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Digital Ocular Fundus Imaging: A Review

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Abstract

Ocular fundus imaging plays a key role in monitoring the health status of the human eye. Currently, a large number of imaging modalities allow the assessment and/or quantification of ocular changes from a healthy status. This review focuses on the main digital fundus imaging modality, color fundus photography, with a brief overview of complementary techniques, such as fluorescein angiography. While focusing on two-dimensional color fundus photography, the authors address the evolution from nondigital to digital imaging and its impact on diagnosis. They also compare several studies performed along the transitional path of this technology. Retinal image processing and analysis, automated disease detection and identification of the stage of diabetic retinopathy (DR) are addressed as well. The authors emphasize the problems of image segmentation, focusing on the major landmark structures of the ocular fundus: the vascular network, optic disk and the fovea. Several proposed approaches for the automatic detection of signs of disease onset and progression, such as microaneurysms, are surveyed. A thorough comparison is conducted among different studies with regard to the number of eyes/subjects, imaging modality, fundus camera used, field of view and image resolution to identify the large variation in characteristics from one study to another. Similarly, the main features of the proposed classifications and algorithms for the automatic detection of DR are compared, thereby addressing computer-aided diagnosis and computer-aided detection for use in screening programs.

Digital Imaging

The availability of digital cameras – from dedicated photographic cameras to cell phones – has quickly decreased the use of film-based imaging. The development of medical imaging too has undergone a rapid transition in the same direction, one of enhancement.

Some imaging modalities, e.g. computed tomography, scanning laser ophthalmoscopy (SLO) and optical coherence tomography rely on digital imaging, in contrast to, fundus photography and fluorescein angiography which appeared quite early, do not.

The first photographic images of the ocular fundus were obtained by the end of the 19th and the beginning of the 20th centuries, and the concept of a fundus camera dates back to that time [1]. As stated in a recent review [2]: 'The primary role of ophthalmic imaging however, goes well beyond documentation in its ability to aid in the diagnosis of a broad range of eye conditions'. Additional continuous efforts have been made to achieve the best possible fundus images [3].

This review focuses on digital imaging of the human eye fundus and its impact on clinical use. It does not attempt to provide an exhaustive description of all digital imaging modalities with application to the human ocular fundus; instead, it concentrates primarily on fundus photography.

It establishes a link between traditional (analog/nondigital) and digital imaging, and addresses intrinsic differences, advantages and disadvantages of each.

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Eye fundus imaging is useful to document its status and to assess any changes from a healthy condition. Imaging may focus on the structure or on a particular functional aspect of the retina (e.g. color fundus photography or fluorescein angiography, respectively), or on a correlation of the two [1, 4].

Besides the diagnosis of ocular diseases, retinal imaging also allows the detection, diagnosis and management of hypertensive and cardiovascular diseases [1, 5]. The importance of easy access to the retinal microcirculation is clear: 'The retinal microvasculature is unique in that it is the only part of the human circulation that can be directly visualised non-invasively in vivo, readily photographed and subject to digital image analysis' [6].

The different options for fundus imaging are manifold (e.g. computed tomography, magnetic resonance imaging, ultrasound imaging, infrared thermography [7], hyperspectral imaging [8], color Doppler imaging [9], or photoacoustic ophthalmoscopy [10] and blood flow magnetic resonance imaging [11] in the rat retina). We also refer to red-free photography, color fundus photography, stereofundus photography, SLO and angiography. These modalities share 2D imaging of the ocular fundus in contrast to 3D imaging by optical coherence tomography [12, 13–17] or volumetric information from confocal SLO (CSLO). Each modality provides specific structural (e.g. color fundus photography) or functional information (e.g. fluorescein angiography) on the ocular fundus. This review only addresses 2D imaging.

Among the advantages of digital imaging are the ease and speed of access to data (images) [18]; fast and exact duplication, archiving and transmission [19–21], and immediate access to the results. The imaging procedure can be repeated if the quality of the initial result is inadequate [20; 21]. Although film-based images can be digitized (to compute macular pigment density distribution from two different wavelength-based images [22] or to assess the status of the optic nerve [23]), immediate access to the images is not possible, as it is necessary to develop the film first. This delay prevents the photographer from verifying the results and therefore correcting any problem in the acquisition process, which can be easily achieved in digital imaging at no additional cost.

The digitization of fundus photographs was addressed by Cideciyan et al. [24], who proposed a nonlinear restoration model incorporating four components: the eye, the fundus camera, the film and the scanner. Scholl et al. [25] found digitized images to be useful for grading age-related maculopathy and age-related macular degeneration.

Three advantages were enumerated in favor of digital imaging [26]. First, '... digital imaging permits the photographer to judge instantly the quality of the captured image, and to take better pictures if necessary'. Second, '... more time is needed for mounting and identifying 35-mm slides, which occupy substantial storage space and have to be catalogued manually for retrieval'. Third, '... the total costs per stored image are lower than for 35-mm film, and damage to the environment is lower because of the avoidance of chemical processing, which is necessary for film-based photography'.

The '... easier access and improved ability to manipulate large volumes of data ... enable more innovative approaches ...', as in the case of the quantification of fluorescein angiograms [27].

Because of easy transmission, digital imaging is a clear advantage for imaging in remote locations and population screening [28–33] although the security of the information can be a concern [34]. Images can be sent to reading centers for manual or automatic screening [35]. In fact, several groups worldwide are pursuing research to find the best and most accurate automatic systems for disease grading. In addition, research is also under way in order to extract as much information as possible from digital images to provide information on the structure and function of the human retina and to improve knowledge on the changes in the diseased retina at the earliest possible stage.

As stated in an editorial by Bressler [36]: 'Findings from new imaging techniques may not represent a significant scientific advance if the new procedures have not been shown to provide advantages that outweigh disadvantages in comparison with existing technology'. This important and obvious statement emphasizes the balance between gains and losses in information and knowledge. The large differences in image resolution (number of pixels) and its relationship to the field of view (FOV) in ocular fundus images, as compared to film-based fundus images are of particular importance in this regard.

Even so, as Dhawan et al. [37] have noted, the role of computerized medical imaging is clear: 'Computerized medical imaging and analysis methods using multiple modalities have facilitated early diagnosis, treatment evaluation, and therapeutic intervention in the clinical management of critical diseases'. Research and development in medical imaging, not restricted to the eye, have been reviewed [38, 39].

Since color fundus photography and fluorescein angiography play a special role, a small introduction should be made here.

In color fundus photography, contrast filters are used to modify the spectral range of the illumination source. In this way, the visibility of several structures can be enhanced.

Red light is poorly absorbed by the retinal pigment epithelium, thus revealing the choroid and the choroidal pattern. Green light, however, is absorbed by blood and reflected by the retinal pigment epithelium, providing a good contrast for visualizing the retinal vascular network, hemorrhages, drusen and exudates. Because of these features, green (red-free) photographs are routinely taken in addition to fluorescein angiograms. Moreover, blue light allows for better imaging of anterior retinal layers. It is absorbed by blood and by the retinal pigment epithelium, providing a dark background against which top layers of the retina are imaged [2]. Elsner et al. [40] and Fernandez et al. [41] have reported on the effects of wavelength on human fundus imaging.

Based on this imaging modality, stereo imaging is particularly useful as it enhances the visual sense of depth. Due to the motion (shifting) of the camera, beams from the two images fall in opposite slopes of the cornea, enhancing the stereoscopic effect [2].

In addition, mydriatic and nonmydriatic fundus images offer different advantages. Cameras that can capture images through small, nonmydriatic pupils are tailored for the physiological dilation that occurs in a darkened room. This feature makes them suitable for remote primary care units and for screening programs. In contrast, mydriatic retinal photographs are significantly more sensitive than nonmydriatic photographs [42]. Conversely, mydriatic cameras can provide better fundus images but require dilated pupils and are primarily used in ophthalmology clinics and research centers [2].

