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障害者対策総合研究事業

補償光学適応走査型レーザー検眼鏡を用いた
糖尿病網膜症の病態解析と早期発見、
早期治療に関する研究
平成25年度 総括研究報告書

(3年計画の2年目)

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I. 総括研究報告書

厚生労働科学研究費補助金（障害者対策総合研究事業）
総括研究報告

補償光学適応走査型レーザー検眼鏡を用いた糖尿病網膜症の病態解析と早期発見、早期治療に関する研究

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【研究要旨】

糖尿病網膜症（DR）は、網膜微小循環の障害がその本体である。人眼における微小循環動態の解明が可能になれば、病気の早期発見やより良い治療法の確立に有用であり、その技術の確立は社会的失明予防の観点からも重要である。本研究では補償光学の技術を適用した共焦点走査型レーザー検眼鏡（AO-SLO）を用いて非侵襲的に健常者および糖尿病網膜症患者におけるAO-SLOデータの収集、データ処理技術の検討、血流動態の解析を行った。正常群、DR群、非糖尿病網膜症（NDR）群の3群の比較の結果、血球速度はDRが正常、NDRと比較し有意に早く、NDRと正常の間に差は認められず、また分岐のない毛細血管においてDRでは有意に速度変化する血管が多かった。赤血球列の伸び率はDRとNDRに差がない一方で、正常群と両群との間には有意な差があり、糖尿病網膜症の早期発見の手掛かりとなり得ると考えられた。

A. 研究目的

補償光学の技術を適用した共焦点走査型レーザー検眼鏡（Adaptive Optics Scanning Laser Ophthalmoscopy: AO-SLO）を用いて非侵襲的に糖尿病網膜症（DR）における傍中心窩網膜毛細血管網の血流動態をとらえ、病初期の微小循環障害を解明していく。このように糖尿病網膜症の早期発見、早期治療を実現することによって、患者には将来的な見通しや生活習慣改善のアドバイスを提供できるようになり、失明の予防や視機能の温存が可能になると考えられる。さらに、汎網膜光凝固術や硝子体手術などの重傷化した糖尿病網膜症に対する現在の高額な治療を減らすことが可能となり、医療費の削減につながると期待される。

B. 研究方法

対象は健常者、DR患者、非糖尿病網膜症（NDR）患者。京都大学眼科外来に設置されたAO-SLOを用いて対象の傍中心窩網膜毛細血管網を撮影、保存した。すでに開発済みの血流解析専用ソフトウェアを用いて、固視微動に伴う動画の位置ずれおよび歪みを補正した後、耳側および鼻側の $1.4^{\circ} \times 2.8^{\circ}$ の範囲に対して、動画から血管造影を構築、時空間画像を用いた血球速度の算出、赤血球列の伸長率を測定した。毛細血管においても、血球速度は心拍動の影響を受けるため、すべての対象について、パルスオキシメーターを用いて心拍動を記録、すべての血球に対して脈波との同期処理を行った。

（倫理面への配慮）

本研究はヘルシンキ宣言に従い、京都大学医学部医の倫理委員会にて研究計画は承認されている。ボランティア、患者の協力を得てデータ収集を行う場合は、被検者へ十分に説明を行い文章で同意を得てから行うものとし、また、対象症例の個人のプライバシーは厳重に保護し、データは匿名化して保存している。また個人が特定されるような形でのデータの公表は行わない。AO-SLO は非侵襲的であり、有害事象の発生は予想されない。

C. 研究成果

1) 赤血球列の分岐のない毛細血管における速度変化

正常被検者 20 人 20 眼、NDR 17 人 17 眼、DR (mild, moderate NPDR ; nonproliferative DR) 8 人 8 眼が解析可能であった。赤血球列の分岐のない毛細血管における速度変化は正常が全体の 5%、NDR が 29.4%、DR が 50.0% で、DR で有意に多かった。

2) 血球速度の解析

赤血球列の速度は DR ($1.62 \pm 0.37\text{mm}$) が正常 ($1.26 \pm 0.22\text{mm}$)、NDR ($1.31 \pm 0.21\text{mm}$) と比較し有意に早く、NDR と正常の間に差は認められなかった。拍動に伴う血球速度の変化の検討において、Pulsatility Index (PI) は $PI = (V_{\max} - V_{\min}) / V_{\text{mean}}$ で定義されるが、3 群間に有意差は認められなかった。

3) 赤血球列の伸長率

伸長率は DR (0.39 ± 0.19) と NDR (0.39 ± 0.19) に差がない一方で、正常群 (0.67 ± 0.20) と両群との間には有意な差を認めた。

D. 考察

AO-SLO で得た血球速度データを心拍動と同期させることで正確な速度の算出に成功した。

正常群、DR 群、NDR 群の 3 群の比較の結果、血球速度は DR が正常、NDR と比較し有意に早く、NDR と正常の間に差は認められなかった。これは心拍動同期前に得た前年の結果とは矛盾するものである。そこで、血球の流れやすさに注目し、赤血球列の分岐のない毛細血管における速度変化、赤血球列の伸長率を調べたところ、DR では有意に速度変化する血管が多く、これが前年度の結果との間に生じた矛盾を説明する因子と考えられた。DR で速度上昇が起こる理由としては以下の原因が挙げられる。傍中心窩毛細血管網には赤血球列が流れやすい毛細血管 (erythrocyte aggregate preferred path; EAPP) と流れにくい血管 (plasma gap capillary; PGC) が存在すると考えられ (Arichika S. PLoS One 2014)、PGC は血流の EAPP における急激な変化を緩和する役目 (relief valve) があると考えられる。NDR と NPDR の違いは血管の形態変化の有無と推察され、NPDR における PGC の閉塞は隣接する EAPP における流速の上昇を、EAPP の閉塞は隣接する PGC における赤血球列の plugging を誘発すると考えられる。また赤血球列の伸び率は糖尿病では低下していたが、これは糖尿病では、恒常的に赤血球列の形成が起こっているために、相対的に 1 コずつ分離して流れる赤血球の数が少なくなっているためと想像される。伸長率は糖尿病網膜症の早期発見の手掛かりとなり得ると考えられた。

E. 結論

AO-SLO は非侵襲的に網膜微小循環を可視化することができる。糖尿病網膜症において、毛細血管レベルの血流変化を評価できる可能性がある。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

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Ⅱ. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書 籍 名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
宇治彰人、大音壮太郎、板谷正紀、有近重太、吉村長久	Image Quality Improvement in Adaptive Optics Scanning Laser Ophthalmoscopy Assisted Capillary Visualization Using B-spline-based Elastic Image Registration.	PLoS One	8(11)	e80106	2013
有近重太、宇治彰人、板谷正紀、大音壮太郎、吉村長久	Noninvasive and direct monitoring of erythrocyte aggregates in human retinal microvasculature using adaptive optics scanning laser ophthalmoscopy.	Invest Ophthalmol Vis Sci	54(6)	4394-4402	2013
有近重太、宇治彰人、大音壮太郎、宮本和明、吉村長久	Adaptive optics-assisted identification of preferential erythrocyte aggregate pathways in the human retinal microvasculature.	PLoS One	9(2)	e89679	2014

Ⅲ. 研究成果の刊行物・別刷

Image Quality Improvement in Adaptive Optics Scanning Laser Ophthalmoscopy Assisted Capillary Visualization Using B-spline-based Elastic Image Registration

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Abstract

Purpose: To investigate the effect of B-spline-based elastic image registration on adaptive optics scanning laser ophthalmoscopy (AO-SLO)-assisted capillary visualization.

