zanzottera EC, Messinger JD, Ach T, et al. The Project MACULA Retinal Pigment Epithelium
728 Grading System for Histology and Optical Coherence Tomography in Age-Related Macular
729 Degeneration. Invest Ophthalmol Vis Sci 2015;56:3253-68.

730
731 41. Gocho K, Sarda V, Falah S, et al. Adaptive optics imaging of geographic atrophy. Invest
732 Ophthalmol Vis Sci 2013;54:3673-80.

733
734 42. Wolf-Schnurrbusch UE, Wittwer VV, Ghanem R, et al. Blue-light versus green-light
735 autofluorescence: lesion size of areas of geographic atrophy. Invest Ophthalmol Vis Sci
736 2011;52:9497-502.

737
738 43. Delori F, Greenberg JP, Woods RL, et al. Quantitative measurements of autofluorescence
739 with the scanning laser ophthalmoscope. Invest Ophthalmol Vis Sci 2011;52:9379-90.

740
741 44. Greenberg JP, Duncker T, Woods RL, et al. Quantitative fundus autofluorescence in healthy
742 eyes. Invest Ophthalmol Vis Sci 2013;54:5684-93.

743
744 45. Muller PL, Gliem M, Mangold E, et al. Monoallelic ABCA4 Mutations Appear Insufficient to
745 Cause Retinopathy: A Quantitative Autofluorescence Study. Invest Ophthalmol Vis Sci 2015;56:8179-
746 86.

747
748 46. Duncker T, Greenberg JP, Ramachandran R, et al. Quantitative fundus autofluorescence and
749 optical coherence tomography in best vitelliform macular dystrophy. Invest Ophthalmol Vis Sci
750 2014;55:1471-82.

751
752 47. Duncker T, Stein GE, Lee W, et al. Quantitative Fundus Autofluorescence and Optical
753 Coherence Tomography in ABCA4 Carriers. Invest Ophthalmol Vis Sci 2015;56:7274-85.

754
755 48. Duncker T, Tsang SH, Woods RL, et al. Quantitative Fundus Autofluorescence and Optical
756 Coherence Tomography in PRPH2/RDS- and ABCA4-Associated Disease Exhibiting Phenotypic
757 Overlap. Invest Ophthalmol Vis Sci 2015;56:3159-70.

758

49. Gliem M, Muller PL, Finger RP, et al. Quantitative Fundus Autofluorescence in Early and Intermediate Age-Related Macular Degeneration. JAMA Ophthalmol 2016;134:817-24.

50. Ueda-Arakawa N, Ooto S, Tsujikawa A, et al. Sensitivity and specificity of detecting reticular pseudodrusen in multimodal imaging in Japanese patients. Retina 2013;33:490-7.

51. Schmitz-Valckenberg S, Alten F, Steinberg JS, et al. Reticular drusen associated with geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 2011;52:5009-15.

52. Smith RT, Sohrab MA, Busuioc M, Barile G. Reticular macular disease. Am J Ophthalmol 2009;148:733-43 e2.

53. Tan AC, Fleckenstein M, Schmitz-Valckenberg S, Holz FG. Clinical Application of Multicolor Imaging Technology. Ophthalmologica 2016;236:8-18.

54. Pang CE, Freund KB. Ghost maculopathy: an artifact on near-infrared reflectance and multicolor imaging masquerading as chorioretinal pathology. Am J Ophthalmol 2014;158:171-8 e2.

55. Ben Moussa N, Georges A, Capuano V, et al. MultiColor imaging in the evaluation of geographic atrophy due to age-related macular degeneration. Br J Ophthalmol 2015;99:842-7.

56. Alten F, Clemens CR, Heiduschka P, Eter N. Characterisation of reticular pseudodrusen and their central target aspect in multi-spectral, confocal scanning laser ophthalmoscopy. Graefes Arch Clin Exp Ophthalmol 2014;252:715-21.

57. Drexler W, Morgner U, Ghanta RK, et al. Ultrahigh-resolution ophthalmic optical coherence tomography. Nat Med 2001;7:502-7.

58. Spaide RF, Koizumi H, Pozzoni MC. Enhanced depth imaging spectral-domain optical coherence tomography. Am J Ophthalmol 2008;146:496-500.

59. Staurenghi G, Sadda S, Chakravarthy U, Spaide RF. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN*OCT consensus. Ophthalmology 2014;121:1572-8.

