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Detection of familial adenomatous polyposis with orthogonal polarized spectroscopy of the oral mucosa vasculature

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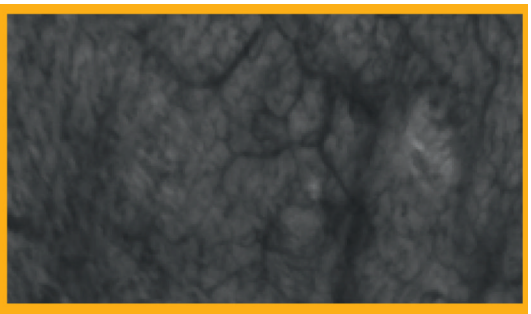
Abstract



Typical image of the oral mucosa.

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disease characterized by the development of multiple colonic polyps at younger age with a near 100% lifetime risk of colorectal cancer. The determination of FAP is made after extensive clinical evaluation and genetic testing of at risk individuals. We investigated a novel spectro-polarimetric imaging system capable of capturing high-resolution images of the oral mucosa at different wavelengths in an attempt to distinguish patients with FAP from controls. Results of a clinical trial show that the system is capable of separating FAP positive individuals from controls by measuring the individuals' oral vascular density and complexity.

Keywords

colon cancer; oral mucosa; reflectance; vessel density

1. Introduction

An inherited predisposition is one of the most important risk factors for colorectal cancer (CRC) and is implicated in 20 to 30% of all cases [1, 2]. One of the most common inheritable colorectal cancer syndromes is familial adenomatous polyposis (FAP). FAP is caused by a germline mutation of the APC (Adenomatous Polyposis Coli) gene, conferring a multiplicity of adenomas at younger age and a near 100% risk of colorectal cancer by the sixth decade of life if a preventative colectomy is not performed. Presently, the recognition

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of the majority of individuals at increased risk for inherited forms of CRC occurs only after evaluation of family history revealing multiple generations with CRC and other tumours, which usually occurs at the time of diagnosis of CRC in the proband. Currently, there are no definitive phenotypic markers for many of the inherited forms of CRC to identify high-risk individual presymptomatically although several studies have shown that these individuals often develop benign soft tissue and bony tumors, desmoid tumors, extraintestinal cancers, and [3] hypertrophy of the retinal pigment epithelium [4].

Furthermore presymptomatic genetic testing is expensive and not entirely conclusive, as approximately 20% of individuals with apparent familial CRC have no detectable mutation [5], in this paper these individuals will be classified as No Mutation Found (NMF).

Light reflectance spectroscopy was utilized to analyze vascular abnormalities and vessel structure in the oral mucosa of patients with another form of hereditary colorectal cancer, Hereditary nonpolyposis colorectal cancer (HNPCC) [6]. Utilizing this technique, investigators concluded that there was a measurable difference in the light reflectance patterns from the oral mucosal tissues of HNPCC patients compared to controls, with reflectance values in the 590–700 nm wavelength range significantly lower for individuals with HNPCC. However, Carrara et al. [7] performed a similar clinical test and showed that there was no considerable difference in oral mucosal reflectance between HNPCC carriers and controls. In a different study De Felice et al. [8] showed that increased oral vascular network complexity was related to gene mutation carrier status and appeared to be a consistent phenotypic marker for HNPCC.

The analysis of the geometrical characteristics of micro-vascular networks of the oral mucosa was successfully applied to other hereditary conditions such as Ehlers-Danlos syndrome, [9], Down syndrome [10], and achondroplasia [11, 12], to name a few.

The main goal of this study was to investigate an inexpensive complement and potential alternative to genetic testing by imaging oral mucosa vascular reflectance (OMR) and oral mucosa vascular density and complexity (OMVD).

2. Materials and methods

In order to provide accurate imaging and reflectance analysis of vessels inside the lower lip of test subjects, a device was assembled consisting of a scientific camera, imaging and polarizing optics, a computer, and a liquid crystal tunable filter (LCTF). Two sources configurations were used in testing, one where the light source was illuminating at a 15° angle to the sample normal and one where the light source was co-axial, Figure 1. Test conducted on patients using both configurations yielded identical results. A program written in Matlab® (Mathworks Natick, Massachusetts) controlled the system. The LCTF (VariSpec, Cambridge Research and Instrumentation, Inc., Woburn, MA), had a spectral range of 500 nm to 700 nm with increments of 5 nm.

Magnifying optics (Stereo Microscope, Bausch & Lomb, Rochester NY) with 100 mm working distance and 0.7× to 3× magnification, allowed for a low distortion high resolution imaging of the mucosa vascular network. An 8-bit camera (Lumenera Corp., Ottawa, Canada) was connected to the optics through a 0.5× eye-piece optical adaptor (Qioptiq Inc. Fairport, NY), finally a ring illuminator (Edmund Optics Barrington, NJ) was positioned on the acquisition side of the optical assembly. A polarizer (Edmund Optics, Barrington, NJ) was added to the front of the illuminator and aligned perpendicularly to the LCTF. The imaging system was tested and calibrated to determine the most effective gain and exposure time. Camera exposure time was 250 ms and image size was 1392 × 1040 pixels. Tests conducted with 1951 USAF Resolution Targets (Edmund Optics Barrington, NJ) showed

that the system had a maximum resolution of 17.96 lp/mm or 5.5 μm and a distortion of less than 1%. The imager field of view was ~15 mm. This layout is similar to the one used in other studies of superficial vasculature [13, 14]. Cross polarization imaging not only eliminates specular reflection from the air-tissue interface but also minimizes the acquisition of single scattering photons remitted by the superficial tissue. Only photons that have undergone multiple scattering events [15] are allowed back through the polarizer and to the camera. Some of these photons will travel through the superficial vasculature on their way back to the imager in a process of transillumination. The high absorption of haemoglobin in the vasculature increases the contrast of these vessels compared to the avascular background [16].

During image acquisition test subjects were asked to hold their lower lip in a downward position with both hands as the imaging device was aligned and focused. Patient movement was minimized through the use of a chin rest, which stabilized the subject in a standardized position. Furthermore a mechanical spacer was used, the spacer was simply a hollow tube with a removable support on the patient end, the support was sterilized after each imaging session.