Methods: AO-SLO videos were acquired from parafoveal areas in the eyes of healthy subjects and patients with various diseases. After nonlinear image registration, the image quality of capillary images constructed from AO-SLO videos using motion contrast enhancement was compared before and after B-spline-based elastic (nonlinear) image registration performed using ImageJ. For objective comparison of image quality, contrast-to-noise ratios (CNRS) for vessel images were calculated. For subjective comparison, experienced ophthalmologists ranked images on a 5-point scale.

Results: All AO-SLO videos were successfully stabilized by elastic image registration. CNR was significantly higher in capillary images stabilized by elastic image registration than in those stabilized without registration. The average ratio of CNR in images with elastic image registration to CNR in images without elastic image registration was 2.10 ± 1.73 , with no significant difference in the ratio between patients and healthy subjects. Improvement of image quality was also supported by expert comparison.

Conclusions: Use of B-spline-based elastic image registration in AO-SLO-assisted capillary visualization was effective for enhancing image quality both objectively and subjectively.

Citation: Uji A, Ooto S, Hangai M, Arichika S, Yoshimura N (2013) Image Quality Improvement in Adaptive Optics Scanning Laser Ophthalmoscopy Assisted Capillary Visualization Using B-spline-based Elastic Image Registration. PLoS ONE 8(11): e80106. doi:10.1371/journal.pone.0080106

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Competing interests: This study was supported in part by Canon Inc. Canon Inc. has provided a prototype of AO-SLO system to the authors' institution. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

Imaging technology in optical coherence tomography (OCT) has advanced rapidly and contributed greatly to progress in ophthalmology during this decade[1,2]. Improvement of OCT image quality has been achieved not only by increased retinal scan speed, as represented by the advancement of OCT generations from time-domain OCT to spectral-domain OCT (SD-OCT)[3–7], but also by image processing techniques to reduce speckle noise using multiple-aligned OCT scans[8,9]. Although multiple B-scan averaging can visualize microstructure in OCT more clearly than single B-scans, this technique is based on high-speed SD-OCT scanning, by which B-scans are generated with minimal scanning distortion variation from one scan to another.

More recently, confocal adaptive optics scanning laser ophthalmoscopy (AO-SLO) has enabled imaging of retinal cells such as photoreceptors and blood cells[10–12]. Adaptive optics (AO) can compensate for aberrations in ocular optics, allowing clear visualization of individual cone photoreceptors in the living eye. As in OCT, multiple scan averaging has been reported as useful in AO-SLO imaging, with averaged AO-SLO images demonstrating photoreceptor microstructure more clearly than single scans[13–15]. However, unlike SD-OCT, averaging of AO-SLO images is challenged by the relatively narrow and magnified scanning area. An interframe position gap produced by eye motion exists between scan frames, and intraframe eye motion produces distortions in frames[16]. Thus, averaging often results in blurry images. To overcome these issues, hardware and software for eye tracking have been

developed that can stabilize the frames, such as cross-correlation methods[17] and the KLT (Kanade-Lucas-Tomasi)-SIFT (Scale-invariant feature transform) algorithm[14], and excellent photoreceptor imaging results have been reported using these systems[18].

In addition to photoreceptor observation, AO-SLO video allows noninvasive monitoring of the movement of blood components without contrast dyes[11,19]. Noninvasive visualization of retinal capillaries using stabilized AO-SLO videos was very effective in analyzing the parafoveal capillary network of diabetic patients[20–22]. Because the technique requires no contrast dyes, angiogram can be safely obtained even in patients with minimal changes of that retina, which would be helpful in the early detection of retinal diseases and may also be a powerful tool for researching the pathogenic mechanisms of retinal diseases. Although the effect of video stabilization on vessel visualization was not analyzed in these studies, it was thought to be profound because the capillary image was constructed as a trajectory of small blood cells using multiple frames.

In this study, we applied B-spline-based elastic image registration on AO-SLO videos to interpolate the images for AO-SLO video stabilization using public domain software ImageJ. B-splines have proven very useful in modeling deformations in many biomedical imaging problems and ensure high-quality interpolation[23–27]. The effect of elastic image registration on AO-SLO-assisted capillary visualization in normal eyes and various retinal diseases was then evaluated objectively and subjectively.

Methods

This study was approved by the Institutional Review Board and the Ethics Committee at Kyoto University Graduate School of Medicine and performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant after a detailed explanation of the nature and possible consequences of the study procedures.

Subjects

Twenty-four healthy subjects with no history of ocular or systemic diseases (mean \pm standard deviation [SD] age, 35.2 \pm 8.1 y) and 25 patients (mean age \pm SD, 56.7 \pm 13.1 y) diagnosed with various eye diseases at Kyoto University Hospital (5 patients each with diabetic retinopathy [DR], idiopathic macular telangiectasia [MacTel], epiretinal membrane [ERM], central serous chorioretinopathy [CSC], and glaucoma) were recruited in this prospective cross-sectional study.

Adaptive Optics Scanning Laser Ophthalmoscopy Imaging

The AO-SLO system developed by Canon Inc. was employed for this study[28], with 840-nm imaging light wavelength and 32-Hz frame rate. The imaging light exposure level was set to less than the maximum permissible exposure indicated by the American National Standards Institute[29]. The scan area at the retina was 2.8 \times 2.8° and sampled at 400 \times

400 pixels. AO-SLO videos were acquired randomly from 1 eye of each subject after pupil dilation with one combined application of tropicamide (0.5%) and phenylephrine hydrochloride (0.5%) and recorded for 2 s per scan area; 6–12 scan areas were collected per subject to cover the parafoveal area. AO-SLO imaging was focused on the photoreceptor layer to enable detection of moving bright objects in the capillaries, which may correspond to leukocytes or plasma gaps as described previously[30].

Video Processing

Prior to linear and nonlinear registrations, all images were desinusoided in preprocessing to cancel the difference in reflective values between the center and near edge of each image caused by sinusoidal movement of the resonant scanner.

Video Stabilization. For each subject, a 2-s video was randomly chosen for analysis. All videos were first stabilized by linear image registration to align frames, followed by nonlinear image registration to correct image distortion produced by intraframe eye motion. The image quality of constructed capillary images was compared before and after nonlinear image registration. All digital images were processed by a single operator (A.U.) using ImageJ (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/index.html>). Grayscale ranged from 0 (black) to 255 (white).

Linear Image Registration. To align position gaps induced by eye motion across sequential frames, the Stackreg plug-in for ImageJ was used[31]. Stackreg is based on an automatic sub-pixel registration algorithm that minimizes the mean square difference of intensities between a reference image and source images. Rigid-body transformation, by which source images are transformed with translation and rotation to match a reference image while maintaining the distance between any pair of landmark points in the images, was applied to all videos. After registration, videos were cropped to eliminate the margin lacking a retinal image, which was a by-product of registration.

