Quantifying Phenotypic Traits in Retinal Coronary Angiography: Automated Extraction of Retinal Vascular Networks and Localization of Optic Discs in Fundus Images

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***Abstract*—Numerous retinopathies are related to the dysfunction of retinal vasculature, especially micro-vessels. Extensive research in ophthalmology has singled out critical roles of vascular morphology, and the functional dynamics of blood flow in diseases. Advances in angiography has yielded a myriad of applications for computational methods that design efficient tools to complement retinal imaging and microscopy in analytic ophthalmology. In this paper, we propose a novel mathematical approach for the design of quantitative tools that enable researchers, as well as automated vision-based systems, to perform pattern recognition, and feature extraction in retinal vasculature. The present feasibility-stage implementation of these new algorithms demonstrates the power and versatility of the set of tools we provide for the detection of morphological pathology, as well as the theoretical study of retinal neurovasculature anatomy when regarded as a complex (dynamic) system. In contrast to current state-of-the-art methods that rely on bottom-up algorithms to deal with noise and trace the vessels, we propose a top-down scheme to overcome noise and capture morphological features such as center-lines, radii, and the edge locations of circulatory blood vessels. This approach is comprised of three components. First, the algorithms for detection and measurement of the vasculature morphological structures in two-dimensional fundus images are implemented. These algorithms combine advanced kernel-based methods to extract blood vessels, and are further enhanced by variants of Canny Edge Detection algorithms. Second, a fully automated approach is provided to identify the optic disc in healthy/diseased fundus images, eliminating current bottle-necks requiring extensive human expertise. Third, we construct a hierarchical network of geometric (topological) structures of the extracted vessels, rooted in the optic disc. A notable application of our methods is to capture complex vasculature structures in noisy, blurred, and light-reflecting fundus images. Another advantage of our approach is the automation of *in vivo* quantification of complex phenotypic traits of retinal neurovasculature, which are expected to play an important role in emerging computational models for mapping genotype-phenotype relations and personalized medicine.**

I. Introduction

Analysis and quantifying medical images forms an essential step in delineating practical issues in relation to the diagnoses of systems. Extracting appropriate features to represent the content and structure of an image by precisely capturing anatomical and pathological features of the retinal tissue is the goal of quantifying fundus images. Segmentation of blood vessels and quantifying phenotypic traits, such as width, length, and distinguishing between regions of lesions, plays an important role in the diagnosis of vasculitis, malformations, vein occlusion [12], exudates, diabetes [19], glaucoma [13], and many other retinal diseases exhibiting a vascular phenotype.

Currently, almost every medical imaging technique (ultrasound, X-ray, MRI, CT, etc...) can be used to capture high resolution, two- or three-dimensional, blood vessel images. However, the complexity of the vasculature structure, unavoidable noise in the system, and faded images, challenges scientists to come up with precise, efficient, and practical approaches.

Decades of intensive research have brought a vast array of tools and methods. Comprehensive reviews and comparison of many of these accomplishments are mentioned [2], [3]. Li Wang [3] recently proposed a multi-resolution, Hermite polynomial-based model to analyze two-dimensional images, and construct a tree-type data structure of the blood vessels.

Using fuzzy methods is currently in vogue due to its ability to achieve noise removal and ease of enhancement in combination with other probabilistic methods [4], [6]. Statistical and kernel-based methods have also been proposed to overcome uncertainty in images [7], [9]. Additionally, template matching approaches have been examined [8], [10], [11]. Nonetheless, greater advancements are needed to handle unaddressed patterns of noise, reflection of light, and complex structural arrangements in images. Extensive variation in vessel width (especially in the case of arterial stenosis and aneurysms) remains an obstacle for quantifying phenotypic traits. Experts tend to have subjective variability in their identification of subtle features, creating an urgency for the ability of automated

methods to quantify phenotypic traits. Our own research [1], high throughput *in vivo* phenotyping, is needed to collect the time-series that encodes the dynamic variation of morphologies, and can predict the onset of angiogenesis in diabetic and other high-risk patients.