Analysis of the data was conducted in post-processing with Matlab® on a Pentium 4 laptop (Hewlett Packard® Pavilion).

In this study, 89 individuals were tested; 41 images were acquired from the inside of each patient's lower lip and stored as uncompressed files. After each test, patients relaxed their lip for about 1 to 2 minutes before the next set of images were captured.

De Felice et al. [2] and Carrara et al. [9] both reported higher reflectance above 625 nm for control subjects than those positive for HNPCC, furthermore they noted that reflectance below 575 nm was the same for both populations. Following their example, we conducted a type of analyse which is relied on self-normalization mechanism. Two images were selected, one at 650 nm (R1) and one at 550 nm (R2), the latter is an isosbestic wavelength [14], while at 650 nm, vessels of the oral mucosa are virtually invisible. Beyond the task of normalizing data by this algorithm, we can monitor if there is a difference in reflectance between positive and negative groups above 625 nm. This difference can be detected by imaging at 650 nm. And since both groups reported the same reflectance below 625 nm, 550 nm is a baseline. A new image was created dividing the R2 image by the R1 image (R2/R1); a region of interest (300 × 600 pixels) was selected in the middle of the resulting image and the mean and standard deviation were calculated. Generally, we tried to avoid large arteries and veins that could skew the average reflectance results.

The R2/R1 image was also utilized to calculate oral mucosa vessel density. An algorithm originally designed by Sofka et al. [17] for tracing retinal vessels was used to obtain a binary image of the mucosa vessels. Normalized images were processed the vessel-tracing program (Likelihood Ratio Vesselness (LRV)) [17], which produced three output files: images with traced vessels (Fig 2.II), binary image where each pixel is one for vessel location and zero for background (Fig 2.III), and one text file with information about the number and location of vessel branches. This method combines a match-filter response, confidence measure and vessel boundary measure and is capable of detecting low contrast and narrow vessels while eliminating false positives due to nonvascular structures. The extended template of the multiscale matched filter helps to preserve vessels that are only a pixel wide and usually low contrast. The confidence measure emphasizes the shape of the intensity surface, which helps detect low contrast vessels. The vessel boundary is useful in distinguishing between offset edges near tissue abnormalities and true vessels. Vessel branching is also reported (red lines in Figure 2).

Two types of analysis were performed. First the number of vessel branches and their weight were measured (number of points assigned to each branch). This information was achieved directly from the output text file from vessel tracing program.

Secondly we calculated the Kolmogorov complexity [18, 19] of the binary image obtained after tracing, Figure 2III.

This step was achieved following the example of De Felice et al. [8] a Lempel-Ziv algorithm [20] was used as an acceptable measurement [19] of Kolmogorov complexity. For a detailed explanation of the algorithm we direct the reader to the paper by Kaspar and Shuster [21]. Kolmogorov complexity is the quantification of the number of different patterns in a string of data. This concept can be applied to imaging particularly when an image is a simple binary, as in our case. The main process of the used algorithm is to separates similar data into different classes; the higher the number of used patterns the higher is the complexity of the image.

The amount of pressure exerted on the lip during measurement was initially thought to cause local ischemia, potentially interfering with the accuracy of vessel tracing. To assess whether this effect might impact results we conducted a simple test wherein subjects were asked to apply different levels of pressure on the spacer. A pressure sensor (Phidgets, Calgary, Alberta, Canada) was embedded on the spacer and connected to a data acquisition card and monitor unit (Fluke, Everett, WA, USA), Figure 1. Four different levels of increasing pressure were monitored (lowest pressures are normal conditions of individuals). For each acquired image we selected a region of interest of 70 × 70 pixels. The oral mucosal vessel density (OMVD) and oral mucosal reflectance (OMR) were calculated in the selected area and the results were correlated to the applied pressure. Results from four individuals confirm that pressure does not measurably interfere with the analysis of vessel density and reflectance since very little variation of OMVD and OMR (maximum OMVD deviation is $0.08 \cdot 10^{-3}$ and maximum deviation for OMR is $0.6 \cdot 10^{-3}$) are noticeable as the pressure increases from 0.2 N/m^2 – to 1.7 N/m^2 – (Figure 3). A second investigation was directed at clarifying if positioning of the imager onto the patient mucosa could potentially give different results of OMVD. For this reason we observed OMVD at different parts of the oral mucosa of three volunteers from the control group. Three separate images (300 × 300 pixels) were taken from each of three different oral mucosa sections, shown in Figure 4. First, images from the frenulum area (Section 2 in Figure 4) were taken, followed by images on right and left sides. Left and right images were at a distance of 12 mm from the centre of the frenulum. Results showed in Figure 4 left hand side shows no significant difference of OMVD for the different sections of oral mucosa.

In order to determine how different levels of contrast and vessel blurring affect the accuracy of the vessel tracing a series of phantom images were created where vessel contrast was sequentially reduced. Vessel tracings from three different contrast levels (high contrast = 100%, medium contrast = 56%, and low contrast = 9%) are shown in Figure 5. Though the lower contrast images have more imperfections after tracing, the long-range vessel tracing looks much the same. In addition, the OMVD values for these three images were very similar (0.102, 0.098, and 0.098). From this it was concluded that poor contrast does not cause poor vessel tracing and that minor imperfections in vessel tracing do not cause a significant change in the OMVD value.

3. Results

Thirty-three patients with gene positive FAP from 29 unrelated pedigrees, 45 population controls 5 FAP gene negative patients, and 6 NMF patients, were recruited at the

Gastroenterology department of the Johns Hopkins School of Medicine, exclusion criteria for control patients included anyone with a history of polyps, colon cancer or a first degree relative with colorectal cancer or multiple polyps. Additionally, none of the patients were smokers. Typical images for positive and negative individual are shown in Figure 6 below, values of OMVD were 0.24 and 0.22 respectively.

Vessel branching, OMVD, and OMR, were also observed in a subset of patients with greater than 20 colorectal adenomas with no mutation found (NMF) in the APC and MYH gene, the known causes of oligopolypsosis.