Elastic (Nonlinear) Image Registration. After linear image registration, videos were corrected for scanning distortions that were uncorrectable by linear registration and stabilized using the ImageJ plug-in bUnwarpJ, an algorithm for elastic and consistent image registration (Figure 1 and the text of Digital Content S1, which describes the development of the bUnwarpJ algorithm as an ImageJ Plug-in in detail)[32,33]. Deformation of bUnwarpJ was based on B-spline models, and the source image was elastically deformed in order to look as similar as possible to the target (reference) image. Because the bUnwarpJ plug-in was designed for registration between just 2 images and does not perform consecutive processing for registration between the reference image and other remaining frames, bUnwarpJ was run 63 times to analyze each 64-frame (2-s) video. We then created a macro that automates a series of ImageJ commands, and videos were registered automatically (Digital Content S2). Our macro was programmed to use the first frame as a fixed reference frame for image warping by bUnwarpJ.

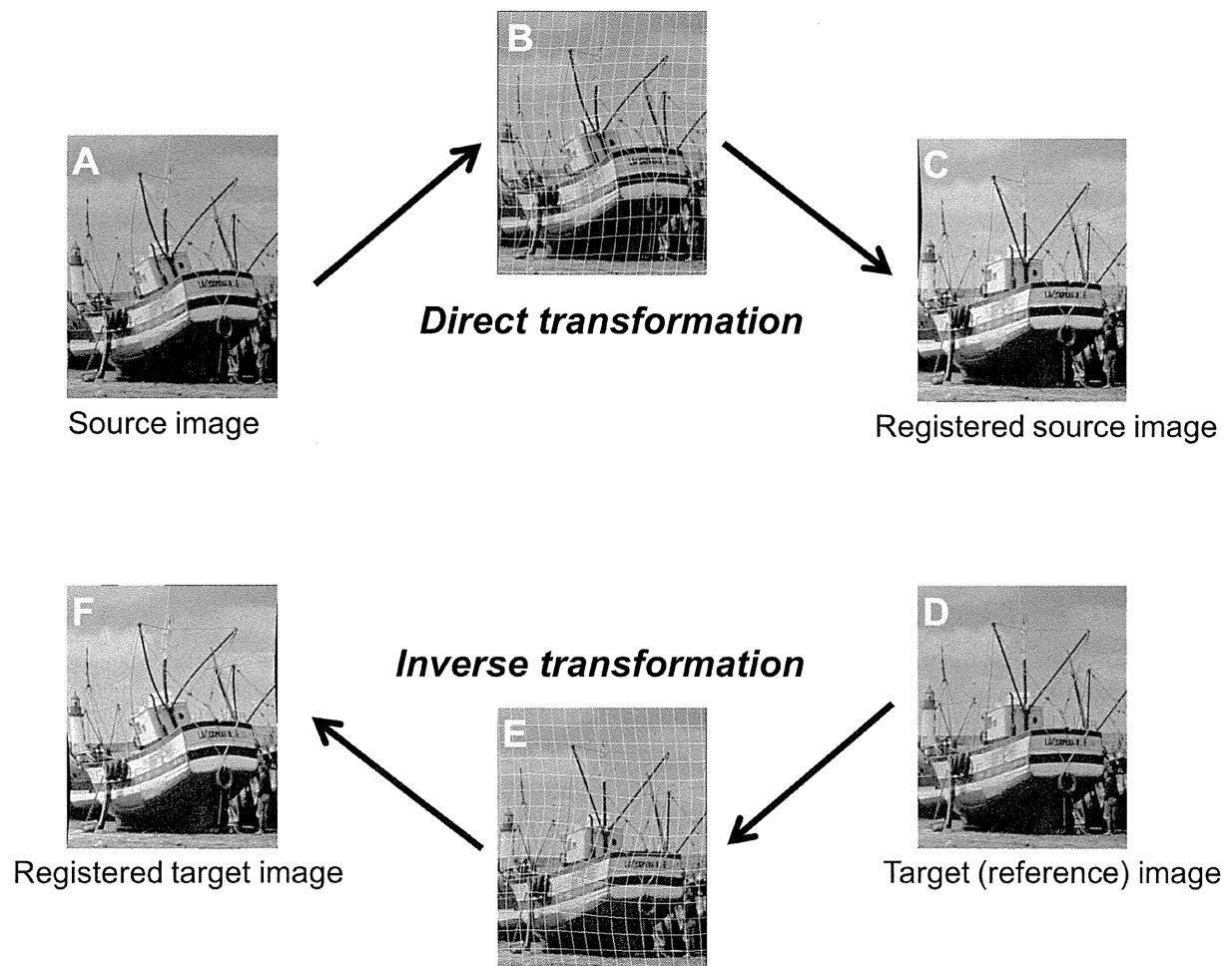


Figure 1. Elastic and Consistent Image Registration. (A) Source image. (B) Deformation grids on (A). Deformation of bUnwarpJ is based on B-spline models. Source image is elastically deformed in order to look as similar as possible to target (reference) image (D). (C) Registered source image. (D) Target (reference) image. (E) Deformation grids on (D). Simultaneously, target image (D) is elastically deformed in order to look as similar as possible to source image (A) to reduce registration error and obtain better correspondence compared to registration without the consistency factor.

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Capillary Visualization. The capillary images were constructed as projections of the moving objects in sequential frames using the motion contrast-enhancement technique reported by Tam et al.[20] Pixels were divided between sequential frames, and the variance of pixels among all division images in each x-y position was calculated to visualize contrast-enhanced capillary images. For each video processed by linear image registration, capillary visualization was performed both before and after elastic image registration, classified as the E(-) and E(+) groups, respectively, for comparison. A macro was also created to facilitate capillary visualization (Digital Content S3).

Assessment of the Effect of Elastic Registration on Capillary Visualization

The effect of elastic image registration on capillary visualization was assessed by comparing the quality of visualized capillary images between the E(-) and E(+) groups objectively and subjectively.

Contrast-to-Noise Ratio Comparison. To objectively compare image quality, contrast-to-noise ratio (CNR) was calculated for regions of interest (ROI) and compared between pairs of E(-) and E(+)-group images[8,34]. ROIs examined in this study consisted of a line selected on the vessel and areas selected in space surrounding the vessel. To match the position of ROIs between E(-) and E(+) images, we established ROIs on the first frame of the registered videos and copied