Film versus Digital Fundus Images

Digital imaging developed in the mid 1960s following the space program of the National Aeronautics and Space Administration [43]. Its application to the medical field led to the use of imaging modalities that were not available previously, such as computed tomography, ultrasound imaging and magnetic resonance imaging.

A characteristic of digital images, in particular of ocular fundus images, is resolution. Resolution is normally expressed as the number of pixels present in the image. Although resolution correlates with the potential of the image to capture details of the objects present in the FOV, it does not convey information on image quality. To capture small details, a 'sufficient' number of pixels is required and is expressed as pixel density. As summarized by Prasad and Roy [43]: 'The number and density of pixels must be high enough to produce a faithful representation of the subject ...'.

Conventional 35-mm films contain silver elements that are packed to a density equivalent to approximately 2,500 lines per inch, corresponding to a frame resolution in a digital image of about 4,096 × 2,736 pixels [43]. The recommended resolution (100 lines per millimeter) depends on the film used and development process (1–100 cycles per millimeter for a response above 10% for a typical transparency film used for imaging diabetic retinopathy, DR [20]). In Fujichrome Velvia 100 films, resolution ranges from 80 to 160 lines per millimeter (http://www.fujifilm.com/, accessed May 13, 2011) depending on contrast. In this way, a link is established between the intrinsic resolution of a 35-mm film and the resolution of the sensor used by a digital camera.

Nonetheless, the quantitation process is usually not mentioned. Although the most common is 8 bits per color channel, i.e. 256 different levels per color channel in the saved images, 10 and 12 bits per channel are usually available at the detector level [44].

Also, grainy films have been replaced by a charge-coupled device (CCD), and seldom by a complementary metal oxide semiconductor [45]. While digital cameras initially used only one CCD sensor, newer cameras use three CCD sensors. This enhancement allows current cameras to separate information that is generated for each of the red-green-blue color channels for each pixel.

In this way, a digital (red-green-blue color) image with a resolution similar to that of the film-based one should be 4,096 × 2,736 × 3 bytes (33,619,968 bytes, i.e. about 32 megabytes), using 8-bit color channels only. On the other hand, according to Mead et al. [19], a digital image of 1,300 × 1,300 pixels (for a 45° FOV fundus image) is enough to detect microaneurysms.

Images of this resolution (4,069 × 2,736 pixels) present two major problems. At the acquisition step, through the time required in transmitting the image from the sensor (camera) to the computer. In the case of fluorescein angiograms, it prevents sequences of images to be taken in a short period of time, e.g. in documenting the filling phase. At the archiving and transmission step, large hard-disk computer space is needed in addition to long transmission time through the network, e.g. to a reading center, remote hard-disk drive or data warehouse.

Research has shown [46] that a resolution of 50 pixels per degree may provide diagnostic power comparable to

film-based images to detect DR. Thus, 2,500 pixels should be required to produce a 50° FOV retinal image, which is much higher than frequently found in the literature.

Evaluation of DR based on film usually makes use of a small FOV. Two 30° FOV 35-mm stereoscopic images are commonly used; these allow the assessment of lesions of the retinal capillaries such as microaneurysms. In contrast, digital imaging of the human ocular fundus is usually performed over a larger FOV, with 45°, 50° and 60° FOV being common. In addition, digital fundus images usually present lower resolution compared with film-based images on top of lossy compression file formats [20].

Different fundus camera makers follow different paths regarding these issues. Some produce large image files (uncompressed) and then compress them using a lossless compression, hence preserving all of the information on the acquired image. Others use lossy compression techniques, which reduce image files to a small fraction of their original size, at the cost of losing information. In either case, virtually all centers that apply digital ocular fundus imaging use a much smaller image size for routine purposes and larger images (resolution and image file size) for research purposes.

To cope with digital images, standard protocols for archiving, communication and the like have been proposed. The Picture Archiving and Communication System is an image-based information system for the acquisition, storage, communication, archiving, display and remote manipulation of medical images. The standard of Digital Imaging and Communications in Medicine, originally developed for radiological images, is now used in different areas of medical imaging [47].

Table 1 summarizes the differences between several studies in relation to digital versus nondigital ocular imaging. Clear differences are noticeable in the number of eyes or patients used; the number of photographs per eye, resolution, and FOV, and image type or compression. Seven studies do not mention the sensor type used. Five make no reference to the image resolution (number of pixels), and nine make no reference to the image file type used.

Influence on the Diagnosis

Several studies on ocular fundus photography have established the gains and losses in diagnosis and screening feasibility with the transition from film-based imaging to digital imaging.

Henricsson et al. [48] compared the performance of digital images with slides in detecting and grading DR. Digital color and red-free images and 35-mm slides were obtained using the Topcon Imagenet System 1.53 system and Topcon TRC-50 VT fundus camera, Kodachrome 64 color film system, respectively. They obtained 50° FOV images of 640 × 480 pixels in true color and 50° FOV color 35-mm film slides. Exact agreement was found between grading obtained from color slides and digital color images in 82% of the cases. Exact agreement increased to 85% when red-free images were used as an adjunct to digital color images. Henricsson et al. reported that 'Good to excellent agreement was found between the grading of colour slides and digital colour images', although the '... tendency [was] towards undergrading of the digital colour images ...'.

In a study by Liesenfeld et al. [28], images from 129 patients with diabetes were screened for DR by slit lamp examination using two-field 50° FOV nonstereo digital fundus photographs and 35-mm transparencies of the same field. The authors concluded that 'Telescreening for diabetic retinopathy by an assessment of two-field 50° non-stereo digital images is a valid screening method'. In addition, in contrast to 35-mm transparencies, no digital images were lost.

Lim et al. [18] compared digital images through undilated pupils with 35-mm slide images through dilated pupils for the detection of DR. A modified Canon CR6-45NM camera equipped with a Sony DXC-970MD digital camera was used to obtain 45° FOV digital images. Image resolution was 640 × 480 pixels. The 35-mm film fundus images were obtained through dilated pupils using a Zeiss 30° fundus camera. The authors concluded that 'Nonmydriatic digital fundus imaging for detection of diabetic retinopathy has a low sensitivity rate and a high specificity rate and is less clinically useful than standard dilated 35-mm fundus slide images'.

One year later, a study with similar conditions was published by Bursell et al. [49], who evaluated the ability to determine clinical severity of DR, the timing of the next retinal evaluation and the necessity of referral to an ophthalmologist by comparing stereoscopic nonmydriatic digital-video color retinal images to Early Treatment Diabetic Retinopathy Study (ETDRS) standard seven-field 35-mm stereoscopic color fundus photographs. Their conclusion was slightly different. These authors found 'substantial agreement ($\kappa = 0.65$)' for DR assessment and 'excellent ($\kappa = 0.87$)' agreement for suggested referral to an ophthalmologist. Digital images (45° FOV) were 640 × 480 pixels in size and true color (24 bits – 8 bits per color channel). In addition, compressed Joint Photographic Experts Group (JPEG) images were pro-