The values for oral mucosal vascular density and oral mucosal reflectance in five patients with multiple polyps and no mutation found in either the APC or MYH gene, were compared to those of patients with FAP and controls.

Sensitivity and specificity of each metric were obtained by receiver operator curve (ROC) using an off the shelf statistical software package (GraphPad Prism 5, GraphPad Software Inc., La Jolla California).

For OMR the average values of R2/R1 images were analyzed. Mean values, standard deviations and overlap between positive and negative groups are shown in Figure 7a. For this particular test the area under the ROC curve was 63.2%. Both groups were tested using Kolmogorov-Smirnoy test [21] to investigate whether their distribution function (PDF) was normal, a condition for performing a student T-test. Our finding showed that both groups have normal PDF with 3% significance levels. A student T-test showed a p-value = 0.18. This analysis indicates that discrimination of positive patients from controls is not statistically significant when using OMR as a marker.

The density and complexity of the oral mucosa superficial vasculature was studied by counting the number of branching points in the images as well as through a Kolmogorov complexity analysis. The result for the branching test is shown in Figure 7b below.

This test showed that FAP positive patient could be separated from negative and controls with a sensitivity and specificity of 78% and 73% respectively, the area under the curve of this test ROC curve was 80.6. A student T-test was also performed and showed a p-value = 0.04 showing that FAP gene positive population had statistically higher number of branches than FAP gene negatives and controls across all ages.

OMVD a measure of vessel complexity was finally performed; results are shown in Figure 8. Sensitivity of 90.9% and specificity of 90.0% were obtained. The area under the ROC curve is 90.4%, while a student T-test had a p-value = $2.45 \cdot 10^{-5}$ indicating that OMVD is a marker with less than 10% error for discriminating between FAP gene positive, FAP gene negative patients and controls.

The threshold between the positive and control groups is approximately 0.23 for OMVD.

The summary of all results for OMR, OMBR, and OMVD are shown in Table 1 below.

Oral mucosal vascular density was statistically significantly higher in patients with multiple polyps and no mutation found compared to controls, $p = 0.00086$, but was not different from those with FAP, $p = 0.83$. All six patients in the NMF group had OMVD values greater than the cut off level, indicating they were positive for this phenotypic marker [22].

4. Discussion

Orthogonal polarization spectroscopy was applied to the screening of individuals with FAP gene mutation. Two main techniques were tested, one based on the intensity of the spectroscopic remittance (OMR) and a second based on the calculation of the oral mucosa vessel density (OMVD) and branching number. The OMR results show no separation between positive and negative groups, Figure 7a, while the calculation of the number of branching points and OMVD (Figures 7b and 8) appears to be a valid marker for FAP within a general unrelated population. The better separator seemed to be the OMVD parameter, its threshold of separation was 0.23 with FAP positive having higher vascular density than the negative group. OMVD was also able to diagnosed six NMF individuals as positive. This finding needs to be tested with a larger population for statistical significance but if proven correct could have great implications for this imaging technique. In fact, this subset could represent patients in which standard commercial genetic testing technology cannot identify deleterious mutations in the APC or MYH gene but could be identified through OMVD.

Since more vessels are present in FAP patients than controls, one would expect also a lower total reflectance from the FAP populations (due to the high absorbance of haemoglobin in the visible range of the spectrum), unfortunately we were not able to prove such finding with present tools, possibly due to the large variability in the mucosa diffused scattering. Comparing data distribution (Figure 8) and relative change for each individual (Figures 3 and 4) showed that OMVD is independent from imaging location on the oral mucosa and pressure of lips on spacer.

5. Conclusions

This paper proposed a new marker for the screening of individuals with Familial Adenomatous Polyposis using a simple imaging technique. The results show that measurement of the mucosa vessel structure can separate FAP positive from general controls. In particular vascular density had a sensitivity of 90% and specificity of 90.9%. Other groups have shown that reflectance spectroscopy could be utilized to distinguish individuals with a different genetic mutation (HNPCC) from general controls [2, 4] but in our testing OMR was not successful in achieving significant discrimination for the FAP gene positive population.

Given the simplicity of the system necessary to obtain OMVD results, we believe this technique could be applied in the screening of large populations as a preventive tool for CRC risk assessment.

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Biographies

Ali Basiri graduated from the Electrical Engineering department of Shahid Beheshti University in Iran, 2006. His B.S. thesis topic was calculating the Electromagnetic Field

around the Stator of a Motor Using Constant Current Using the Finite Element Method. He received his master degree in Electrical Engineering 2008 from The Catholic University of America. His research was related to satellite communication: Investigation of Interferences in satellite communication by using Sub-beam approach. Ali started his Ph.D. under the supervision of Dr. Ramella-Roman in 2008. His current research topics includes: oximetry of the retina and new methods for assessment of blood perfusion and oxigenation in the skin.

Daniel L. Edelstein directs the Hereditary Colorectal Cancer Registry at the Johns Hopkins School of Medicine. He received his Masters in Biotechnology from Johns Hopkins. His primary field of research focuses on hereditary colorectal cancer syndromes, specifically, Lynch syndrome and familialadenomatous polyposis. His major areas of investigation include the analysis of new phenotypic markers for hereditary colorectal cancer and the association of colonic and extracolonic neoplasia in these syndromes.

Jenna Graham received a Bachelors of Biomedical Engineering from Catholic University in May of 2011. She interned at The Johns Hopkins University Applied Physics Lab during the summers of 2009, 2010, and 2011 working in the areas of biomaterials and robotics. She is currently pursuing a Ph.D. in Biomedical Engineering at Johns Hopkins University and will conduct research in the area of cell and tissue engineering.

Afshin Nabili received his Masters in Biomedical Engineering from the Catholic University of America in 2009. Afshin is currently working as a laboratory supervisor for the school of engineering at CUA. He started his Ph.D. in 2009 and his current research topic includes: elderly fall detection mechanism, fall prevention, and fall damage minimization system.