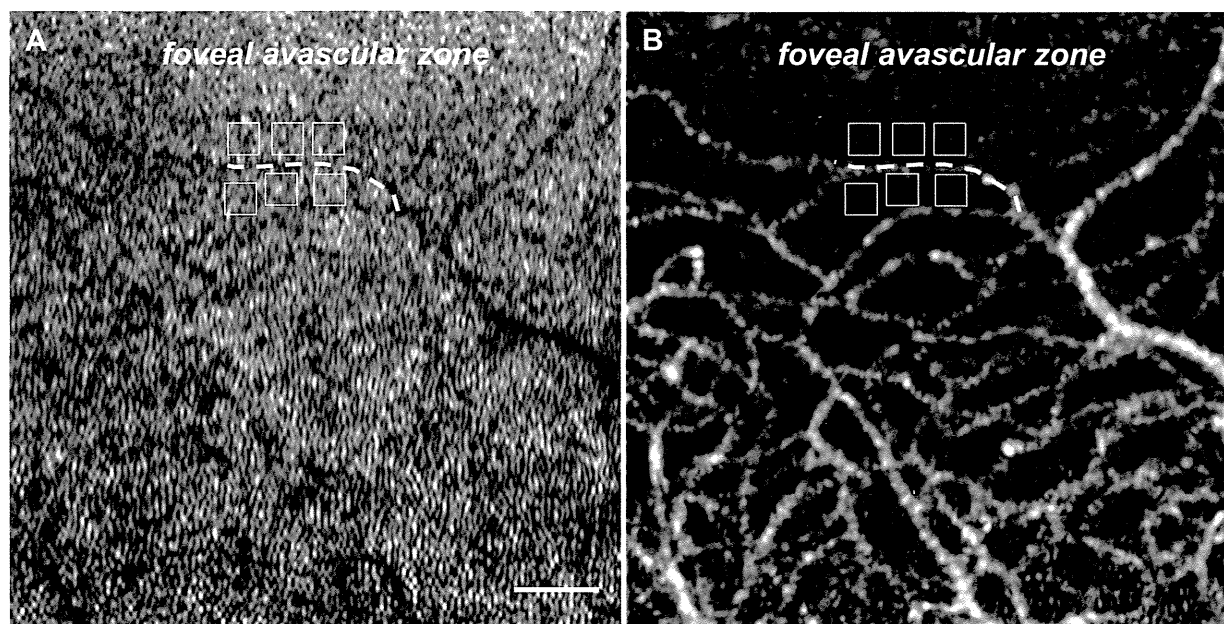


Figure 2. Calculation of Contrast-to-noise Ratio. (A) First frame of video obtained using adaptive optics scanning laser ophthalmoscopy (AO-SLO). Regions of interests (ROI) were set on the first frame of the video. ROIs examined in this study were a line selected on the vessel shadow (yellow dotted line) and areas selected on space surrounding the vessel (white squares) for each subject. Scale bar, 100 μm . (B) Capillary image constructed from the video in (A) after elastic registration. ROIs on (A) were copied to the image with capillary visualization.

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them to the images with capillary visualization (Figure 2). Briefly, the line was established along a vessel shadow. Six 20×20 -pixel areas were selected adjacent to the selected capillary shadow on the cone mosaic pattern and then copied to the brightly visualized capillary image using ROI Manager, a built-in function of ImageJ that records the exact location of the ROIs, and the mean gray values of the selected capillary and vessel free space were calculated. CNR was calculated as follows:

$$CNR = (f - b) / \sqrt{\delta_f^2 + \delta_b^2} \quad (1),$$

where f and b are the mean gray values of the ROI set on visualized capillary (foreground) and the 6 ROIs set on vessel free space (background), respectively, and δ_f and δ_b are the standard deviation from the foreground and background mean values, respectively.

Expert Comparison. For subjective comparison, 6 experienced ophthalmologists masked to image information performed independent expert comparisons of pairs of E(-) and E(+) images [8,35]. A comparative image quality score for clarity of capillary images was assigned to each image pair as follows: 5 = markedly better capillary image in E(+); 4 = slightly better capillary image in E(+); 3 = equal capillary images in E(+) and E(-); 2 = slightly better capillary image in E(-); and 1 = markedly better capillary image in E(-).

Measurement of Processing Time Required for Elastic Image Registration

The processing time required for elastic image registration using bUnwarpJ was measured to assess its ease of use and future applicability. Image processing was performed using a Microsoft Windows 7 64-bit operating system with 64-bit central processing unit (Corei7, 2.80 GHz).

Statistical Analysis

Statistical analysis was performed using StatView version 5.0 (SAS Inc., Cary, NC). All values are presented as the mean \pm standard deviation. Paired t tests were used to determine CNR differences between the E(-) and E(+) groups. Comparison of the ratio of CNR values in the E(+) and E(-) groups and the mean score assigned by experts between patients and normal subjects was performed using one-way analysis of variance, with post-hoc comparisons tested by the Scheffe procedure. P values < 0.05 were considered statistically significant.

Results

Effect of Elastic Registration on Capillary Visualization

All AO-SLO videos tested in this study were successfully stabilized by elastic image registration with ImageJ, and distortion that was wavy and uncorrectable by linear image

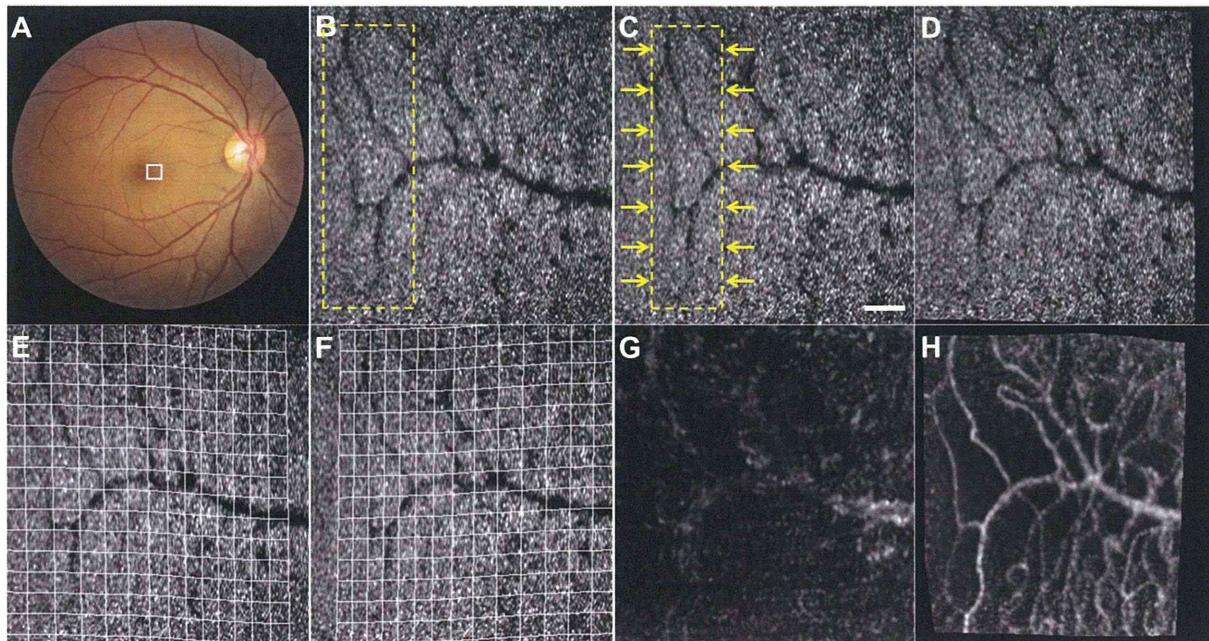


Figure 3. Effect of B-spline-based Elastic Registration on Capillary Visualization. (A) Color fundus photograph of the right eye of a 34-year-old woman with no history of ocular or systemic diseases. (B, C) Two consecutive frames obtained by adaptive optics scanning laser ophthalmoscopy (AO-SLO), which correspond to the area outlined in white in (A) showing scanning distortions that were uncorrectable by linear registration. High-intensity dots representing the cone mosaic pattern and dark shadow of the vessels are observed. Although the squares outlined by a dashed line in (B) and (C) indicate an identical area of the retina, the area in (B) appears to be wider than the area in (C). Scale bar, 100 μ m. (D) Registered image of (C), which was elastically deformed to appear as similar as possible to (B). (E, F) Deformation grids on (B) and (C), respectively. (G) Capillary image constructed from unregistered AO-SLO video recorded for 2 s. (H) Capillary image constructed from AO-SLO video after B-spline-based elastic registration. The capillary is more brilliant than that of (G) constructed without registration.