In this paper, we propose a new top-down method in which a kernel-based method was used to project the images to a higher-dimensional space. Using this projection, we avoid dealing with lesions, various types of noise, and reflected light. Thereafter, we applied a local to global model to extract the edges of the vessels and their bifurcated segments. A Canny-type algorithm was applied to fill the gaps along the longitudinal vessel, while closeness and varying widths of vessels were considered. Through application of the Canny based edge detection algorithm, we constructed a multi-resolution topological structure from the vessels. Whereas the blood vessels originate from the optic disc [5], we use this topological structure to identify it. In addition to the geometrical correlation between blood vessels and the optic disc, the impact of the density of blood vessels to measure the size of the optic disc and fovea has already been demonstrated [19].

In the next section, we describe our methodology and algorithms in detail. In the *Identifying the Optic Disc* section, we evaluate our algorithms by comparing them with a current standard.

II. Methodology

Measuring morphological traits of retinal blood vessels plays an important role in the screening of numerous ocular diseases. The mysterious structure of the retinal blood vessels has motivated researchers to study this topic from a computational point of view. The identification of fractals as the mathematical structure underlying vasculature has opened new branches of research [9], [10]. Utilizing fractals as a data structure for storing vessels, to study their distribution, has been examined and yielded promising results [11], [12], [13]. In this study, the method for storing vessels and finding the density of their distribution (i.e. locating the optic disc) is inspired by their fractal structure. The first part of this section explains the algorithm for extracting the vessels and generating the hierarchal structure rooted in the optic disc. The second illustrates the ability of the hierarchical structure to identify the optic disc.

A. Vasculature Structure

Using statistical learning and kernel machines in data mining is a well known approach. Vapnik introduced a new branch of data clustering approaches; whereby applying kernel machines, complex data structures could be clustered [16], [17]; 'Foundation of Analysis'. In this work we used kernel methods to project complex data objects to higher-dimensional spaces in order to efficiently distinguish meaningful information (pixels) from noise [14], [15].

1) Kernel Mapping: A preliminary step towards extracting the vasculature structures from the fundus images is to increase their respective pixel contrast. Mapping the images, via a kernel, allows us to individually project the color intensities of the images' respective pixels to higher and lower levels of saturation, providing greater separation of pixel values effectively "sharpening" the images. Figure 1 compares an original and kernel-mapped fundus image.

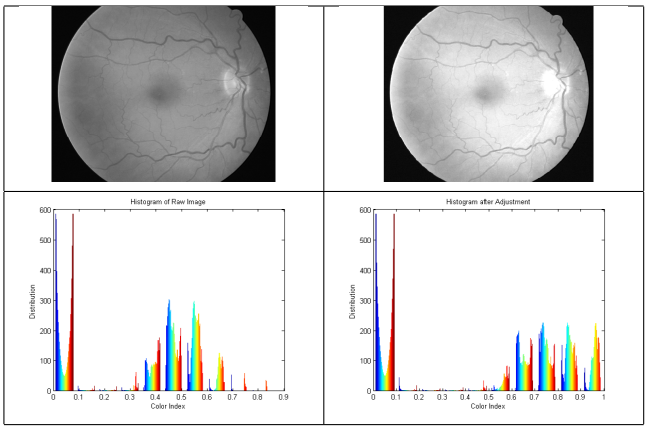

Fig. 1. The first column shows the original fundus image with its associated color intensity histogram. The second column shows the image and its histogram after adjusting the color indexes. As shown in the histograms, the range of color indexes (x-axis) and their intensities (y-axis) provides a quantifiable difference between the original and adjusted image.