Francis M. Giardiello's primary field of research focuses on cancer in the gastrointestinal tract. His major areas of investigation include assessment of the risk of neoplasia in gastrointestinal conditions, the study of the intestinal polyposis syndrome phenotypes with emphasis on the association of these disorders to colorectal and extraintestinal neoplasm,

evaluation of phenotypic-genotypic interaction in the inherited colorectal cancer syndromes, chemoprevention clinical trials in the inherited colorectal cancer syndromes, and evaluation of biomarkers of colorectal cancer.

Jessica C. Ramella-Roman received an Electrical Engineering degree (Laurea) from the University of Pavia in Italy in 1993 and a Master and Ph.D. degree in Electrical Engineering from Oregon Health Science University in Portland, Oregon in 2004. She was a Post Doctoral Fellow at the Applied Physics Laboratory of the Johns Hopkins University before joining The Catholic University of America as an Assistant Professor of Biomedical Engineering in 2006. Her current research interests include polarized light imaging and modeling, and the use of spectroscopic methodologies for measurements of skin and retinal oxygenation.

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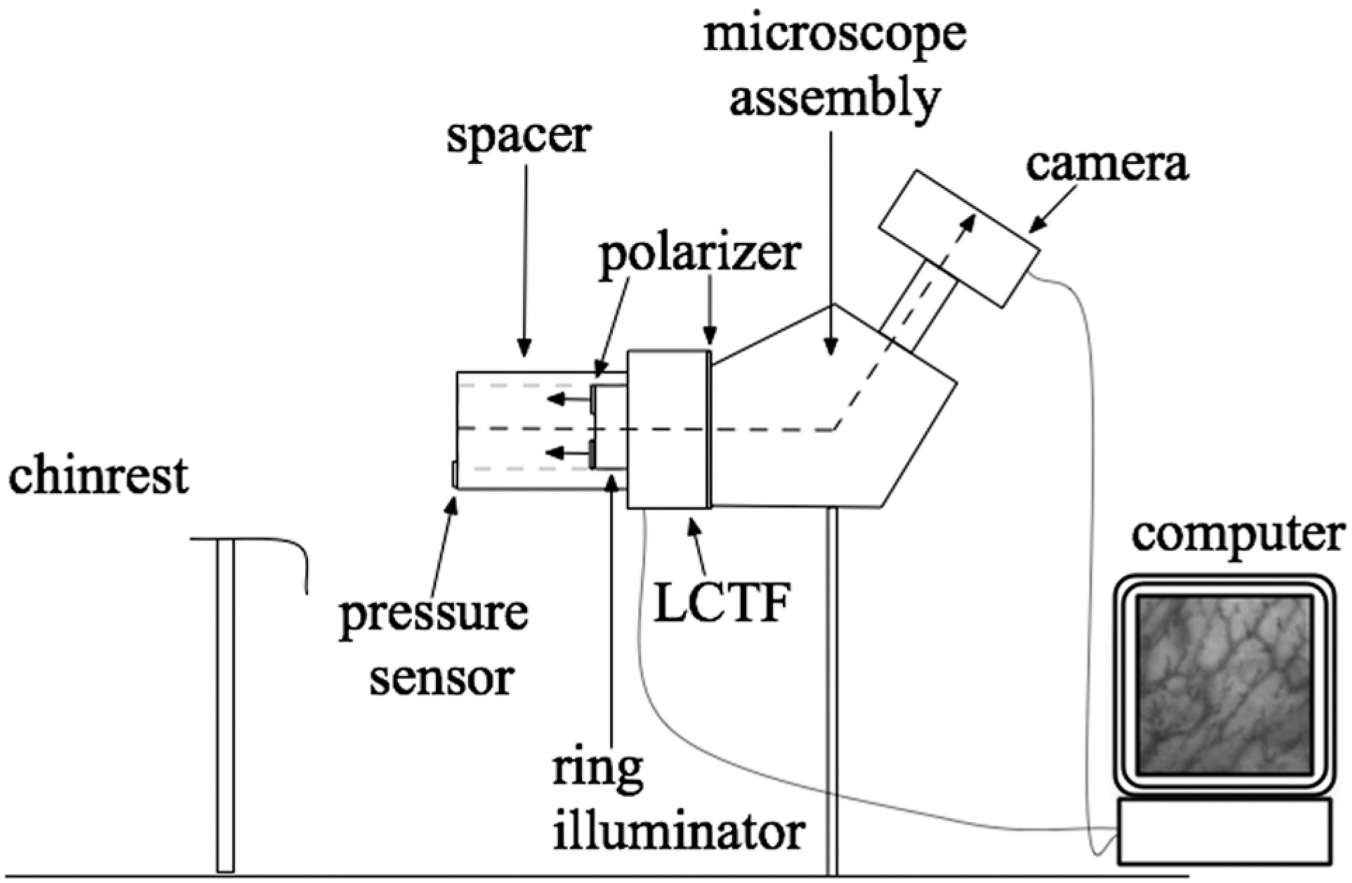

Figure 1.
The imaging system. The pressure sensor on the chin rest was utilized only during the testing phase. The schematic is not to scale.