Table 1. Digital versus nondigital ocular imaging

Study	Eyes (subjects), n	Digital image	Fundus camera	Digital camera	FOV	Resolution	File type	Nondigital imaging	Fundus camera (film)	FOV	Conclusions
Henricsson et al. [48] 2000	– (279)	1 × C and 1 × RF	Topcon 'Imagenet System 1.53'	Sony DXC 930	50° and 50°	640 × 480 and 640 × 480		7-field 35-mm color (ETDRS) M	Topcon TRC-50 VT (Kodachrome 64 ASA)	50°	Good to excellent agreement for detection and grading of DR RF as adjunct modality seems to facilitate the detection of DR lesions
Liesenfeld et al. [28] 2000	– (129)	2 × NS M	Topcon TRC 50X		50°	768 × 576		Slit lamp biomicroscopy and 2× NS 35-mm slide images	Topcon TRC 50X (Kodak Ektachrome 100 ASA)	50°	Nonstereo digital images are a valid screening method for DR
Lim et al. [18] 2000	40 (22)	3 × NM	Canon CR6-45NM	Sony DXC 970MD	45°	640×480		35-mm slide images M	Zeiss (Kodak Ektachrome 64 ASA)	30°	Nonmydriatic digital images are less clinically useful for detection of DR
Bursell et al. [49] 2001	108 (54)	3 × NM ST	Topcon TRC NW-5S	Sony 970-MD	45°	640 × 480	Compressed JPEG (10:1)	7-field 35-mm ST color (ETDRS) M	Zeiss FF4 (Kodachrome 64 ASA)	30°	Value of the nonmydriatic digital imaging for the determination of clinical DR
Razvi et al. [50] 2002	400 (200)	M	Canon 45NM	Sony HAD 3CCD	45°		Compressed JPEG	Direct ophthalmoscopy			Advantage of combining digital imaging and ophthalmoscopy in eye screening
Rudnisky et al. [44] 2002	207 (105)	M ST	Zeiss FF450	Kodak/ Canon DCS560	30°	3,040×2,008	Uncompressed TIFF	Contact lens biomicroscopy			High-resolution stereoscopic digital imaging biomicroscopy is both sensitive and specific for diagnosis of clinically significant macular edema
Herbert et al. [51] 2003	288 (145)	1 × NM	Topcon TRC NW5-S	Sony 3-Chip	45°	800 × 600	Compressed JPEG	Slit lamp biomicroscopy			Single-digital fundal image is insufficient for screening purposes
Leeuwen et al. [26] 2003	137 (91)	M ST	Topcon TRC-50EX	Sony HAD 3CCD	35°	~800 × 600	Uncompressed TIFF	35-mm slide images M	Topcon TRC-50EX (Kodak Ektachrome 64 ASA)	35°	Digital images are as good as 35-mm film for grading of age-related maculopathy
Massin et al. [52] 2003	147 (74)	5 × NM	Topcon TRC-NW6S	Sony DXC-950 P	45°	800 × 600		7-field 35-mm ST color (ETDRS)	Canon CF 60 UV	30°	Nonmydriatic digital images are suitable for DR screening
Sabti et al. [53] 2003	92 (51)		Canon CF 60 UV		30° and 60°						Digital images provide an efficient method for diagnosing and classifying sight-threatening DR, particularly proliferative DR

Table 1 (continued)

Study	Eyes (subjects), n	Digital image	Fundus camera	Digital camera	FOV	Resolution	File type	Nondigital imaging	Fundus camera (film)	FOV	Conclusions
Lawrence [54] 2004	– (151) and – (103)	1× NM 3× M	Topcon TRC-NW5SF and Topcon TRC-NW6S		45° and 45°	640 × 480 and 800 × 600		7-field 35-mm ST color (ETDRS)	Topcon TRC-50VT	30°	The 800 × 600 digital image system is an accurate method of detecting DR, provided there is adequate pupillary dilation and three retinal images are taken
Pirbhai et al. [55] 2005	223 (118)	M NS	Topcon TRC 50IX			1,024 × 1,024		Fluorescein angiography			Digital images for screening exudative AMD were highly sensitive, specific, and showed high positive predictive and negative predictive value in confirming or excluding the presence of neovascularization
Saari et al. [29] 2004	108 (70)	2× C M and 1× RF M	Topcon TRC 50 IA		50° and 50°	768 × 576 and 1,320 × 1,032		Modified ETDRS classification			Digital 50° RF and 2 × 45° or 50° digital color images are suitable for DR screening The hand-held camera (MediTell) does not fulfill the needs for DR screening
		2× C M	Canon CR6–45NM		45°	2,160 × 1,440					
			MediTell		20°	768 × 576	JPEG				
Schiffman et al. [56] 2005	222 (111)	15 fields	DigiScope		55° to 60°	930 diagonal pixels (19°)	Compressed JPEG 2000 (9:1 to 15:1)	7-field 35-mm ST color (ETDRS)		30°	System may be useful to screen for DR
Somani et al. [57] 2005	203 (103)	3× C M ST	Topcon TRC-NW6S	Nikon D100	45°	3,008 × 2,000	Compressed JPEG (16:1)	3× C 35-mm film slide M ST	Zeiss FF450 (Kodak Ektachrome)	30°	Good correlation for the identification of moderate to advanced AMD
Chun et al. [58] 2007	231 (120)	1× C NM	Canon CR6-45NM	Sony DXC390 3CCD	45°	800 × 480	Compressed JPEG (7:1)	Slit lamp biomicroscopy and indirect ophthalmoscopy M			A single 45°, nonmydriatic, digital image is not reliable as the sole modality for DR screening
Lopez-Bastida et al. [59] 2007	1,546 (773)	2× NM	Topcon TRC-NW6S		30° and 45°			Slit lamp biomicroscopy and indirect ophthalmoscopy M			Nonmydriatic camera is an effective option in community based screening programs for DR
Hubbard et al. [60] 2008	605 (0)		<several>	<several>	30°	(1)	Compressed JPEG (20:1)	Digitized color slide films (2)	<several> (Kodak Ektachrome 100 ASA)		In AREDS2, the best digital images matched the best film

C = Color; RF = red-free; ST = stereo; NS = non-stereo; M = mydriatic; NM = nonmydriatic.

(1) Images were received in different resolutions, but all were saved as compressed JPEG with a resolution of 2,912 × 2,480 pixels. (2) Slide films were digitized at 3,400 × 2,300 pixel resolution.

duced. They also concluded that 'This image validation study demonstrates the value of the JVN [Joslin Vision Network] system for nonmydriatic electronic retinal imaging and the determination of clinical diabetic retinopathy compared with gold standard ETDRS seven standard field 35-mm stereoscopic color 30° fundus photography'.

Leeuwen et al. [26] compared the quality and reliability of grading age-related maculopathy in 137 eyes of 91 patients using stereo digital images and stereo 35-mm color transparencies. Both 35-mm film and digital images of 35° FOV were obtained with a Topcon TRC-50EX fundus camera equipped with a Sony HAD 3CCD color video camera. Digital image resolution was 800 × 600 pixels. The authors concluded that digital images were as good as 35-mm film for grading age-related maculopathy.

Similarly, Massin et al. [52] compared the results of fundus photography through a nonmydriatic digital camera with results of ETDRS retinal photographs for the detection of DR. Forty-five-degree color fundus photographs of the eyes (147 eyes) of 74 patients were taken using a Topcon nonmydriatic camera without pupil dilation (Sony 3CCD DXC-950P digital camera). Digital images were captured at 800 × 600 pixel resolution in true color (24 bits). ETDRS 35-mm color slides were taken with a Canon CF 60 UV camera and were used as reference images for the detection of DR. Massin et al. concluded that '... photographs taken by the Topcon TRC-NW6S non-mydriatic camera, without pupillary dilation, are suitable for DR screening'.

Lawrence [54] evaluated the accuracy of two digital imaging systems, with two different resolutions, in detecting DR. A group of patients (n = 151) was imaged with a 640 × 480 pixel resolution (45° FOV) while another group of patients (n = 103) was imaged with an 800 × 600 pixel resolution (45° FOV), in addition to the seven-field ETDRS (used as gold standard). The author concluded that the 800 × 600 resolution system '... offers an accurate method of detecting diabetic retinopathy, provided there is adequate pupillary dilation and three retinal images are taken'.