2) Canny Edge Detection: Canny Edge Detection involves pre-filtering the image through convolution with a simple Gaussian filter to eliminate noise that might otherwise interfere with the edge detection process. Selection of a small, versus a large, filter window directly affects the observable and statistical smoothing applied to the image, and helps to reduce unavoidable noise from image acquisition. After smoothing, standard kernels G_x and G_y are applied in both the x and y directions of the image to determine edges by calculation of the image's gradient $|G|$.

$$G_x = \begin{pmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{pmatrix}$$

$$G_y = \begin{pmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{pmatrix}$$

$$|G| = \sqrt{G_x^2 + G_y^2}$$

$$\theta = \arctan(\frac{|G_y|}{|G_x|})$$

The detection of the edges after applying the 3 x 3 kernels to each pixel is determined by the angle θ and stored for comparison to determine the "strong" versus "weak" edges of the image, as specified by a double threshold intrinsic to the image based on maximum and minimum pixel values.
For example:

1) Round the θ value to the nearest multiple of 45 degrees, corresponding to the directional choices for the pixel's eight adjacent pixels. (0 = right, 45 degrees = upper-right, 90 degrees = upper-center, 135 = upper-left, 180 = left, etc. . .)
2) Compare the gradient value for each pixel based on the positive and negative θ value to obtain the next piece of the edge based on the gradient thresholding values.
3) If the pixel under examination, relative to its eight adjacent pixels, is largest within this threshold it is preserved as a "strong" edge. If it is "weak", as long as it is connected to a "strong" edge it is preserved. If it is neither of these, it is marked for removal.

The Canny algorithm applies a double threshold to label edges corresponding to "strong" and "weak", by referencing the value of the gradient as described in (3). It is these thresholds which ultimately determine edges detected as "strong" (i.e. pixels in the neighborhood described in (1) referring to the pixel of interest's gradient value) or "weak". Figure 2 shows the results of the edge detection algorithm with different threshold values.

3) Dilation: Dilation is a set operation performed over a discrete neighborhood of size n. The structuring element can be thought of as a geometric shape that overlaps and extracts the maximum pixel value lying within its boundaries; performing this operation iteratively pixel by pixel and replacing the pixel of interest with the maximum pixel value within the neighborhood.

Dilating the detected edges by $n = 1$ extends them towards filling the vessels and segmenting the vasculature structures from surrounding regions. Figure 3 shows an example of dilated edges in comparison with the original image.

B. Identifying the Optic Disc

Despite the optic disc being located in the observably blind region of the eye, known as "the blind spot", studying discs is important for diagnosing vascular disorders. The optic disc is also the gateway between the nervous and visual systems [18]. Since all blood vessels are directed towards the optic disc, extracting the geometrical distribution of the vessels is the first step towards the optic disk's location [5]. Differences in color indexes of an optic disc, relative to its surrounding regions in fundus images, has motivated researchers to develop color-index image analysis tools. However, since there is a distinct similarity between color indexes of the optic disc and exudative lesions, these color-index image analysis tools are not appropriate for quantifying affected images. In this study, we used a geometric-based algorithm to define a feasible region for the location of the optic disc and to exclude exudate regions from it. Moreover, we enhanced this algorithm, where appropriate, with image analysis tools to improve its accuracy.

Figure 4 shows the color indexes of the two-dimensional images. These graphs show the differences between the color distributions in the optic discs relative to their surrounding areas. Notice that the gradient of the surface around the optic disc is zero. To further emphasize the importance of analyzing color indexes, consider the contour plots of these images in Figure 5.