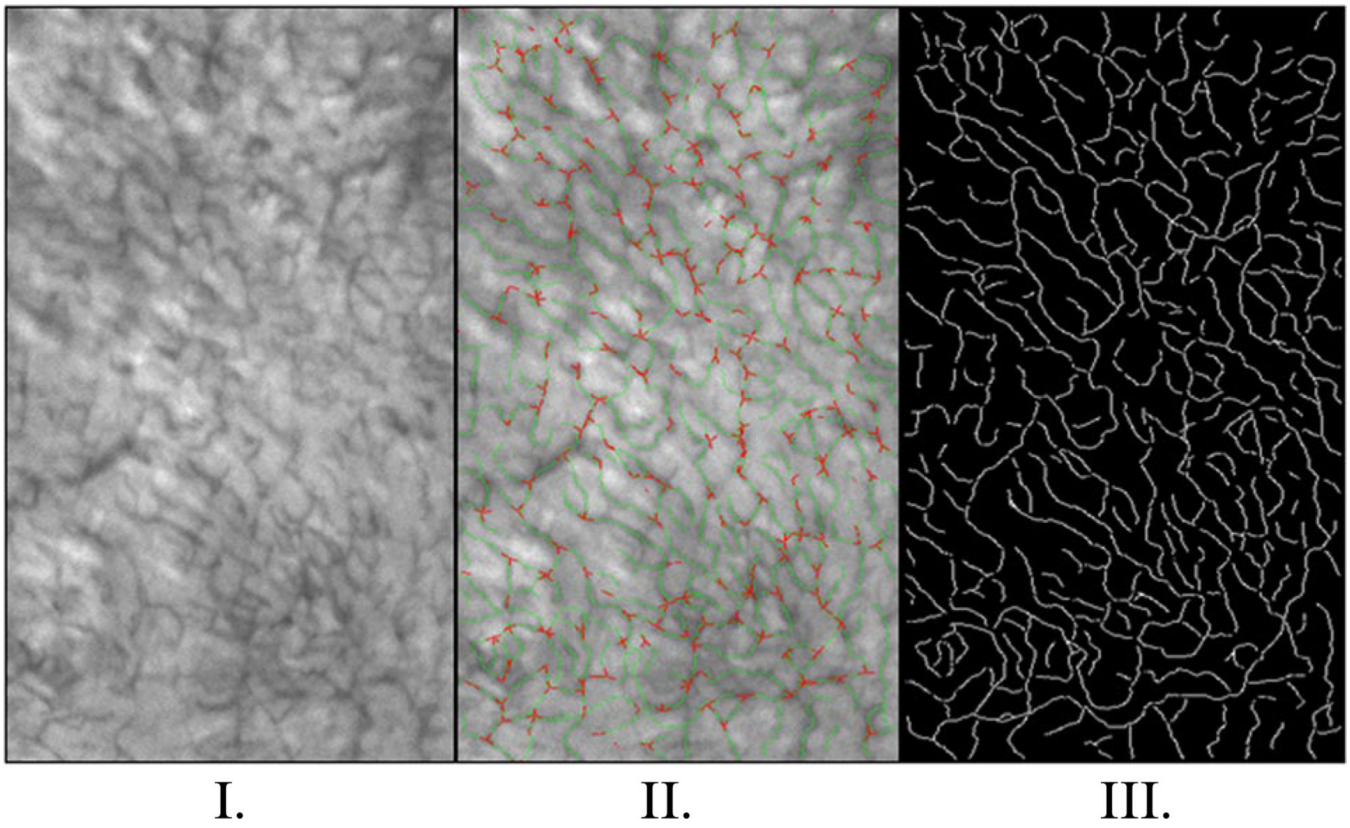

Figure 2.
(online color at: www.biophotonics-journal.org) Section I represents the raw image. Section II is the image after it has been traced using the Sofka method, traced green lines are vessels and red lines represent junctions. Section III displays only vessels and was input into the complexity measuring algorithm.

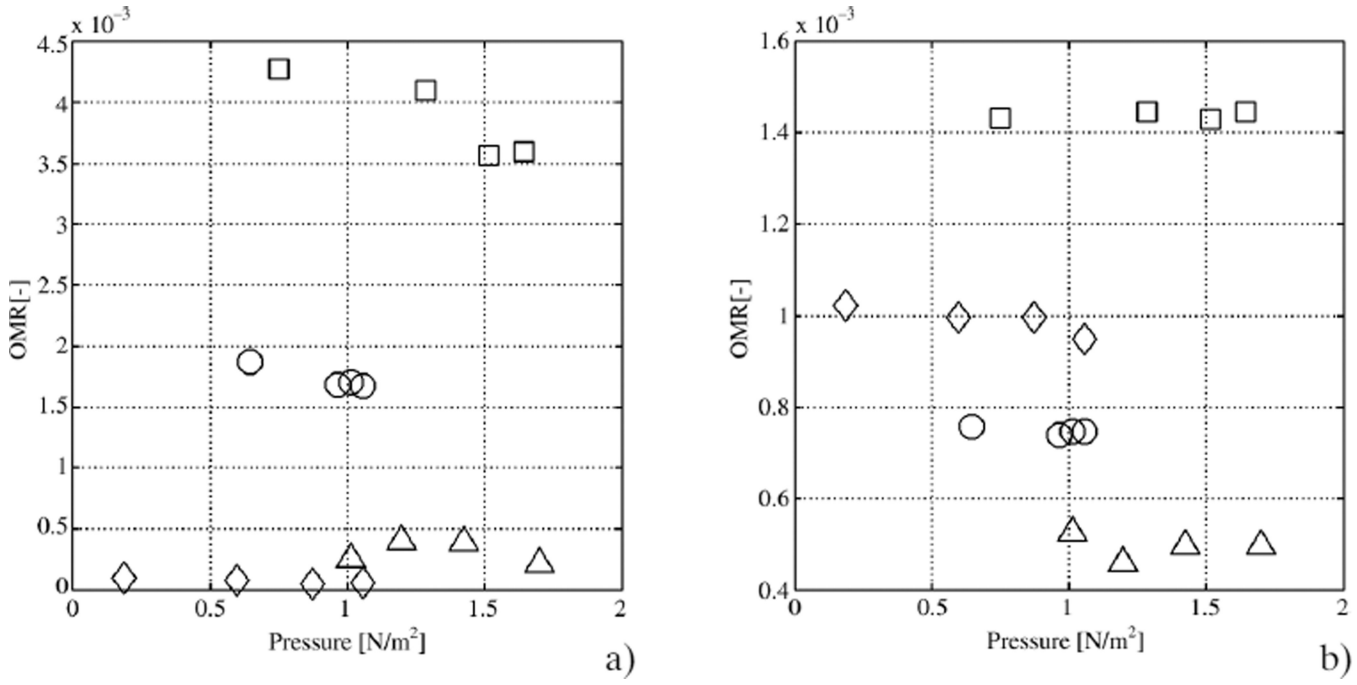

Figure 3.
(**a**) OMR for four different pressures in four individual tests. (**b**) OMVD for four different pressures in four individual tests. Each symbol is a representative of each different volunteer.