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registration was considerably diminished (Figure 3 and Digital Content S4). Bright moving objects in the shadows of capillaries on the cone mosaic pattern, considered to be leukocytes or plasma gaps, were easier to observe in stabilized videos than in videos only subjected to linear image registration.

Table 1 shows CNRs for ROIs set on pairs of E(-) and E(+) capillary visualized images in normal subjects and patients with various disease. CNR was significantly higher ($P < 0.0001$) in the E(+) group than the E(-) group (1.92 ± 0.57 vs. 1.24 ± 0.59 , respectively) overall. CNR was also significantly higher in the E(+) group than in the E(-) group in normal subjects and each disease group. In no pair of ROIs was the CNR lower in the E(+) group than in the E(-) group. The average ratio of CNR in the E(+) group to CNR in the E(-) group was 2.10 ± 1.73 overall, and no significant difference in this ratio was observed between patients or normal subjects ($P = 0.379$).

The mean score assigned by experts was 3.99 ± 0.69 overall, 4.21 ± 0.69 in normal subjects, 3.92 ± 0.58 in DR patients, 3.88 ± 0.87 in MacTel patients, 3.40 ± 0.59 in ERM patients, 3.72 ± 0.42 in CSC patients, and 3.96 ± 0.79 in glaucoma patients, and the mean score did not significantly

differ between patients and normal subjects ($P = 0.306$) (Table 1).

Processing Time Required for Elastic Image Registration

Mean processing time required for elastic image registration was 626 ± 190 s in total, with no significant difference between patients and normal subjects ($P = 0.258$). Mean processing time was correlated with the area ($R = 0.385$; $P = 0.006$) and width ($R = 0.399$; $P = 0.004$) of cropped videos, but not their vertical length ($R = 0.169$; $P = 0.248$).

Case 1

The parafoveal area of the left eye of a 53-year-old man with no history of ocular or systemic diseases was examined by AO-SLO, and capillary image was constructed from video recorded for 2 s (Figure 4). Capillaries were brilliantly visualized using elastic image registration (Figure 4F), comparably to early-phase fluorescein angiography (FA) images obtained using Heidelberg Retinal Angiography 2 (Heidelberg Engineering)

Table 1. Differences in Contrast-to-noise Ratio between Images Constructed with or without Elastic Image Registration.

Characteristic	Normal	DR	MacTel	ERM	CSC	Glaucoma	Total
No. eyes	24	5	5	5	5	5	49
Men/women	14/10	3/2	3/2	3/2	5/0	5/0	33/16
Age (y)	35.2 ± 8.1	50.2 ± 7.0	59.4 ± 10.6	65.2 ± 10.6	50.2 ± 12.7	56.6 ± 20.6	46.2 ± 15.4
Processing time (s)	614 ± 198	667 ± 41	530 ± 240	794 ± 211	654 ± 180	544 ± 106	626 ± 190
CNR (E-)	1.30 ± 0.57	1.86 ± 0.37	0.90 ± 0.48	1.02 ± 0.55	1.28 ± 0.62	0.84 ± 0.64	1.24 ± 0.59
CNR (E+)	1.98 ± 0.56	2.18 ± 0.44	1.90 ± 0.52	1.66 ± 0.50	1.98 ± 0.93	1.53 ± 0.31	1.92 ± 0.57
P value	< 0.0001	0.0331	0.0113	0.0279	0.0428	0.0164	<0.0001
CNR(E+)	1.95 ± 1.55	1.17 ± 0.14	2.93 ± 1.96	2.36 ± 2.07	1.57 ± 0.35	3.21 ± 3.16	2.10 ± 1.73
CNR(E-)	4.21 ± 0.69	3.92 ± 0.58	3.88 ± 0.87	3.40 ± 0.59	3.72 ± 0.42	3.96 ± 0.79	3.99 ± 0.69
Expert score							

DR = diabetic retinopathy; MacTel = idiopathic macular telangiectasia; ERM = epiretinal membrane; CSC = central serous chorioretinopathy; CNR (E-) = contrast-to-noise ratio of images constructed without elastic registration; CNR (E+) = contrast-to-noise ratio of images constructed with elastic registration.

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(Figure 4D), while capillary images constructed without elastic registration were rather noisy and blurred (Figure 4E).

Case 2

A 44-year-old woman with proliferative diabetic retinopathy was referred to us. Panretinal photocoagulation was performed 10 y previously. Her visual acuity was 20/20 in her right eye and 20/20 in her left eye. Parafoveal area of the right eye was scanned by AO-SLO, and capillary image was constructed with and without elastic image registration (Figure 5). In the registered capillary image, microaneurysm as well as caliber variation was depicted clearly in the registered capillary image, and the same findings were also detected in FA.

Case 3

A 46-year-old man with a 6-year history of mild blurring of vision in the right eye was diagnosed with MacTel type 1 (Figure 6). Visual acuity was 20/20 in his right eye. Photocoagulation scar was observed temporal to the fovea. The OCT scan showed intraretinal cystoid spaces[36,37]. AO-SLO video was successfully registered, and the constructed capillary image showed microaneurysm. Capillary was visualized more clearly and sharply in images constructed with elastic registration than in images without elastic registration.

Case 4

A 65-year-old woman with mild ERM had visual acuity of 20/20 in the right eye (Figure 7). AO-SLO image showed "microfolds" (multiple thin, straight, hyporeflective lines in the

photoreceptor layer) as previously reported by Ooto et al.[38] The capillary image was rather noisy in the unregistered image and visible more clearly in the registered image. In both registered and unregistered images, vessel images overlapping with microfolds were obscured by white striped artifacts. These artifacts were not detected in images from patients other than ERM patients and were considered unique to ERM.

Case 5

CSC was diagnosed in the left eye of a 42-year-old man. Visual acuity was 20/50 in the left eye (Figure 8). OCT showed serous retinal detachment and irregularity of the retinal pigment epithelium at the fovea. AO-SLO image of the fovea showed a dark area and many dark patches representing lost or damaged cones, and the pattern of remaining cones was irregular[39]. Vessel shadows near the fovea assimilated into the dark area and were not visible. Capillary images constructed from the video were clearer in the registered image than in the unregistered image, especially in the area with dark patches. A spotty area was seen in the region containing dark patches, and these spots might be due to imperfect alignment.