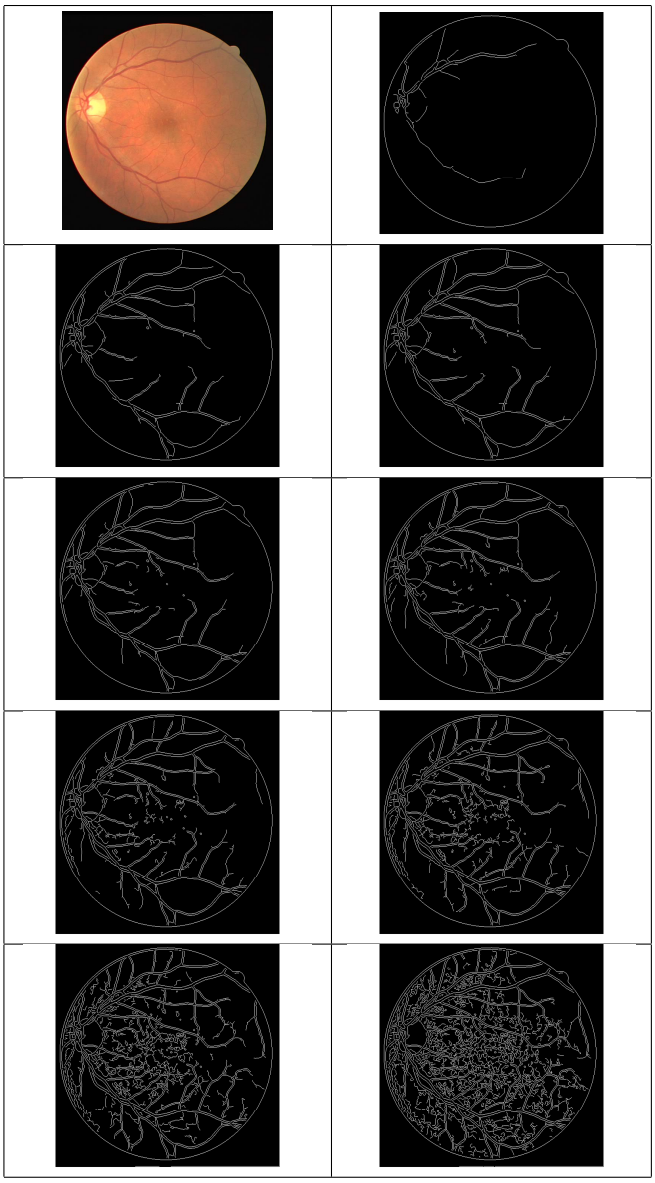

Fig. 2. Multi-resolution of vessel edges: the Canny Edge Detection algorithm with different threshold values. The threshold values decrease generally, and with respect to each other (i.e. upper versus lower threshold), left to right, and top to bottom.

Previously, some healthy fundus images were considered. Next, let us consider some pathological subjects. Figure 6 shows four examples of affected fundus images and their respective color intensity surfaces. As depicted by the color intensity surfaces (second column), identifying the optic disc based on the analysis of color indexes is insufficient. However, analyzing the multi-resolution structure of the blood vessels provides satisfactory results. The results of the analysis of the multi-resolution structures are shown in the first column of Figure 7, where in the second column one can observe a strong correlation between these structures and the location of

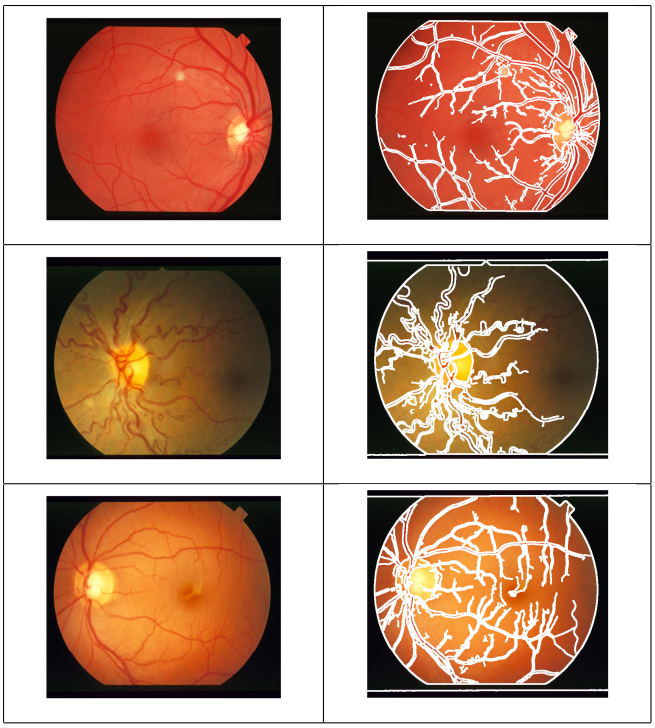

Fig. 3. The detected edges were dilated pixel by pixel over a neighborhood of radius $n = 1$.

the optic disc.