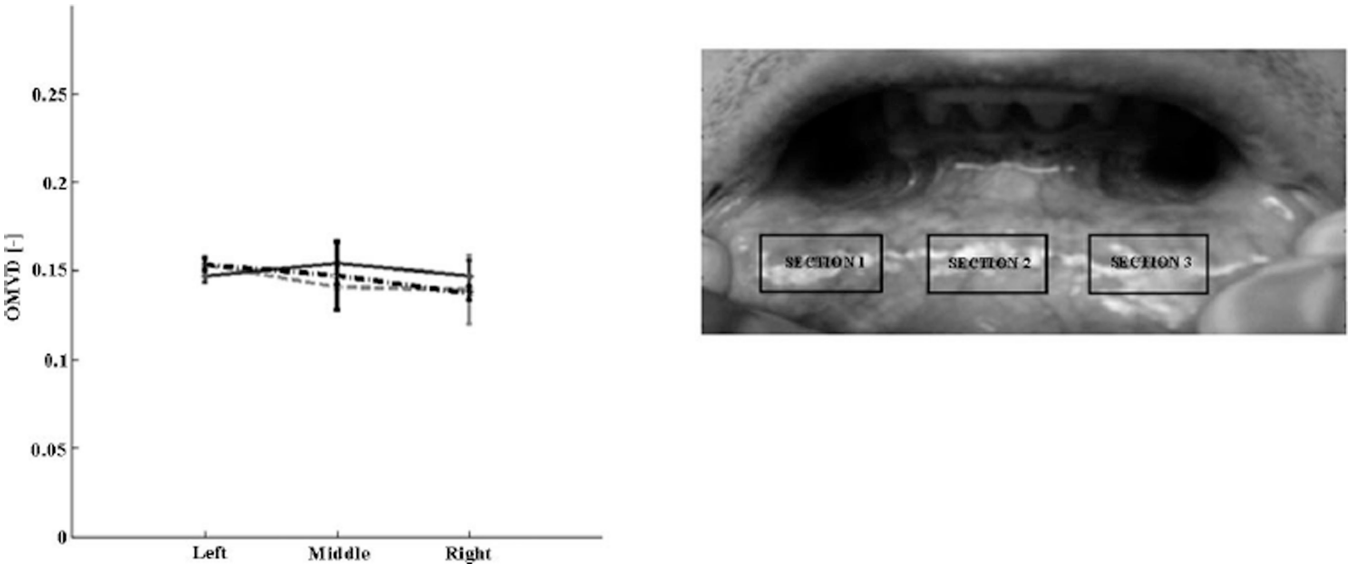

Figure 4.
Left hand side, OMVD results for three sections of oral mucosa on three volunteers. Right hand side, three portions of the oral mucosa imaged with our system.

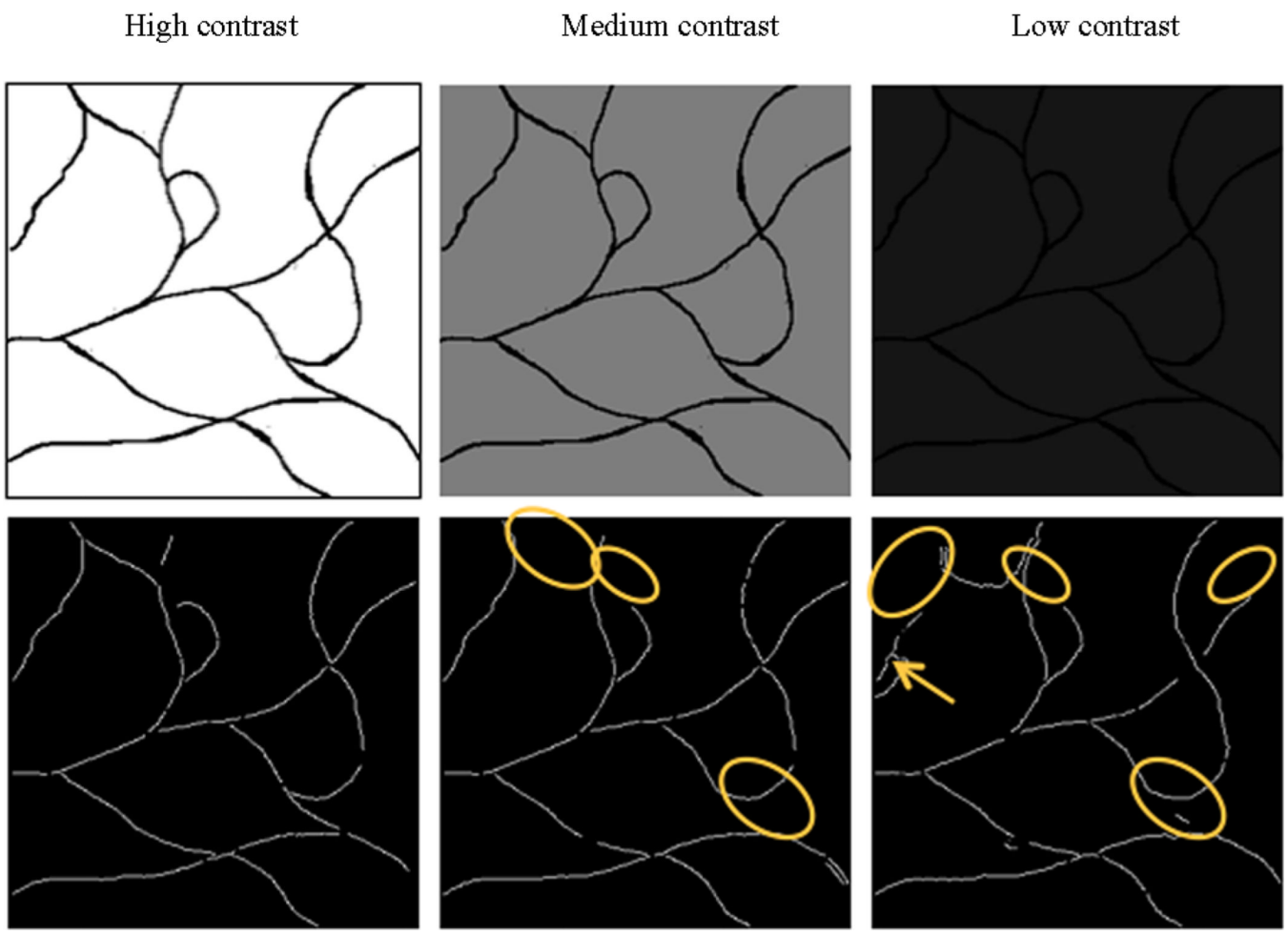

Figure 5.
(online color at: www.biophotonics-journal.org) Vessel tracings from phantom images with high, medium, and low contrast. Minor tracing imperfections are highlighted with circles and arrows in the medium and low contrast images.