Discussion

In this study, we applied B-spline-based elastic registration to AO-SLO videos and demonstrated that the registration enhanced the quality of capillary images constructed from AO-SLO video both subjectively and objectively in normal eyes and eyes with various retinal diseases. CNRs for ROIs set on capillary visualized images were approximately twice as high in the E(+) group as in the E(-) group, and the mean score assigned by experts was 3.97 ± 0.70 , suggesting that the registration played an important role in capillary visualization. Visualization of retinal capillaries using AO-SLO was very effective in analyzing microstructures such as microaneurysm and neovascularization, and it may be useful for early detection in patients with diabetes and other retinal vascular disorders[21,22]. The technique requires no contrast dyes, and angiograms can be obtained from the patient without adverse effects. Therefore, it enables frequent acquisition of capillary images from the same patient and may be useful in the evaluation of ophthalmologic or medical treatments in the future.

Capillaries were visualized using motion contrast enhancement as previously reported[20]. Because the technique is based on calculating sequential division images, which are obtained by dividing the gray values of pixels between successive frames, precise registration is required to cancel the image of the mosaic pattern outside of the vessel shadow and extract only the motion of blood cells. In our protocol for capillary visualization, comparatively short (2-s) recording time and wide recording field ($2.8 \times 2.8^\circ$) were adopted to minimize the inter-frame position gap between scan frames produced by eye motion. Although the protocol was also convenient for simultaneous acquisition of the wide-field capillary network, inadequate registration would fail to cancel the background mosaic pattern by pixel and result in spotty areas on the image as in the example shown in Figure 8.

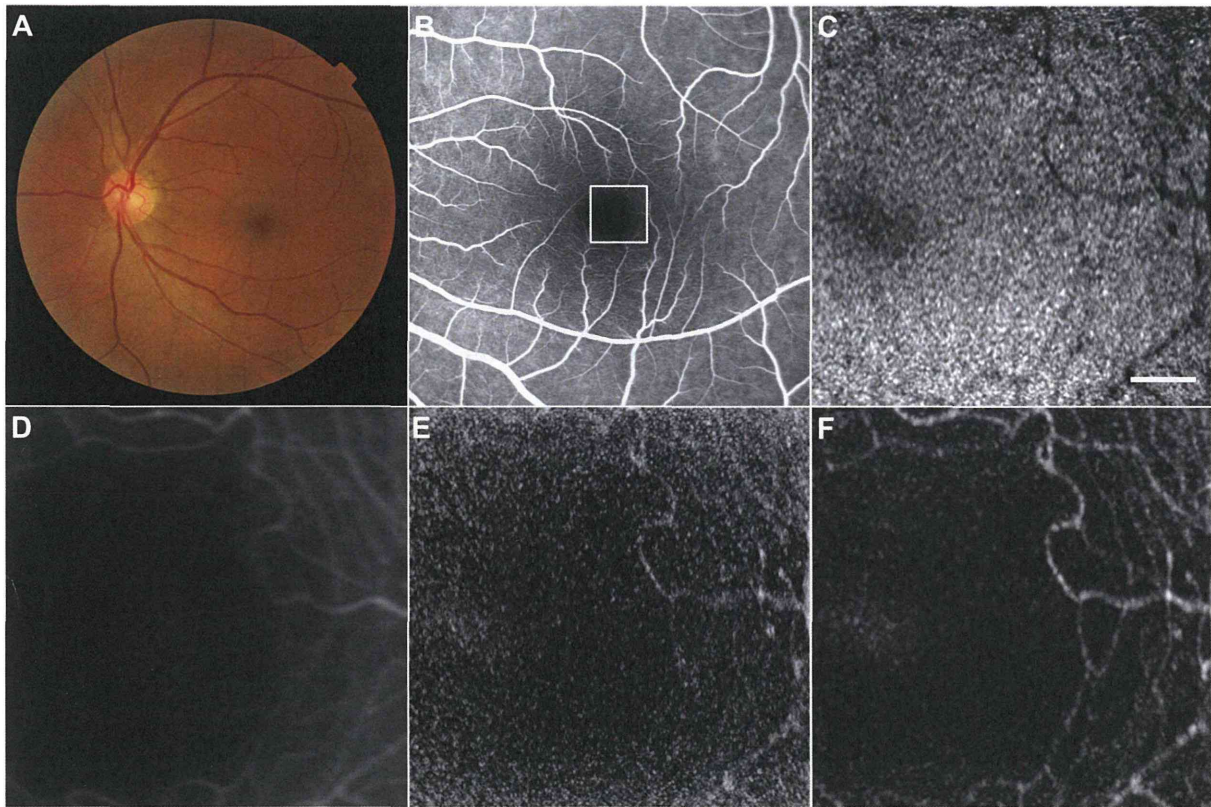


Figure 4. Imaging Results from Case 1. (A) Color fundus photograph. (B) Early-phase fluorescein angiography (FA) image. (C) First frame of the adaptive optics scanning laser ophthalmoscopy (AO-SLO) video, which corresponds to the area outlined in white in (B). (D) FA image. Magnified view of the area outlined in white in (B). (E) Capillary image constructed from unregistered AO-SLO video. (F) Capillary image constructed from AO-SLO video after B-spline-based elastic registration. Scale bar, 100 μ m. Note that the capillary image is brilliantly visualized and comparable to early-phase FA image, while the capillary image constructed without elastic registration (E) is rather noisy and blurred.

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B-spline-based deformation models are control grids composed of B-spline curves, which are smooth curves defined by the given control points[32,33]. Using the deformation models, pixels in an image from AO-SLO video can be matched to pixels on the reference image to obtain interpolated images warped by a spline function. B-spline-based elastic image registrations have been utilized widely in many biomedical imaging problems and have proven suitable for the type of deformations encountered with magnetic resonance imaging[23,24,26,27,40]. Other than the registration method applied here, several approaches have been reported to date. Arathorn et al. reported cross-correlation methods, whereby the frames were cut in rectangles and displaced in the x and y directions to correlate them to a reference frame[17,18]. Since the distortions created by intraframe eye motion are mainly due to the raster pattern of scanning, which is relatively slower than scanning line, breaking up the frames into strips parallel to the fast scanning mirror direction is reasonable. In fact, our results on the processing time required for elastic image registration

support the adequacy of cross-correlation methods. The mean processing time was correlated with the width but not vertical length of cropped videos, suggesting that many more distortions are present in the horizontal direction, which is perpendicular to the fast scanning mirror direction in our system, than in the vertical. In the KLT-SIFT algorithm[14], stable point features were extracted from AO-SLO images using the SIFT algorithm[41], and features were tracked from frame to frame using the KLT algorithm, which has low computational complexity and is considered faster than traditional techniques[42], followed by second-order polynomial transformation to remove distortions. Using KLT-SIFT, features on 30-Hz AO-SLO video can be tracked in real-time.