In the work of Saari et al. [29], three digital fundus cameras were assessed for DR screening. Digital color images and red-free retinal images were obtained with a Topcon TRC 50IA, a Canon CR6-45NM and a Meditell (a hand-held digital color video camera) in 70 patients with diabetes and control subjects. A total of 427 images were evaluated. Mydriatic ophthalmoscopy and color and red-free images were taken as reference standards. For all types of digital imaging, the pupils were dilated. Two-color 50° FOV images of 768 × 575 pixel resolution, one red-free image and one black-and-white image of 1,320 × 1,032 pixel resolution were obtained with a Topcon TRC 50IA camera. Two 45° FOV color images of 2,160 × 1,440 pixel resolution were obtained with a Canon CR6-NM fundus camera. The hand-held digital video camera was used to capture 20° FOV color images of 768 × 576 pixels that were saved as JPEG images. According to the authors, one digital 50° red-free and two 50° or 45° color images are suitable for DR screening. The hand-held digital video camera, however, did not achieve this goal.

The DigiScope system, which was developed to be used in primary-care physicians' offices, was presented by Zeimer et al. [46]. Schiffman et al. [56] used the DigiScope to compare digital retinal imaging obtained with seven-field color fundus photography for the detection of DR. Images from the DigiScope were used in the JPEG 2000 image file format with a compression ratio of 9:1 to 15:1. In this study, 15 slightly overlapping fields provided a 55–60° FOV centered on the maculae of 222 eyes (111 patients); each of the 15 fields corresponded to 930 diagonal pixels for a 19° FOV [46]. The agreement found between the DigiScope and the seven-field photography '... indicates that the DigiScope may be useful to screen for diabetic retinopathy'.

A total of 203 eyes of 103 patients with a diagnosis of age-related macular degeneration (AMD) were enrolled in the study of Somani et al. [57], who compared the sensitivity and specificity of stereoscopic digital photography of the dilated pupil with a 45° FOV nonmydriatic camera with those of 35-mm slide film photography in the identification of AMD. Digital images were saved as compressed JPEG image files. Images obtained were also of 45° FOV with a nonmydriatic fundus camera (Topcon TRC-NW6S equipped with a digital camera Nikon D100 of 3,008 × 2,000 pixel resolution). Captured tag image file format (TIFF) files of 17.2 megabytes (of true color) were thereafter compressed to JPEG images of 1.1-megabyte file size. Stereoscopic images were viewed through liquid crystal display shutter glasses on a 21-in monitor of 1,024 × 768 pixels, i.e. at a lower resolution than the image acquired. On the other hand, a Zeiss FF450 fundus camera using Ektachrome Kodak film slides was used to obtain 30° film photographs of the optic disk and macula. The results of the study allowed the authors to conclude that 'High-resolution stereoscopic, mydriatic, 45° digital images captured with a nonmydriatic camera and JPEG compressed correlate well with stereoscopic slide

film photographs in the identification of moderate to advanced AMD (AREDS level 3a or greater)'. Although a good correlation was found, it was observed only for moderate to advanced AMD stages.

Hubbard et al. [60] analyzed the brightness, contrast and color balance of digital versus film retinal images to propose a model tailored for the evaluation of AMD. They considered 3-megapixel systems, at 30° FOV, as acceptable to image AMD retinas where '... drusen as small as 32 μm diameter ...' can be found although resolutions over a 6-megapixel system are preferred [60]. They concluded that 'In AREDS2, the best digital images matched the best film. Overall, however, digital provided lower contrast of retinal detail'.

Apart from the work done by Liesenfeld et al. [28], additional comparisons between digital imaging and other diagnostic techniques of direct observation performed during the last decade were analyzed.

Razvi et al. [50] reported on the advantage of combining digital imaging and ophthalmoscopy in eye screening. They found that this combination was superior to either digital imaging or ophthalmoscopy alone in detecting DR. Using a standard 45° FOV Canon 45NM retinal camera equipped with a Sony HAD 3CCD color video camera, they imaged 400 eyes of 200 patients with type 1 or type 2 diabetes and found that 'Screening systems using digital imaging in which images are collected and later viewed without patient present will miss the added benefit of added ophthalmoscopy as highlighted in this study'.

A similar study, which was performed by Rudnisky et al. [44], compared high-resolution stereoscopic digital photography to contact lens biomicroscopy for the diagnosis of clinically significant macular edema (CSME). A total of 207 eyes of 105 patients had complete data sets for both diagnostic modalities. The digital images were evaluated (at least) 2 months thereafter by a masked grader.

Special care was taken in digital imaging acquisition and storage. A 30° FOV Zeiss FF450 fundus camera equipped with a 'Kodak/Canon DCS560' digital camera, a 6-megapixel digital camera (image resolution of 3,040 × 2,008 pixels) were used. In addition, images were saved uncompressed (TIFF image file format), thus preserving the information captured in 17.4-megabyte image files. Images were later viewed using 3D viewing software on a computer monitor with a screen resolution of 1,024 × 768 pixels only. This fact was addressed by the authors in the discussion. They concluded that 'High-resolution stereoscopic digital photography is both sensitive and specific when identifying CSME and correlates well with the accepted standard of contact lens biomicroscopy for the diagnosis of CSME'.

Herbert et al. [51] compared the detection of DR in digital images with slit lamp biomicroscopy. Digital images of 45° FOV were obtained using a Topcon TRC NW5-S with an 800 × 600 pixel Sony camera. The images were saved as JPEG images compressed with '... loss of quality, in this system estimated at 10%'. The authors concluded that '... a single digital fundal image is insufficient for screening purposes'.

Sabti et al. [53] assessed the correlation between fundus digital image and clinical examination. In addition, they assessed the possibility of developing a screening program for the early detection of sight-threatening DR using a Canon CF 60 UV fundus camera. Fifty-one patients (92 eyes) were enrolled in this study. All patients underwent digital fundus photography of 30° and 60° FOV. The authors concluded that '... digital images provide an efficient method for diagnosing and classifying sight-threatening DR, particularly proliferative diabetic retinopathy (PDR)'. They also noted that '... agreement between the digital fundus camera and clinical examination by an ophthalmologist for diabetic maculopathy detection, though substantial statistically, was not very satisfactory'.

Pirbhai et al. [55] evaluated mydriatic nonstereo digital color fundus photographs as a screening tool for the identification and classification of exudative AMD. A total of 223 fundus images were obtained from 118 patients. Fundus photographs were taken at the time of fluorescein angiography with a Topcon TRC 50IX at a resolution of 1,024 × 1,024 pixels. There was no information on the sensor used. The authors stated in their conclusion that 'As a screening tool in exudative AMD, digital color fundus photographs were highly sensitive, specific, and showed high positive predictive and negative predictive value in confirming or excluding the presence of neovascularization'. In addition, 'Very few treatable lesions are missed using telemedicine in age-related macular degeneration'.

The conclusions of Chun et al. [58] differed from those of Saari et al. [29] and Lawrence [54]. Chun et al. aimed '... to evaluate a digital imaging system for diagnosing and grading diabetic retinopathy (DR) and cystoid macular edema (CME)'. To this end, an ophthalmologist practicing at a distance graded 231 nonmydriatic color fundus images from 120 patients (45° FOV); the results were compared with dilated ophthalmoscopy performed by a retinal specialist. The level of agreement was 'moderate' (κ = 0.44 and 0.60, respectively) for both DR and clini-

cally significant macular edema. The authors therefore concluded that 'A single 45°, nonmydriatic, digital image is not reliable as the sole modality for DR screening'. Nevertheless, they suggested that the 0.38-megapixel (800 × 480 pixels) low-resolution camera might be partially responsible for the moderate agreement, and that '... digital fundus image review may serve as a useful method to screen for DR in patients with limited access to an ophthalmologist'.

In a study performed by Lopez-Bastida et al. [59], 773 patients (1,546 eyes) diagnosed with type 1 or type 2 diabetes underwent screening for DR in a prospective observational study assessing the effectiveness of a nonmydriatic digital camera (45–30° FOV photographs) as compared to the reference method for screening DR. Digital images were obtained with a nonmydriatic digital retinal camera Topcon TRC-NW6S, but there was no information regarding image resolution. The authors considered '... digital retinal imaging with a non-mydriatic camera as an effective option in community-based screening programmes for diabetic retinopathy'.