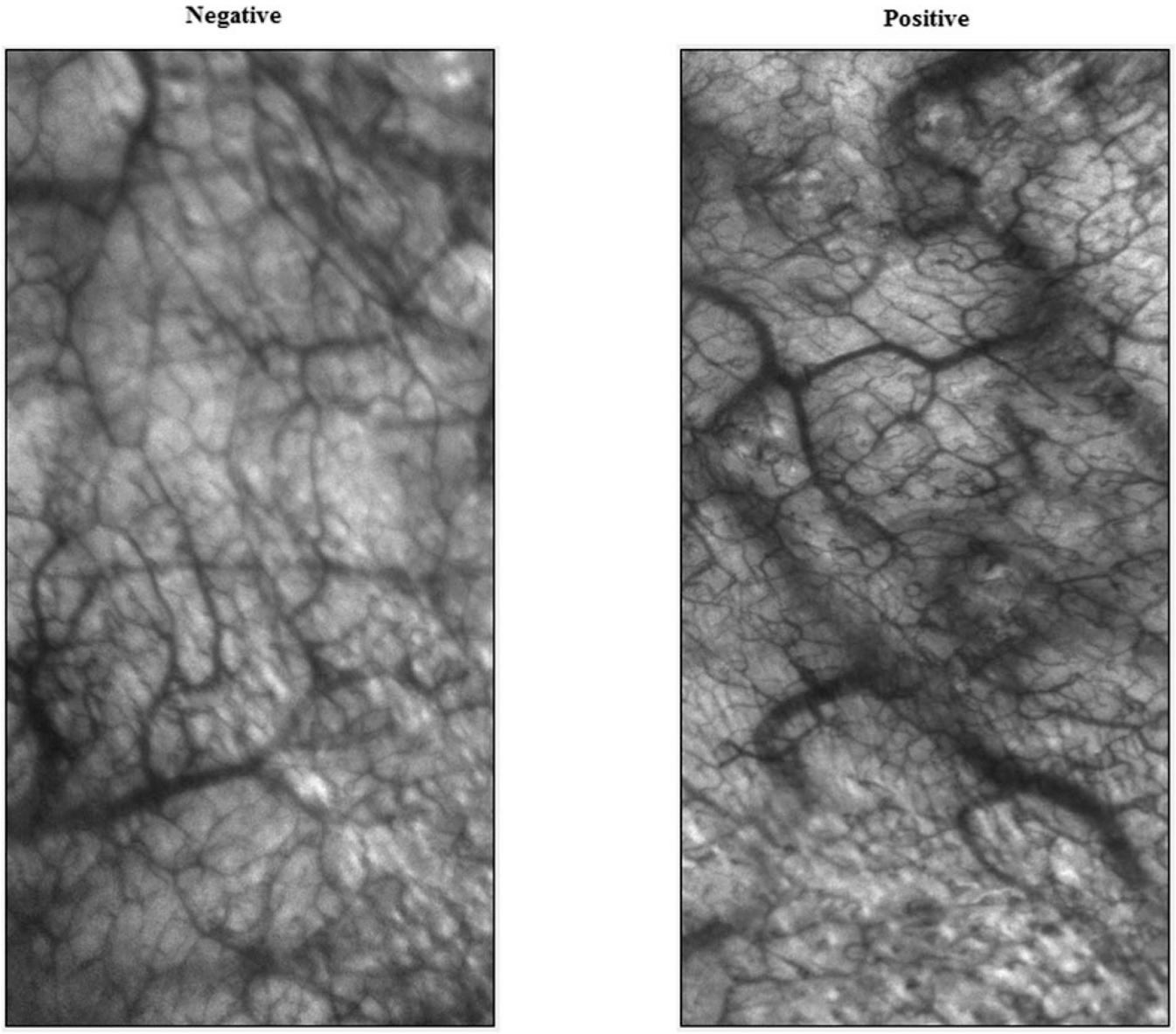

Figure 6.
Typical images of the oral mucosa for two individuals, negative for FAP right hand side (OMVD = 0.22) and positive for FAP left hand side (OMVD = 0.24). Images are 600 × 300 pixels size.