To evaluate our methodology, we applied these algorithms to two datasets: STructured Analysis of the Retina (STARE) [20], and Digital Retinal Images for Vessel Extraction (DRIVE) [21]. These datasets included fundus images of both healthy and pathological subjects. We compared the results of our algorithm with the manually extracted vessels provided by Adam Hoover [22]. As mentioned previously, instead of tracking vessels in our algorithm, the automated extraction and measurement of morphological traits is emphasized.

A direct comparison of automated and segmented vasculature structures to manually extracted vessels is shown in [23]. Moreover, some of the fundus images show different levels of hierarchical vasculature structure. To evaluate the second part of our methodology, we compared it to two well-known applications for identifying optic discs [5], [6]. The results from the application of our algorithm on the two datasets are available at [23].

CONCLUSION

In this paper, we have presented a novel computational method to quantify retinal blood vessels and identify optic discs in two-dimensional fundus images. This methodology consists of a kernel-based algorithm to extract vasculature structures. Taking advantage of the Canny Edge Detection algorithm, our methodology constructs a hierarchical structure of the blood vessels. This algorithm accurately quantifies vessel structure, length, and can capture dynamics in width by precisely detecting the edges of vessels. Moreover, analyzing

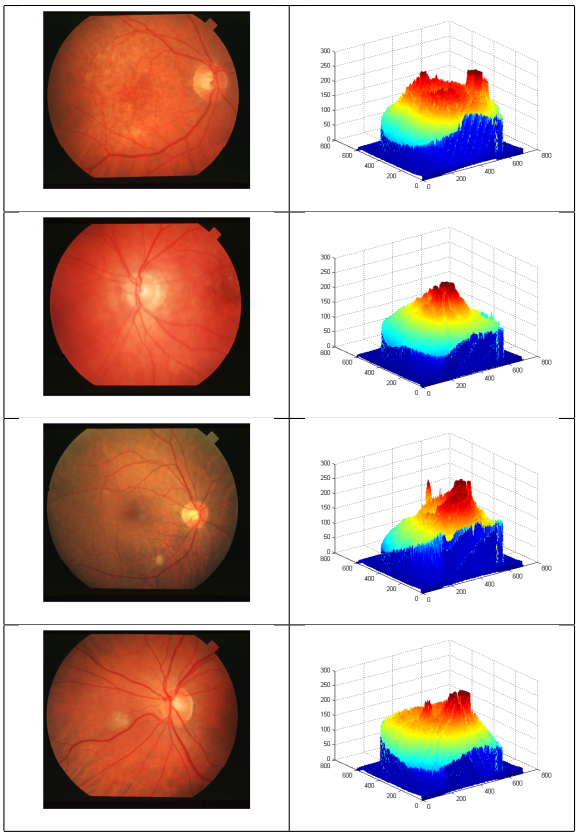

Fig. 4. The x and y-axis form the xy-plane of the image. The z-axis shows the color indexes of the image corresponding to its xy-coordinate. The optic disc is the flat region on the surface and differentiation of the color densities in these regions are zero in contrast with other parts of the image. This is an important feature to identify the optic disc.

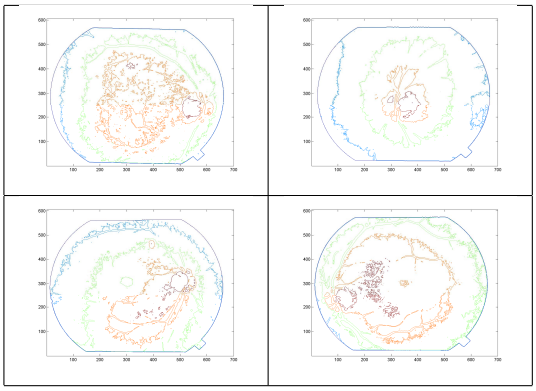

Fig. 5. These are the contour plots of the fundus images, left to right and top to bottom correspond to the images in Figure 4. The optic disc regions have the highest color indexes and are indicated by dark red. Other structures have lower color indexes, indicated by their colder colors relative to the optic disk.