60. Steinberg JS, Auge J, Fleckenstein M, et al. Longitudinal analysis of reticular drusen associated with age-related macular degeneration using combined confocal scanning laser ophthalmoscopy and spectral-domain optical coherence tomography imaging. Ophthalmologica 2015;233:35-42.

61. Litts KM, Ach T, Hammack KM, et al. Quantitative Analysis of Outer Retinal Tubulation in Age-Related Macular Degeneration From Spectral-Domain Optical Coherence Tomography and Histology. Invest Ophthalmol Vis Sci 2016;57:2647-56.

62. Khurana RN, Dupas B, Bressler NM. Agreement of time-domain and spectral-domain optical coherence tomography with fluorescein leakage from choroidal neovascularization. Ophthalmology 2010;117:1376-80.

63. Jung JJ, Chen CY, Mrejen S, et al. The incidence of neovascular subtypes in newly diagnosed neovascular age-related macular degeneration. Am J Ophthalmol 2014;158:769-79.

64. Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, et al. Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. Ophthalmology 2014;121:693-701.

65. Nunes RP, Gregori G, Yehoshua Z, et al. Predicting the progression of geographic atrophy in age-related macular degeneration with SD-OCT en face imaging of the outer retina. Ophthalmic Surg Lasers Imaging Retina 2013;44:344-59.

66. Hariri A, Nittala MG, Sadda SR. Outer retinal tubulation as a predictor of the enlargement amount of geographic atrophy in age-related macular degeneration. Ophthalmology 2015;122:407-13.

67. Stetson PF, Yehoshua Z, Garcia Filho CA, et al. OCT minimum intensity as a predictor of geographic atrophy enlargement. Invest Ophthalmol Vis Sci 2014;55:792-800.

68. Moussa K, Lee JY, Stinnett SS, Jaffe GJ. Spectral domain optical coherence tomography-determined morphologic predictors of age-related macular degeneration-associated geographic atrophy progression. Retina 2013;33:1590-9.

69. Hu Z, Medioni GG, Hernandez M, et al. Segmentation of the geographic atrophy in spectral-domain optical coherence tomography and fundus autofluorescence images. Invest Ophthalmol Vis Sci 2013;54:8375-83.

70. Chen Q, Niu S, Shen H, et al. Restricted Summed-Area Projection for Geographic Atrophy Visualization in SD-OCT Images. Transl Vis Sci Technol 2015;4:2.

71. Wu Z, Luu CD, Ayton LN, et al. Fundus autofluorescence characteristics of nascent geographic atrophy in age-related macular degeneration. Invest Ophthalmol Vis Sci 2015;56:1546-52.

72. Wu Z, Luu CD, Ayton LN, et al. Optical coherence tomography-defined changes preceding the development of drusen-associated atrophy in age-related macular degeneration. Ophthalmology 2014;121:2415-22.

73. Schaal KB, Legarreta AD, Gregori G, et al. Widefield En Face Optical Coherence Tomography Imaging of Subretinal Drusenoid Deposits. Ophthalmic Surg Lasers Imaging Retina 2015;46:550-9.

74. Schutze C, Ahlers C, Sacu S, et al. Performance of OCT segmentation procedures to assess morphology and extension in geographic atrophy. Acta Ophthalmol 2011;89:235-40.

75. Abdelfattah NS, Zhang H, Boyer DS, Sadda SR. Progression of Macular Atrophy in Patients with Neovascular Age-Related Macular Degeneration Undergoing Antivascular Endothelial Growth Factor Therapy. Retina 2016;36:1843-5.

76. Drexler W, Liu M, Kumar A, et al. Optical coherence tomography today: speed, contrast, and multimodality. J Biomed Opt 2014;19:071412.

77. [MacularPhotocoagulationStudyGroup]. Subfoveal neovascular lesions in age-related macular degeneration. Guidelines for evaluation and treatment in the macular photocoagulation study. Macular Photocoagulation Study Group. Arch Ophthalmol 1991;109:1242-57.

78. Novotny HR, Alvis DL. A method of photographing fluorescence in circulating blood in the human retina. Circulation 1961;24:82-6.

79. Martin DF, Maguire MG, Ying GS, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med 2011;364:1897-908.

80. Vujosevic S, Vaclavik V, Bird AC, et al. Combined grading for choroidal neovascularisation: colour, fluorescein angiography and autofluorescence images. Graefes Arch Clin Exp Ophthalmol 2007;245:1453-60.