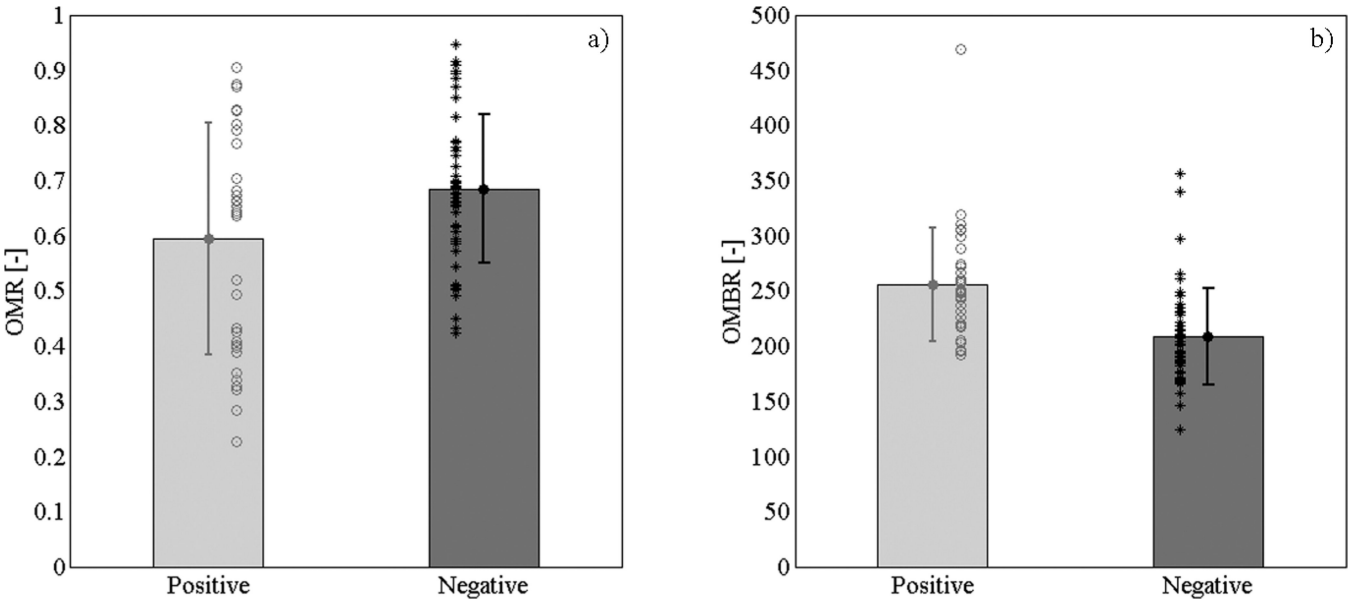

Figure 7.

(**a**) Bar chart showing OMR. The average value and standard deviation for positive and negative group are 0.059 ± 0.21 and 0.0685 ± 0.19 respectively. (**b**) Bar chart showing the value of the number of branching points in the population. The average value and standard deviation for positive and negative group are 255 ± 50 and 209 ± 43 respectively.

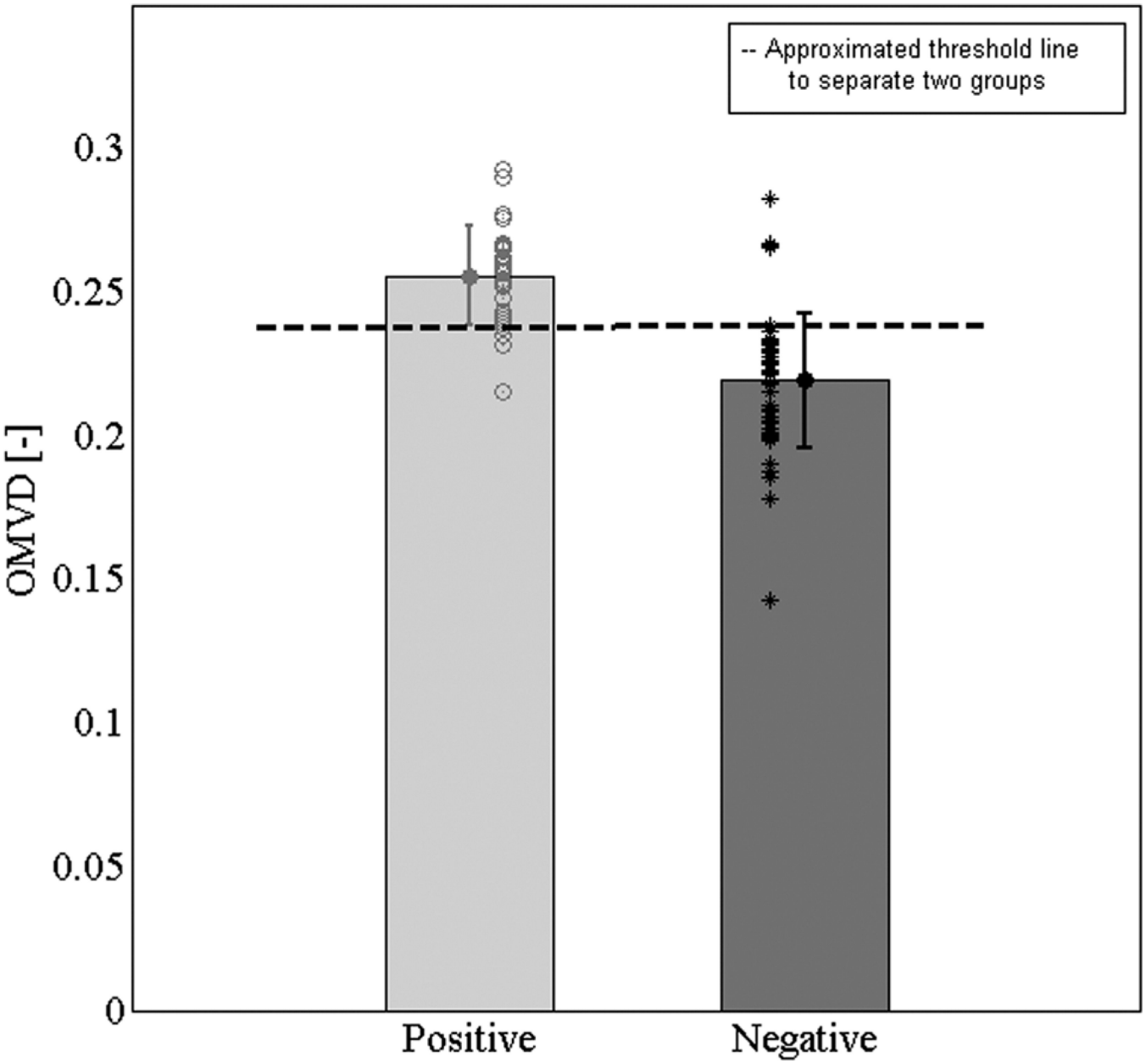

Figure 8.
Bar chart showing the value of OMVD. The average value and standard deviation for positive and negative group are 0.255 ± 0.017 and 0.219 ± 0.23 respectively.

Table 1

Statistical results of OMR, OMVD, and OMBR for positive and negative groups.

	Positive	**Negative**	**Sensitivity %**	**Specificity %**	***P*-value**
Oral nucosal reflectance	0.595 ± 0.21	0.685 ± 0.193	66.67	50	0.18
Oral mucosal Number of Branches	255 ± 50	209 ± 43	78	73	0.04
Oral mucosal Vessel Density	0.255 ± 0.017	0.219 ± 0.023	90.9	90.4	2.45 ± 10^5