Mean processing time required for elastic image registration was approximately 20 s per frame in this study. Compared to the 2 above-mentioned registration methods, which can compute registration on a millisecond time scale per frame, the registration method tested here required substantial computing time. Although our goal is not to track single cones or stabilized

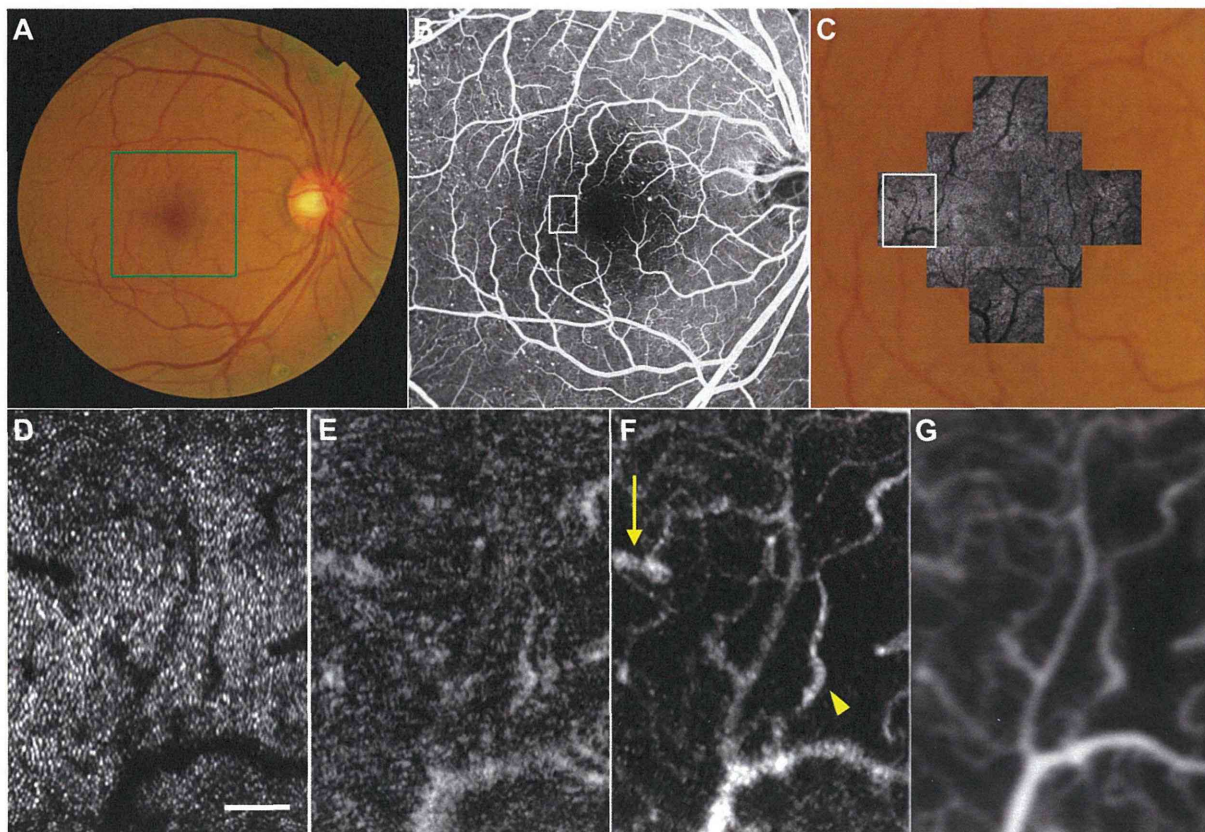


Figure 5. Imaging Results from Case 2. (A) Color fundus photograph. (B) Early-phase fluorescein angiography (FA) image. Microaneurysms are seen as hyperfluorescent dots. (C) Montage of adaptive optics scanning laser ophthalmoscopy (AO-SLO) images overlaid on magnified color fundus photograph corresponding to the area outlined in green in (A). (D) First frame of the AO-SLO video, which corresponds to the area outlined in white in (B) and (C). Scale bar, 100 μm . (E) Capillary image constructed from unregistered AO-SLO video recorded for 2 s. (F) Capillary image constructed from AO-SLO video after B-spline-based elastic registration. Microaneurysm (yellow arrow) as well as caliber variation (yellow arrowhead) are clearly depicted. (G) FA image. Magnified view of the area outlined in white in (B).

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stimulus delivery to the living retina in real-time, 10-min processing time to register a 2-s video is considered to be too long for applying the method widely to future investigations of AO-SLO-assisted blood flow analysis. The use of general-purpose software and the consistent image registration that is included in the energy function of the registration software appeared to factor in the long processing time. However, putting aside the issue of processing time, B-spline-based elastic image registration provides high-quality interpolation because of its precise pixel-wise registration and enables capillary visualization constructed from short-term recording. Development of special tools to integrate software, applications, and systems from different vendors may improve availability and speed.

Public domain software ImageJ and associated plug-ins were used for image processing including image registration and capillary visualization in the current study based on their

ready availability[31,32]. Anyone wishing to try the registration and capillary visualization described here need only download the software and macro texts. Currently, AO-SLO machines are being developed by researchers in many countries, and more groups will participate in this new area of research in the future. We believe that the information described in this paper will support the quick initiation of research on AO-SLO-assisted blood flow analysis, which will in turn facilitate investigations of retinal microcirculation. Using B-spline-based elastic image registration, capillaries were visualized well without contrast dyes, similar to FA, in all diseased eyes except cases of ERM and CSC. In ERM, vessel images overlapping with "microfolds" were obscured by white cloud-like artifacts[38]. In CSC, vessel shadows near the fovea assimilated into the dark area and were not visible[39]. Ultimately, capillaries in these areas could not be visualized. Because AO-SLO was focused on the photoreceptor layer and blood cells were observed as shadings