81. Friedman SM, Margo CE. Choroidal neovascular membranes: reproducibility of angiographic interpretation. Am J Ophthalmol 2000;130:839-41.

82. Slakter JS, Yannuzzi LA, Guyer DR, et al. Indocyanine-green angiography. Curr Opin Ophthalmol 1995;6:25-32.

83. Holz FG, Bellmann C, Rohrschneider K, et al. Simultaneous confocal scanning laser fluorescein and indocyanine green angiography. Am J Ophthalmol 1998;125:227-36.

84. Yannuzzi LA, Negrao S, Iida T, et al. Retinal angiomatous proliferation in age-related macular degeneration. Retina 2001;21:416-34.

85. Spaide RF, Yannuzzi LA, Slakter JS, et al. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. Retina 1995;15:100-10.

86. Giani A, Pellegrini M, Carini E, et al. The dark atrophy with indocyanine green angiography in Stargardt disease. Invest Ophthalmol Vis Sci 2012;53:3999-4004.

87. Pauleikhoff D, Spital G, Radermacher M, et al. A fluorescein and indocyanine green angiographic study of choriocapillaris in age-related macular disease. Arch Ophthalmol 1999;117:1353-8.

88. Mori K, Gehlbach PL, Ito YN, Yoneya S. Decreased arterial dye-filling and venous dilation in the macular choroid associated with age-related macular degeneration. Retina 2005;25:430-7.

89. Coscas G, Lupidi M, Coscas F. Heidelberg Spectralis Optical Coherence Tomography Angiography: Technical Aspects. Dev Ophthalmol 2016;56:1-5.

90. Huang D, Jia Y, Gao SS, et al. Optical Coherence Tomography Angiography Using the Optovue Device. Dev Ophthalmol 2016;56:6-12.

91. Stanga PE, Tsamis E, Papayannis A, et al. Swept-Source Optical Coherence Tomography Angio (Topcon Corp, Japan): Technology Review. Dev Ophthalmol 2016;56:13-7.

92. Rosenfeld PJ, Durbin MK, Roisman L, et al. ZEISS Angioplex Spectral Domain Optical Coherence Tomography Angiography: Technical Aspects. Dev Ophthalmol 2016;56:18-29.

93. Gorczynska I, Migacz JV, Zawadzki RJ, et al. Comparison of amplitude-decorrelation, speckle-variance and phase-variance OCT angiography methods for imaging the human retina and choroid. Biomed Opt Express 2016;7:911-42.

94. de Carlo T, Romano A, Waheed N, Duker J. A review of optical coherence tomography angiography (OCTA). International Journal of Retina and Vitreous 2015;1:5.

95. Spaide RF, Klancnik JM, Jr., Cooney MJ. Retinal vascular layers imaged by fluorescein angiography and optical coherence tomography angiography. JAMA Ophthalmol 2015;133:45-50.

96. de Carlo TE, Bonini Filho MA, Chin AT, et al. Spectral-domain optical coherence tomography angiography of choroidal neovascularization. Ophthalmology 2015;122:1228-38.

97. Kuehlewein L, Bansal M, Lenis TL, et al. Optical Coherence Tomography Angiography of Type 1 Neovascularization in Age-Related Macular Degeneration. Am J Ophthalmol 2015;160:739-48 e2.

98. Jia Y, Bailey ST, Wilson DJ, et al. Quantitative optical coherence tomography angiography of choroidal neovascularization in age-related macular degeneration. Ophthalmology 2014;121:1435-44.

99. Kuehlewein L, Dansingani KK, de Carlo TE, et al. Optical Coherence Tomography Angiography of Type 3 Neovascularization Secondary to Age-Related Macular Degeneration. Retina 2015;35:2229-35.

100. Lindner M, Fang PP, Steinberg JS, et al. OCT Angiography-Based Detection and Quantification of the Neovascular Network in Exudative AMD. Invest Ophthalmol Vis Sci 2016;57:6342-8.

101. Nehemy MB, Brocchi DN, Veloso CE. Optical Coherence Tomography Angiography Imaging of Quiescent Choroidal Neovascularization in Age-Related Macular Degeneration. Ophthalmic Surg Lasers Imaging Retina 2015;46:1056-7.