As illustrated in this section, the use of digital imaging versus film or direct imaging techniques was not readily accepted for diagnosis and screening in ocular fundus imaging. However, recent work with larger data sets and at higher image resolution has shown that the feasibility of digital imaging is comparable to that of film or direct imaging techniques and offers more advantages in telemedicine and data storage.

It is noteworthy that, in contrast to anterior eye imagining, the effect of digital image resolution and compression was not studied for eye fundus imaging [61].

Retinal Image Processing and Analysis

In the above survey of digital versus nondigital imaging, we focused on the relationship between the two techniques and their potential impact on diagnosis according to several studies. In this section, we survey computer-aided detection (CAD) and associated procedures from image improvement (e.g. correction of nonuniform illumination) to structure segmentation (e.g. optic disk and vascular network) and grading (e.g. DR grading). Image- and information-processing techniques are required for the quantitative analysis of images in a CAD system [38], a concept that applies to different medical fields. One aspect, in particular, needs mentioning: the distinction between CAD and computer-aided diagnosis (CADx). CAD focuses on the detection and location of diseased areas while CADx focuses on diagnostic classification or disease recognition [62]. Unfortunately, most papers do not distinguish between them, and commonly use CAD for both meanings [e.g. ref. 63].

We have already mentioned that digital imaging enables easy acquisition and transmission to reading centers. These digital images also need to be analyzed for their ability to detect the presence of any signs of disease and classify a retina as healthy or diseased [35, 64]. In addition, for images of diseased retinas, further analysis may be required to grade the lesions according to disease stage or type.

The large number of images being currently acquired has the potential for overloading grading centers and increasing costs. Therefore, any process that may facilitate or automate grading tasks is of great interest. As stated by Abràmoff et al. [1], the main screening application focuses on the early detection of DR although screening programs exist for the detection of glaucoma and AMD, among others, and these screening programs generate a quite large number of digital images to deal with.

The large majority of systems rely on the identification of '... red or dark and yellow or bright lesions within the retina ...' [65]. On the other hand, venous caliber abnormalities, intraretinal microvascular abnormalities and retinal neovascularization are difficult to detect, and thus the detection rates are low [65].

The areas called 'computer processing and analysis of medical images' are quite broad and range from image acquisition and enhancement to compression and storage [66]. Again, the number of papers and the different approaches taken for each of these subjects prevent a full discussion of these topics here. Therefore, some representative papers have been selected to provide a global overview of this research area.

Automated Detection of DR

A computer-based image analysis and statistical classification was published by Ege et al. [67]. The authors described a tool for the automatic analysis of color fundus digital images of 50° FOV with a resolution of 640 × 480 pixels from a three-CCD sensor camera. Thereafter, the results were analyzed and classified by the authors, and their performance was compared by three different classifiers: a Bayesian, a Mahalanobis and a K nearest neighbor (KNN) classifier. Four abnormalities were considered: microaneurysms, hemorrhages, exudates and cotton wool spots, for which the Mahalanobis distance

classifier yielded the best results, with sensitivities of 69, 83, 99 and 80%, respectively.

A method for the detection of microaneurysms in 50° FOV red-free fundus images was presented by Hipwell et al. [68]. Two red-free images were obtained per eye using a Topcon TRC-50XT fundus camera, with an image resolution of 1,024 × 1,024 pixels and an 'approximate pixel resolution of 13 μm'. A sensitivity and specificity of 81 and 93%, respectively, were achieved in the detection of images containing microaneurysms.

Walter et al. [69] presented a new algorithm for the detection of exudates. They first located the optic disk and identified exudates on the green channel of the color fundus photograph. A sensitivity of 92.8% was achieved.

A quantitative index of diabetes was proposed by Cheng and Huang [70] based on the fractal dimension of the vascular distribution. The rationale was based on the fact that the fractal dimension of the retinal vascular distribution of patients with severe diabetes appears to be greater than that of a healthy retina.

Larsen et al. [71] evaluated the performance of the RetinaLyze System (Retinalyze A/S, Hørsholm, Denmark), a system intended for the automatic detection of red lesions in color fundus photographs. The system was able to correctly identify 90% of patients with retinopathy and 81% of patients without retinopathy.

Another work from the same group [72] demonstrated a specificity of 71% and a sensitivity of 96% in detecting DR in a larger set of images.

Usher et al. [73] proposed a tool for DR screening from digital color fundus photographs. 95% sensitivity was achieved, with 46% specificity in detecting any retinopathy. The software was able to identify microaneurysms, hemorrhages, exudates, drusen, and other (unspecified) lesions.

In a study by Lalonde et al. [74], the RetsoftPlus software was evaluated as a tool for retinal image analysis. This software was intended to be multipurpose; it included the detection of microaneurysms and exudates, among other functions, as well as image coregistration. The system achieved a sensitivity of 90% and a specificity of 75% in detecting microaneurysms and a sensitivity of 100% and a specificity of 87% in detecting exudates.

Li and Chutatape [75] used principal-component analysis to detect the optic disk in color fundus images of the human retina and a novel approach to detect exudates. In addition, the authors used a fundus coordinate system to provide a better description of features within the retinal images.

A method for the classification of red lesions (microaneurysms and hemorrhages) from color fundus photographs of the human retina was presented by Niemeijer et al. [76]. In this work, a KNN classifier was applied to classify lesions as red lesions, and the system achieved a sensitivity of 100% and a specificity of 87%. The computing time per image was about 15 min.

A fully automated approach for the detection and classification of changes in a time series of color fundus images was described by Narasimha-Iyer et al. [77]. (A survey on image change detection algorithms can be found in Radke et al. [78].) The authors applied a coregistration process to compare changes in the appearance of the eye fundus over time and a Bayesian detection and classification algorithm to classify the differences. The system achieved a performance of 99% correctly classified changes on a set of nonproliferative and proliferative DR fundus images.

Quellec et al. [79] proposed a new scheme for the detection of microaneurysms on color fundus photographs and fluorescein angiograms. The method is based on wavelet decomposition. The Haar wavelet provided the best results, with a sensitivity of 88% and a specificity of 96%.

In the work of Singalavanija et al. [80], a large series of images from healthy retinas and retinas diagnosed with DR was used to test a system capable of detecting the retinal vascular network, optic disk and fovea. In addition, the system was able to detect diabetic features such as exudates, hemorrhages, microaneurysms and cotton wool spots. The system achieved a sensitivity of 75% and a specificity of 83%, respectively.

Larsen et al. [81] presented a retrospective cross-sectional study using digitized 35-mm color fundus slides from a set of patients referred to a DR screening clinic for photocoagulation treatment. Two photographs were used: one centered on the fovea and one centered on the optic disk. The process automatically detected red and bright lesions following detection of the vascular network and the optic disk. The authors reported 100% sensitivity in detecting any abnormality.

An information fusion system for DR computer-aided detection/diagnosis was assessed by Niemeijer et al. [63]. While most systems focus on detecting a particular lesion type, the system studied by Niemeijer et al. aims at the integration of complementary detection systems. The authors concluded that a supervised fusion technique, either alone or associated with a 'likelihood distribution normalization (PPDN)', is 'superior over other fusion methods' for the type of lesions considered [63], with a

receiver operator characteristic area under the curve of 0.881.