this hierarchical structure, and appropriately using standard image analysis tools, we can identify the optic disc. In this manner, we have used two important biological features of angiography to detect the optic disc: the intrinsic geometry of the optic disc with respect to the vessels; and the differences of color indexes of the optic disc relative to its surrounding regions. This methodology precisely distinguishes between

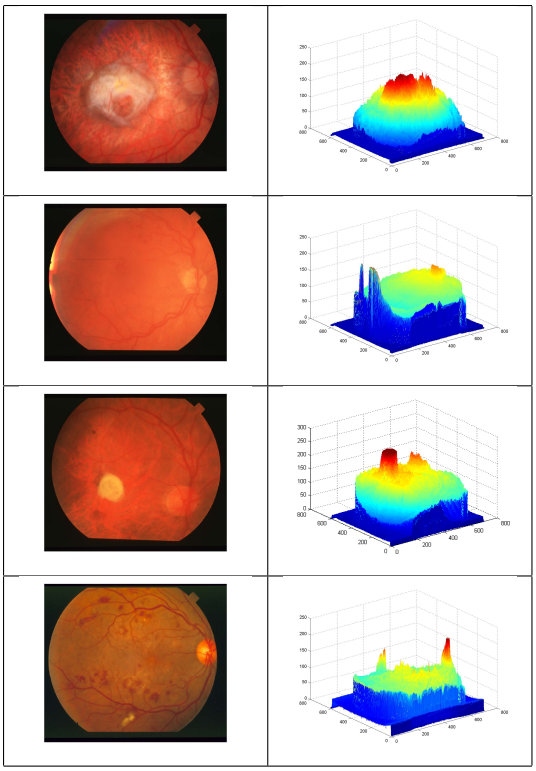

Fig. 6. These images are examples of affected retinal fundus angiography from the STARE datasets.

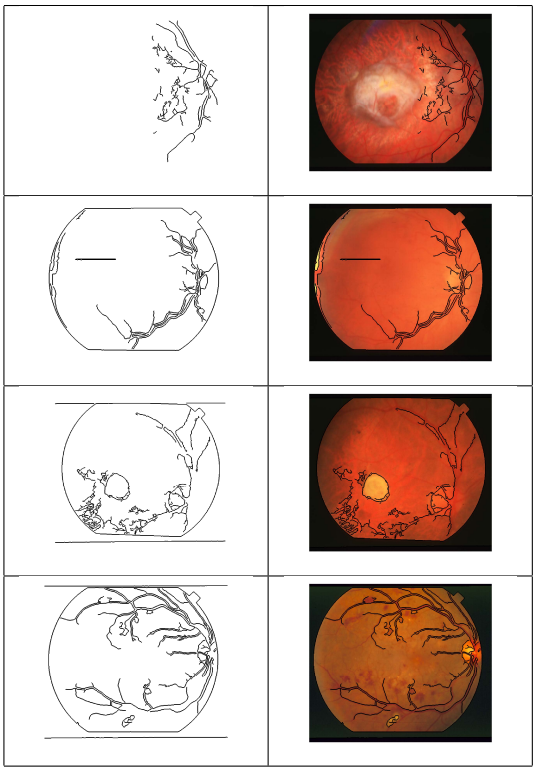

Fig. 7. These images are examples of affected retinal fundus angiography from the STARE datasets. The third image illustrates the necessity of using the geometrical and color-intensity methods together. In Figure 6, the surface of this image has two peaks, one corresponding to the lesion and the other to the optic disc. The intensity peak of the lesion is much higher than that of the optic disk. However, the density of the vasculature structure surrounding the optic disc is much greater than its density surrounding the lesion. Observably, there are no blood vessels inside the lesion. Hence, through the combination of these two observables, it is possible to accurately locate the optic disc.

closely located vessels and their longitudinal gaps. It also separates the lesion regions from the optic disc. We evaluated our methodology on two datasets, where the results showed its robustness and accuracy on normal and pathological retinal fundus images. Efficiency and precision of the algorithms are demonstrated by comparing our results with manually segmented fundus images [22] and comparing them with current well-known algorithms for identifying optic discs [5], [6].

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