102. Roisman L, Zhang Q, Wang RK, et al. Optical Coherence Tomography Angiography of Asymptomatic Neovascularization in Intermediate Age-Related Macular Degeneration. Ophthalmology 2016;123:1309-19.

103. Ferrara D, Waheed NK, Duker JS. Investigating the choriocapillaris and choroidal vasculature with new optical coherence tomography technologies. Prog Retin Eye Res 2016;52:130-55.

104. Pellegrini M, Acquistapace A, Oldani M, et al. Dark Atrophy: An Optical Coherence Tomography Angiography Study. Ophthalmology 2016;123:1879-86.

105. Spaide RF, Fujimoto JG, Waheed NK. Image Artifacts in Optical Coherence Tomography Angiography. Retina 2015;35:2163-80.

106. Staurenghi G, Viola F, Mainster MA, et al. Scanning laser ophthalmoscopy and angiography with a wide-field contact lens system. Arch Ophthalmol 2005;123:244-52.

107. Csutak A, Lengyel I, Jonasson F, et al. Agreement between image grading of conventional (45 degrees) and ultra wide-angle (200 degrees) digital images in the macula in the Reykjavik eye study. Eye (Lond) 2010;24:1568-75.

108. Lengyel I, Csutak A, Florea D, et al. A Population-Based Ultra-Widefield Digital Image Grading Study for Age-Related Macular Degeneration-Like Lesions at the Peripheral Retina. Ophthalmology 2015;122:1340-7.

109. Duisdieker V, Fleckenstein M, Zilkens KM, et al. Long-Term Follow-Up of Fundus Autofluorescence Imaging Using Wide-Field Scanning Laser Ophthalmoscopy. Ophthalmologica 2015;234:218-26.

110. Tan CS, Heussen F, Sadda SR. Peripheral autofluorescence and clinical findings in neovascular and non-neovascular age-related macular degeneration. Ophthalmology 2013;120:1271-7.

111. Domalpally A, Danis RP, Chew EY, et al. Ultra Wide Field Autofluorescence Imaging in Age Related Macular Degeneration- Optos Peripheral Retina (OPERA) Age Related Eye Disease Study 2(AREDS2) Ancillary Study. Invest Ophthalmol Vis Sci 2014;55:3410 [ARVO E-Abstract].

112. Friberg TR, Chew EY, Domalpally A, et al. Peripheral Retinal Findings in color images in Age-Related Macular Degeneration - OPERA, an AREDS 2 Ancillary Study. Invest Ophthalmol Vis Sci 2014;55:4017 [ARVO E-Abstract].

Figures legends

Figure 1: Right eye of a 72 y/o male patient with geographic atrophy (GA) and foveal sparing without any signs of neovascularization. A) Color fundus photography (CFP) shows multifocal GA, drusen and pigmentary changes. B) Fundus autofluorescence (FAF) showing well-demarked areas of hypo-autofluorescence corresponding to GA and, hyper-autofluorescent drusen and reticular pseudodrusen. Of note, despite overall stronger contrast between GA and intact retina when compared to CFP, demarcation of the foveal border of the atrophy is partially obscured by macular pigment. C) Near infrared reflectance (NIR) discloses areas of GA and reticular pseudodrusen. In conjunction with FAF it allows precise demarcation also of the foveal atrophy border. D) Green-light autofluorescence is less affected by macular pigment and thus allows clear delineation of atrophy borders also in close proximity to the fovea. E) Corresponding spectral-domain optical coherence tomography (SD-OCT) scan visualizing cross sectional morphology of the retina. A druse, reticular pseudodrusen and GA with consecutive choroidal signal enhancement can be observed. Scan position as indicated by green line in C. F) SD-OCT sub-RPE slab depicting the areas of GA and allowing for identification of the spared fovea. Position of the scan volume is indicated by the blue frame in C; distance between single line-scans: 120 µm.

Figure 2: Multimodal imaging of the left eye of a 69 y/o patient with atrophy in the presence of neovascular age-related macular degeneration that has been treated with anti-vascular endothelial growth factor therapy. A) Color fundus photography, B) fundus autofluorescence, C) Multicolor imaging, D-F) Fluorescein angiogram, G-I) Indocyanine-green angiography, J-K) Spectral-domain optical coherence tomography B-scans (positions as indicated in C). L) OCT-angiography (position as indicated in C) showing matured vessels.