Abràmoff et al. [82] compared the performance of automated DR detection using two different algorithms on a large scale (over 16,600 patient visits, two fundus images from each eye). In this work, the algorithm that won the 2009 Retinopathy Online Challenge Competition was compared with the algorithm in use, i.e. EyeCheck [82]. The retinas of patients were imaged with nonmydriatic digital retinal cameras (Topcon NW100, Topcon NW200, or Canon CR5-45NM) at 18 different centers. Different settings were used as well: 45° FOV with 640 × 480 pixels, 35° FOV with 768 × 576 pixels, 35° FOV with 1,792 × 1,184 pixels, and 35° FOV with 2,048 × 1,536 pixels. All images were JPEG compressed (at the minimum compression setting available) and were resampled to 640 × 640 pixels before processing. The results showed that '... further improvements in detection performance cannot be differentiated from best clinical practices, because the performance of competitive algorithm development now has reached the human intrareader variability limit'. It should be noted that these algorithms, as most of the ones intended for this sort of application, were optimized to recommend referral of patients with any form of DR to an ophthalmologist, hence the excellent performance achieved.

A prior study (2 years before) by the same group used the same dataset [83]. In that study, the authors had concluded that 'Automated detection of diabetic retinopathy using published algorithms cannot yet be recommended for clinical practice', demonstrating the fast pace in the progression of the techniques to automatically detect DR from eye fundus images.

Other noninvasive approaches to diagnose DR from fundus photographs were evaluated in another study [84], in which DR was detected by focusing on microaneurysms and exudates (counting and location) in color fundus images. The study was thus not tailored for ischemia.

In a recent paper, Quellec et al. [85] focused on the detection of lesions from retinal images, in particular in the detection of microaneurysms (the first sign of DR) and drusen (the hallmark of AMD). A set of optimal filters representing the typical lesions to be detected in addition to negative and positive lesion confounders was developed. Lesions were classified using a classifier (e.g. KNN classifier), whereby a performance similar to previous methods from the same group was achieved although much faster (less than 1 s per image) [85].

Table 2 summarizes the studies performed and included in this review. Of particular interest are the differences between the imaging modalities, the number of images per eye, and the differences in image resolution. Six studies have not disclosed the FOV.

Automated Identification of DR Stages

Cheng et al. [70] reported on a method for the classification of patients with diabetes into four groups of DR: normal, slight, medium and severe DR. In this study, 92 images were analyzed, from which 75 were used as training set. The authors relied on the fractal dimension of the retinal vascular network in addition to 'lacunarity', a parameter that describes the characteristics of fractals having the same dimension but different appearances. A set of classification schemes was analyzed, such as the backpropagation algorithm, the radial basis function network, the genetic algorithms and the combination of multiple classifiers on a voting scheme, which allowed similar results to be achieved.

Later, Lee et al. [86] assessed a system to detect hemorrhages and microaneurysms, hard exudates and cotton wool spots to classify retinas as mild, moderate and severe nonproliferative DR (NPDR). The system was designed for color fundus photographs taken at 45° FOV, two per eye, one centered on the fovea and one centered on the optic disk. Images were captured on film and were digitized to produce images of 512 × 512 pixels. Lee et al. proposed a classification according to the abnormalities detected: (1) no NPDR – no lesions detected; (2) questionable NPDR – lesions automatically detected are not definite; (3) early NPDR – at least one microaneurysm/hemorrhage but no hard exudates or cotton wool spots; (4) moderate NPDR – microaneurysms/hemorrhages and hard exudates or cotton wool spots present, and (5) severe NPDF – presence of 20 or more microaneurysms/hemorrhages in each 4 midperipheral quadrants.

In the work of Yun et al. [87], 124 retinal images were used: 29 from the normal group, 38 from the moderate group, 18 from the severe group and 39 from the proliferative group. Hemorrhages, microaneurysms and the retinal vascular network were segmented using image-processing techniques. A supervised learning technique was applied using the backpropagation algorithm for the training of artificial neural networks. The system achieved a percentage of correctly classified cases of 73% (moderate NPDR) to 100% (normal); the number of test

Table 2. Automated disease detection based on digital ocular fundus imaging

Study	Eyes (subjects), n	Imaging	Camera (sensor)	FOV	Resolution	Main features	Aux features	Classifiers
Ege et al. [67] 2000	– (–)	Digital 1× C	– (3CCD)	50°	640×480	Microaneurysms Hemorrhages/Exudates Cotton wool spots		Bayesian Mahalanobis KNN
Hipwell et al. [68] 2000	– (–)	Digital 2× RF		50°	1,024×1,024	Microaneurysms		
Walter et al. [69] 2002	30 (–)	Digital 1× C (green channel)	Topcon TRC 50IA (Sony 3CCD)	–	640×480	Exudates	OD	
Cheng and Huang [70] 2003	– (–)	Digitized FA		(1)	(2)	Vascular network		Backprojection algorithm Radial basis function network Genetic algorithm Voting scheme
Larsen et al. 2003 [71]	260 (137)	Digitized C		60°	(3)	Red lesions (microaneurysms and hemorrhages)		
Larsen et al. [72] 2003	400 (200)	Digitized C		45°	(4)	Red lesions (microaneurysms and hemorrhages)		
Usher et al. [73] 2004	– (1,273)	Digital 1× C	Topcon TRC-NW5S (Sony)	45°	570 × 570	Microaneurysms Hemorrhages/Exudates Drusen/*Other*	OD	Neural network
Lalonde et al. [74] 2004	46 (–)					Microaneurysms Exudates	OD Macula	
Li and Chutatape [75] 2004	89 (–)	1× C			512 × 512 (5)	Exudates	OD Fovea	
Niemeijer et al. [76] 2005	50+50 (–)	1× C	Topcon TRC-50 (Canon CR5 3CCD)	45°	768 × 576	Red lesions (microaneurysms and hemorrhages)		KNN
Narasimha-Iyer et al. [77] 2006	22 (–)	Digitized 2× C	Topcon TRC 50IA			Differences between image pairs	Vascular network OD, Fovea	Bayesian
Quellec et al. [79] 2006	995 (–)	C and FA			1,280 × 1,008	Microaneurysms		
Singalavanija et al. [80] 2006	600+300 (–)	1× C	Topcon TRC 50IA	50°	570 × 550	Microaneurysms Hemorrhages Exudates Cotton wool spots	Vascular network OD Fovea	
Larsen et al. [81] 2007	– (106)	Digitized 2× C	Canon CF-60UV	60°	(6)	Red lesions Bright lesions	Vascular network, OD	
Niemeijer et al. [63] 2009	15,000 (–)	Digital 4× C	(7)	(8)	(9)	Red lesions (microaneurysms and hemorrhages) Bright lesions (exudates, cotton wool spots and drusen)		KNN

C = Color; RF = red-free; FA = fluorescein angiography; OD = optic disk; (1) Apparent FOV of 50°. (2) FA digitized at 50–800 dpi. (3) Color film digitized at 1,350 dpi to achieve an image resolution of 1,947 × 1,296 pixels. (4) Color film digitized at 1,350 dpi to achieve an image resolution of 1,448 × 1,296 pixels. (5) Several image sources were used. All images were resized to 512 × 512 pixels. (6) Color film digitized at 1,350 dpi. (7) Three cameras used: Topcon NW 100, Topcon NW 200 and Canon CR5-45NM. (8) The FOV varied between 35° and 45°. (9) Image resolution ranged from 768 × 576 to 2,896 × 1,944 pixels.

cases was about one half the number for the training of artificial neural networks in each of the groups.

Acharya et al. [88] applied nonlinear features of the high-order spectra to identify the different stages of DR. Specifically, they proposed to identify mild nonproliferative retinopathy, moderate nonproliferative retinopathy, severe nonproliferative retinopathy and proliferative retinopathy, in addition to healthy cases. A set of 300 retinal photographs (60 photographs of each DR level and 60 photographs of healthy retinas) were acquired using a Zeiss fundus camera with an image resolution of 256 × 256 pixels. Images were corrected by histogram equalization and features were extracted thereafter. A support vector machine classification scheme was applied to classify each photograph into one of the five different groups using 40 images per group as a training set and the remaining 20 images per group as the test set. This processed scheme allowed for a correct classification of 82% of the cases (average; range: 75–90%).