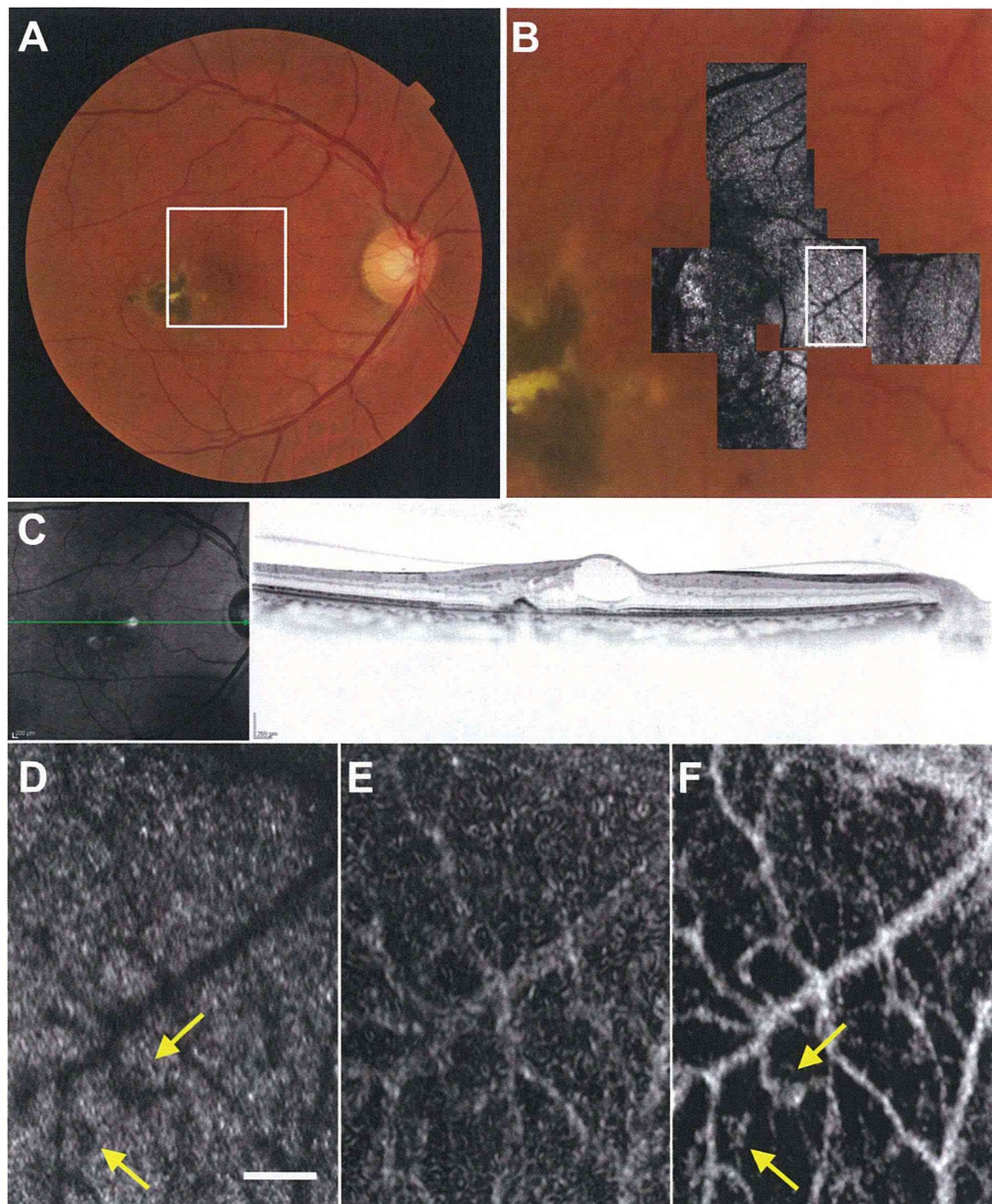


Figure 6. Imaging Results from Case 3. (A) Color fundus photograph. (B) Montage of adaptive optics scanning laser ophthalmoscopy (AO-SLO) images overlaid on magnified color fundus photograph corresponding to the area outlined in white in (A). (C) The optical coherence tomographic scan shows intraretinal cystoid spaces. (D) First frame of the AO-SLO video, which corresponds to the area outlined in white in (B). Dark regions that may correspond to the shadow of microaneurysm on the cone mosaic could be seen along the vessel shadow (arrows). Scale bar, 100 μm . (E) Capillary image constructed from unregistered AO-SLO video. (F) Capillary image constructed from AO-SLO video after B-spline-based elastic registration showing microaneurysm (arrows). The capillary was visualized more clearly and sharply in the image constructed with elastic registration than in the image without elastic registration.

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on the shadows of the bright cone mosaic patterns of photoreceptors, this focusing may be the cause of these

artifacts and loss of capillary images[30]. As previously described, the characteristics of the reflected AO-SLO laser

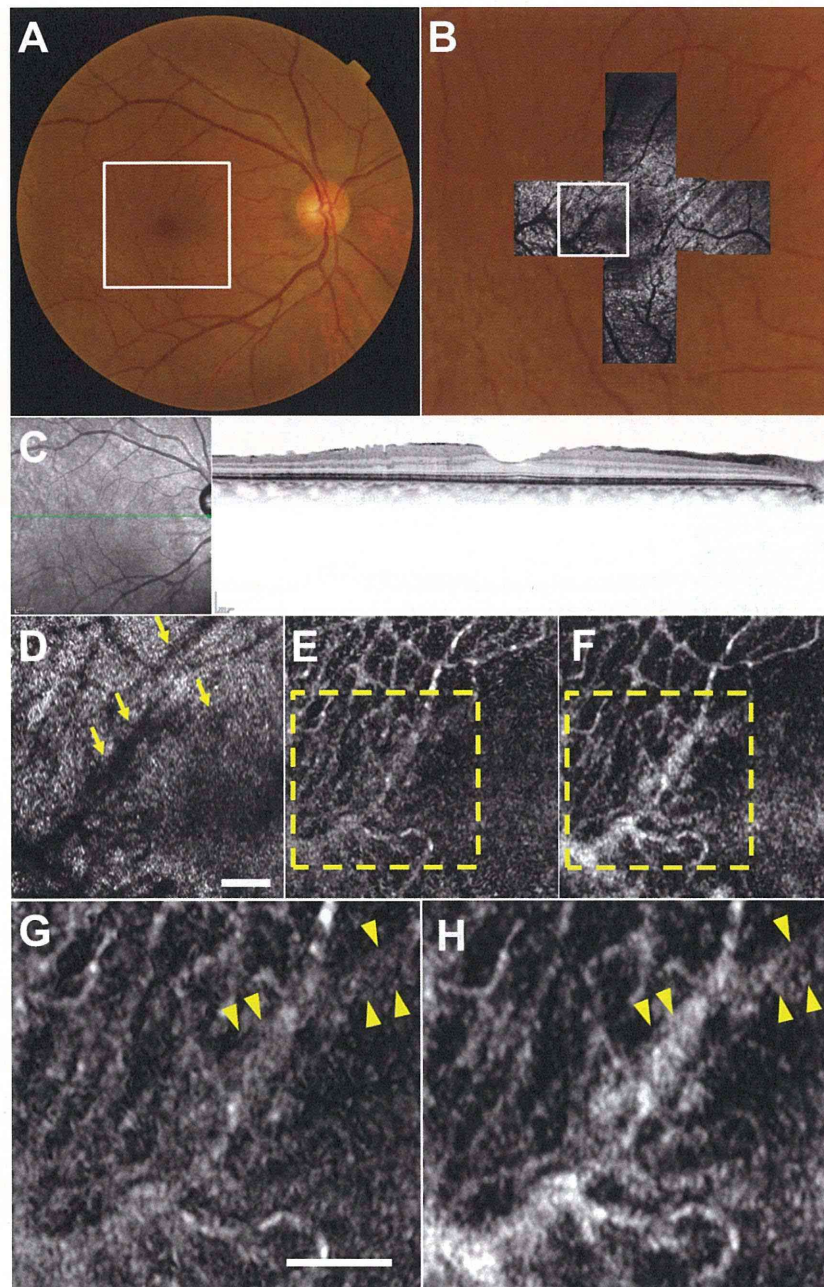


Figure 7. Imaging Results from Case 4. (A) Color fundus photograph. (B) Montage of adaptive optics scanning laser ophthalmoscopy (AO-SLO) images overlaid on magnified color fundus photograph corresponding to the area outlined in white in (A). (C) The optical coherence tomographic scan shows thin epiretinal membrane and retinal folds. (D) First frame of the AO-SLO video, which corresponds to the area outlined in white in (B), shows "microfolds" (arrows). Scale bar, 100 μ m. (E) Capillary image constructed from unregistered AO-SLO video. (F) Capillary image constructed from AO-SLO video after B-spline-based elastic registration. (G, H) Magnified view of the area outlined in yellow in (E) and (F). Scale bar, 100 μ m. The capillary image was rather noisy in (G), and visible more clearly in (H). Note that vessel images overlapping with microfolds were obscured by white striped artifacts (arrowheads) in both images.

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