Approaches using fluorescein angiography were also considered. For example, Reznicek et al. [89] characterized ischemic versus nonischemic retinas of DR patients in vivo.

Segmentation

The segmentation of structures is a fundamental step in retinal image processing and analysis, and thus the retinal vascular network is of paramount importance. First, any change in the vascular network indicates the onset or progression of retinal disorders. Second, important information is gained when associated findings such as microaneurysms are detected. In this way, the vascular network, optic disk, fovea, and microaneurysms are presented as specific subtopics of the segmented structure.

Segmentation refers to the process of identification within the fundus image of the respective structure along with its location and shape. Bartling et al. [90] manually identified the location of the center of the macula and four points on the optic disk border (defining the shortest and longest diameter) to convert length measurement from pixels to metric distance (e.g. pixels to micrometers) to correct for differences in magnification. Thereafter, they used this technique to measure optic disk parameters from digital fundus photographs.

The importance of the retinal vascular network is clearly expressed in the work of Liew et al. [91] as the link between '... a range of retinal microvascular signs and both clinical and subclinical cerebrovascular, cardiovascular, and metabolic outcomes'.

Similarly, Lin et al. [92] refer to the possibility of early diagnosis of cardiovascular diseases based on measurements of arterial vascular trees in the retina; the narrowing of the arterial blood vessels in the retina is an indicator of hypertension and atherosclerosis.

The problem of classifying retinal vessels into veins and arteries following retinal vessel segmentation was addressed by Rothaus et al. [93] with a semi-automated process to propagate a user classification via a vascular graph.

Vickerman et al. [94] proposed a method to quantify vessel diameter, vessel density, vessel branch point density, vessel length density and vessel area density using fluorescein angiography and a semi-automated process to compute arterial and venous trees, they demonstrated that several factors (e.g. vascular endothelial growth factor 165) induce changes in the vascular pattern that are important for the identification of the dominant molecular signaling. The application of this method to the investigation of branching patterns of the arterial and venous trees during the progression of DR can be found in the work of Parsons-Wingerter et al. [95].

Along the lines of extracting information from the retinal vascular network, the measurement of vessel width within the human retina was addressed in the work of Xu et al. [96] using a graph-based method. Fractal analysis is used as well for analyzing the retinal vascular network [97–99].

Moreover, segmentation of the vascular network has applications in retinal montage and tracking [101–102] although other approaches, not explicitly resorting to vessels can be used, as shown, for instance, by Meijering et al. [103].

Vascular Network

Bartsch et al. [104] reported on a method to compute 3D information on blood vessels in the living human eye. Simultaneous fluorescein and indocyanine green angiographies were performed. The former method was used to visualize the retinal circulation, while the latter method allowed visualization of the retina and choroidal vessels in the posterior pole. A prototype CSLO (Heidelberg Retina Angiograph) was used in this study to obtain an image resolution of 256 × 256 pixels. Blind deconvolution was applied to the set of confocal images. Though desirable, no real 3D vascular reconstruction was achieved

although a clear improvement on the axial resolution of the system was obtained.

To determine vessel and nonvessel regions along a vessel profile, a fuzzy C-means clustering algorithm has been applied [105]. According to the authors, the advantages of this approach were a virtually parameter-free process, the lack of an explicit model for the morphology of the vessels, and no use of edge information.

A real-time system able to cope with resolution images of 1,024 × 1,024 pixels at a frame rate of 30 images per second was proposed by Can et al. [106]. The core tracing algorithm was based on a set of six 2D correlation kernels. Seed points were automatically selected.

Another real-time system described by Solouma et al. [107] was intended for real-time systems based on image resolutions of 640 × 480 pixels from 50° FOV. Detection of vessel boundaries was achieved using deformable models. Disclosed performance results were in the order of 300 ms of processing time.

A review on vessel extraction techniques by Kirbas and Quek [108] provided a thorough overview of the complexity of the task. These authors considered six main categories: '(1) pattern recognition techniques, (2) model-based approaches, (3) tracking-based approaches, (4) artificial intelligence-based approaches, (5) neural network-based approaches, and (6) miscellaneous tube-like object detection approaches'. Pattern recognition algorithms were further divided into seven subcategories, while model-based approaches were divided into four subcategories. Even though many techniques were presented, the authors concluded that this was still a potential area for additional research.

Jelinek et al. [109] characterized vessels in the vicinity of the optic disk from color fundus photographs. Blood vessels were therefore tracked from the optic disk boundary using the 2D fitting of a '... physically inspired model to a local region of a vessel'. The segmented vascular network was thereafter classified into arteries and veins based on color and hue by means of a classification algorithm.

Anzalone et al. [110] investigated a segmentation process suited for implementation on a digital signal processor. This two-step approach was applied to red-free fundus images (green channel of the red-green-blue color image). The first step was devoted to vessel enhancement and the second step produced a binary image based on thresholding procedures. The system was able to process 10 images of 400 × 400 pixels per second.

Although a rich body of literature exists on the extraction of tubular structures in medical images, little focus has been given to the delineation of the vascular network as a whole, including its tree structure [92]. To address this problem, Lin et al. improved on a previous vessel segmentation algorithm and grouped extracted vessel segments based on a Kalman filter to ensure their continuity.

A supervised classification process for the segmentation of retinal vessels from fluorescein angiograms was proposed by Vargas and Liatsis [111. This approach is '... based on the eigenvalue decomposition of the Hessian matrix and Fisher's linear discriminant analysis'.

Xu et al. [96] described a method to segment both vessel edges simultaneously using a graph-based approach. An initial vascular network was required to build the graph.

Another supervised method for blood vessel detection was proposed [112] using a neural network to classify pixels from color fundus images of DRIVE [113] and STARE [114] public databases.

Several authors have suggested various methodologies to achieve the segmentation of the vascular network from fundus images. In a brief overview of gradient-based methods, Lam and Yan [115] considered the divergence of vector fields of the image, while other approaches used the gradient co-occurrence matrix [116], the Hessian matrix and clustering algorithms [117], or curvature-based methods [118]. Apart from gradient-based algorithms, several other techniques have been suggested: the use of appropriate wavelet transforms [119, 120], contourlets [121] or filters [122], region-growing methods [123] or deformable contours [124].

Furthermore, recently published work has distinguished arteries from veins. This is important, as it is expected that DR affects arteries and veins differently, depending on disease stage [125]. A Bayesian classifier was proposed [126], whereby the arteries, the veins, the fovea and the retinal background were identified. In other studies [127, 128], a local approach around the optic disk was considered using color, contrast and anatomical features. Li et al. [129] applied a piecewise Gaussian model especially adapted to the central reflex of the vessels as a filter on the image for the classification of retinal vessels. Azegrouz and Trucco [130] presented an automated tracking technique of the central retinal vein in retinal images. In another study, two-feature extraction and two-classification methods were compared to discriminate between arteries and veins based on support vector machines and neural networks [131]. Rothaus et al. [93] also introduced a method that uses a presegmented vascular structure and propagates a user classification based on the vascular graph to classify the entire structure.

Moreover, Muramatsu et al. [132] developed a set of computerized methods for the segmentation of retinal blood vessels to identify major vessel segments and classify them into arteries and veins.

Optic Disk

Two other retinal structures, the optic disk and the fovea, have demonstrated changes [133] in the eye fundus, and several research groups have concentrated on locating the optic disk within eye fundus images. It was noted that two types of 'segmentations' could be found: segmentations that only determine the location of the optic disk, e.g. optic disk center or enclosing circle or ellipse, and segmentations that compute the boundaries of the optic disk.

Walter et al. [69] used the local gray level variation to locate the optic disk and the watershed transformation to find its boundaries.

Foracchia et al. [134] described a method that is based on the fact that major retinal vessels converge at the optic disk, which thus controls the blood supply to the human retina. They proposed a geometrical parametric model based on previously segmented retinal vascular networks. Because it does not specifically search for the optic disk within the image, this process was able to identify the location of the optic disk even if it is outside of the FOV.

Other authors [135] relied on the appearance of the optic disk to identify its location within the ocular fundus image, '... usually appearing as a bright, approximately circular region intersected by blood vessels' although these authors used the Hough transform, a well-known image processing technique, to identify circular forms within an image, following gradient determination using the Sobel differential operator. The optic disk search area was previously restricted to the area of confluence of major retinal vessels, similar to the approach used by Foracchia et al. [134]. Fleming et al. [135] also provided an extensive description of prior approaches in their report.

A similar (although simplified) approach was taken by Sekhar et al. [136] using the Hough transform following morphological operations within the fundus image.

Youssif et al. [137] proposed to locate the optic disk '... based on matching the expected directional pattern of the retinal blood vessels'. Vessels were initially segmented, therefore providing vessel orientation in a method similar to that used by Foracchia et al. [134]. The optic disk was then located by the direction of the optic disk-matched filter (model).

In addition to locating the optic disk, Welfer et al. [138] determined optic disk boundaries. The initial location (region of interest) was based on the main vessel arcade, as in other reports cited here. In addition, a particular approach was used to place the main vessel arcade outside of the retinal vascular network that was initially segmented. Following optic disk segmentation, its boundaries were computed based on morphological mathematical operators and the watershed transformation.

Lu and Lim [139] applied a different approach to locate the optic disk based on its bright appearance in color fundus photographs. Using a set of concentric lines with different directions, they evaluated the image variation along the multiple directions. The optic disk was thereafter located using the orientation of the line segment with the maximum or minimum variation. This approach has the major advantage of not requiring the retinal vascular network to be segmented.

Segmentation of optic disk boundaries based on active contours was used in the work of Marrugo and Millán [140] following optic disk location based on 'color mathematical morphology'.

Fovea

The fovea is a key feature of the ocular fundus. Any changes in the appearance of the fundus gain extra importance if they occur close to the fovea, where the photoreceptors crucial to central vision are located. This importance is clearly demonstrated in the definition of clinically significant macular edema, whose classification is based on the distance between the center of the fovea and the region of macular edema.

Because the fovea is very difficult to identify within the ocular fundus image using an automated system, the related body of research is smaller than for vascular network and optic disk segmentation. The exact center of the fovea is difficult to identify on color fundus photographs even for a human grader. The task becomes easier on high-definition fluorescein angiograms, which show the foveal avascular zone.

Ibañez and Simó [141] applied Bayesian statistical methods to identify the location of the fovea on fluorescein angiograms. In addition, the contour of the fovea was modeled using a unidimensional Markov chain. Two algorithms were used to estimate the contour of the fovea: simulated annealing and iterated conditional-mode algorithms. This procedure was later applied by Simó and de Ves [126].

Fleming et al. [135] searched for the fovea using a template. By computing the correlation coefficient between the image and the template in a region of interest based on the location of the optic disk and the major arcades, they could reduce the number of potential false-positive results. In addition, this region was also delimited based on the distance to the center of the fovea and on the dimension of the optic disk previously estimated for the same image.

Microaneurysms

Microaneurysms are the first visible sign of DR and thus are important features that can be identified in fluorescein angiograms (mostly) or color fundus photographs (more recently) using automated methods.

Spencer et al. [142] used digitized fluorescein angiograms to develop a procedure for the automated detection of microaneurysms using a set of matched filters. The same research group improved their initial approach [143] by applying a region-growing algorithm to delineate each candidate microaneurysm, followed by the analysis of size, shape and energy characteristics of each candidate lesion.

A similar approach was proposed by Mendonça et al. [144]. Fluorescein angiograms were preprocessed and enhanced, and objects were then segmented. Final microaneurysms were validated based on local intensity, contrast and shape.

Hipwell et al. [68] used red-free images. They preprocessed digital ocular fundus images to correct intensity variations in the background and enhance small round features. The use of red-free images, a noninvasive imaging modality, represents a major step towards the noninvasive assessment of the eye fundus.

Similarly, microaneurysms were detected in color fundus images of the human retina by Niemeijer et al. [76, 145]: this was the first step towards automated screening for DR. In their earlier study [76], they referred to "red lesions", including microaneurysms and hemorrhages; later on [145], they distinguished them by size: 'This method allowed for the detection of larger 'red lesions' (i.e. hemorrhages) in addition to the microaneurysms using the same system'.

The importance of identifying microaneurysms for the correct assessment of DR and its progression was stressed by Bernardes et al. [146] and Nunes et al. [147].

Retinal Analysis: Global Importance

It has been suggested that information on the eye fundus is important in a variety of diseases, including heart diseases and stroke, hypertension, peripheral vascular disease and DR [148]. Based on the studies of Can et al. [106] and Catros and Mischler [149], Al-Diri et al. [148] devised a 'junction resolution algorithm' that forms a retinal vascular graph from previously segmented retinal vessels, therefore resolving the connectivity of the vascular network. This connectivity was not fully solved using earlier segmentation algorithms of retinal vascular networks. Using self-organizing feature map, the algorithm assigned segment ends to local sets based on position and alignment, choosing the most plausible solution in terms of geometry.

Other Imaging Modalities

Other digital imaging modalities of special interest to ophthalmology (see Acharya et al. [7] and Alabboud et al. [8]) include fluorescein angiography [150], indocyanine green angiography, fundus autofluorescence and multifocal electroretinography [151].

Fluorescein angiography is especially useful in the management of DR and macular degeneration as it provides information on the retinal circulation and on the status of the blood-retinal barrier. Developments based on high-speed confocal scanning laser ophthalmoscopy brought new insights into the onset and progression of DR from its earliest to its most advanced stages [152–158].

In this imaging modality, sodium fluorescein is intravenously administered to the patient, and the passage of fluorescein is registered throughout the retinal circulation.

In contrast to fundus photography, fluorescein angiography is not based on the reflection of light from the eye fundus. Instead, a light beam of appropriate wavelength is used to excite sodium fluorescein molecules that emit light at a longer wavelength. The peaks wavelengths of absorption and emission are 490 and 520 nm [150] or 490 and 510 nm [159], respectively.

Similarly, indocyanine green angiography [103, 160–164] requires the administration of a dye, but it is tailored for imaging the choroidal circulation because of the wavelength used. With peak wavelengths of absorption and emission in the near-infrared (805 and 835 nm, respectively), indocyanine green angiography allows greater transmission through the retinal pigment epithelium

and blood than the visible wavelength used in fluorescein angiography or color fundus photography [2].

Fundus autofluorescence [165–169] is similar to fluorescein angiography but does not require administration of a dye. It enables accumulation of lipofuscin, which is produced by the oxidation of unsaturated fatty acids [21] and can then be imaged in the retinal pigment epithelium. Although the peak wavelengths of absorption and emission are slightly different from those of sodium fluorescein, the same set of filters can be applied.

Each of these techniques can take advantage of SLO and CSLO techniques [170–173]. Instead of illuminating and imaging the entire area simultaneously, the SLO technique illuminates only a spot in the eye fundus. The final image is then assembled from point images taken sequentially. By adding confocality, the CSLO system allows the performance of SLO at different depths to obtain volumetric information from the ocular fundus.

All these imaging modalities are complementary to ocular fundus photography as they gather different information. However, as stressed in this review, compared with other imaging modalities, digital ocular fundus photography has the unique potential of becoming a prominent and widespread screening tool of ocular diseases (in particular DR), with additional advantages for data storage. Its digital format also represents an advantage for telemedicine and the application of automated processing for disease detection and staging.

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