Visualization of Fundus Vessel Pulsation Using Principal Component Analysis

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PURPOSE. Spontaneous venous pulsation is one of the clinical signs with which to rule out elevated intracranial pressure and papilledema. More subtle pulsatile retinal movements are difficult to observe because of eye movements. Recording a fundus movie and aligning (registering) the images helps, but the images still contain distracting microsaccadic distortions and noise. The authors hypothesized that addressing these latter points should allow observation of minute pulsating features in fundus movies.

METHODS. Principal component analysis (PCA), a basic form of blind source analysis, is applied to recorded fundus image sequences. The authors demonstrate this method in 5-second image sequences acquired with a near-infrared SLO (HRA+OCT Spectralis). The images are first registered to correct for eye drift, then microsaccade-distorted images are rejected, and the remaining image sequence is decomposed into principal components. Finally, a movie is constructed using the first five principal components (these had pulsatile features).

RESULTS. Each of the processing steps (registration, cleaning, PCA filtering) improves the detection of pulsatile features, ultimately allowing clear visualization of spontaneous venous pulsation. Depending on the subject, additional features can be observed: pulsation amplitude of the arterial tree of approximately 10 μm, pulsation of arterioles down to 70-μm diameter, complete venous collapse; overall optic nerve head tissue pulsation, and mechanical links between veins and arteries.

CONCLUSIONS. By disentangling pulsatile motion from other dynamic components of retinal images, unprecedented resolution in physiologic motion of retinal vessel structure is achievable. (Invest Ophtalmol Vis Sci. 2011;52:5457-5464) DOI:10.1167/iovs.10-6806

Spontaneous venous pulsation (SVP) is a clinical sign visible at the optic nerve head in approximately 80% or 90% of patients.1,2 It is observed as a rhythmic variation in vein diameter of one or more vessel segments near or on the optic nerve head. The SVP originates in the complex interaction between pulsatile and steady components of systemic blood pressure, intraocular and intracranial pressure, and vessel architecture, diameter, and stiffness. Several hypotheses detailing these interactions have been proposed,3-15 but the issue has not been satisfactorily resolved.

The clinical relevance of SVP is that of a negative marker when intracranial pressure rises above 14 mm Hg10 and for differentiating early papilledema from pseudopapilledema.14,15 SVP has also been investigated in the context of glaucoma16-23 aortic regurgitation,24 and other pathologic conditions.25,26 Relating to SVP, ophthalmodynamometry measures vascular pressure using arterial and venous collapse induced by an increase in intraocular pressure.27-34

Pulsatile features in addition to SVP have also been observed in retinal arteries. A serpentine movement consists in a lateral movement of the vessels, usually visible in short radius curvatures.35 As in the rest of the body, this pulsation is attributed to the elongation of the vessels consecutive to the systole pressure peak. Some subjects present another movement, namely in segmental shifting, i.e., when pulsation leads not to smooth radius changes but rather to visible rigid and straight segments between vessel bending as a possible sign of arteriosclerosis.25 Eye movements are the primary factor limiting the pulsation observation. Even in well-fixating subjects, the size of eye movements is comparable to pulsatile motion: Pulsation amplitude is at most one vessel diameter for SVP and typically a fraction of a main artery’s diameter for serpentine movements. The amplitude of microsaccades during a fixational task is typically 5 arcmin, corresponding to one-sixth of a 150-μm vessel diameter.

To improve the detectability of small movements, a first step is to decouple the acquisition from the observation. This allows post hoc interpretation and processing of the image sequence, without undue exposure of the patient’s eye. As an obvious processing step, the drift component of the fixational eye movements can be corrected by registering (aligning) the images. Artifacts may remain because of microsaccades concurrent with the acquisition scan, leading to distorted images. They are rarely rejected, although they severely impair motion perception in the reader. Finally, the detection of faint retinal motion is impaired by image noise resulting from safety-related light exposure limitations in image sequences acquired over seconds.

We hypothesize that image sequences contain more information than is currently used and propose a method to improve the visibility of pulsatile features, making them detectable in the first place. Briefly, we record fundus image sequences using near-infrared illumination to maximize patient comfort, thus minimizing any avoidance movements while also eliminating the need for pharmacologic mydriasis; we register the images, eliminate images corrupted by microsaccades, and apply principal component analysis (PCA). Simply speaking, PCA disentangles anatomic signals, signals coding for variations in illumination/detection geometry, and noise. We then use the pulsatile signals to construct movies free of noise and other distracters. Using this technique, we present movies acquired from healthy subjects revealing minute pulsatile features. The development of these processing steps was driven by known properties of our visual system, gearing the outcome to facilitate the detection of
minute motion without being handicapped by adverse mechanisms such as change blindness.\textsuperscript{36}

\textbf{Subjects and Methods}

\textbf{Subjects}

We acquired retinal images from the right eyes of 10 subjects between 21 and 31 years of age who had no known systemic or ocular pathologies. They were screened for intraocular pressure (between 10 and 19 mm Hg), had normal ocular findings on slit lamp and fundus examination, confocal laser scanning ophthalmoscope (cSLO) and optical coherence tomography examinations, and a decimal visual acuity $\geq 1.1$. The study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the local institutional ethics committee. Subjects provided written informed consent after a detailed explanation of the study’s nature and potential consequences.

\textbf{Instrument}

Images were acquired with a confocal scanning laser ophthalmoscope (Spectralis HRA + OCT; Heidelberg Engineering, Heidelberg, Germany) in near-infrared mode (820 nm). The imaged region was set such that the optic nerve head was in the center of the right portion of the image. We added a near-infrared CCD camera to the cSLO and used it for alignment by monitoring the cornea and the iris region from a nasal direction. The cSLO was adjusted so that the pupil plane of the probing beam would match the pupil plane of the eye, and the beam was centered on the eye pupil. This procedure differs from the typical clinical procedure in which the real-time image is optimized for uniform intensity. Two sequences of 45 images each were acquired in each subject at a rate of nine images per second, thus spanning several heart cycles. The subject was asked to refrain from blinking during the sequence and to fixate a custom refraction-corrected low-intensity target. Drug-free mydriasis was achieved by dimming the ambient light. To partially standardize perfusion and intracranial pressure, subjects were seated for at least 20 minutes before recording. Raw data (Supplementary Movies S1a–S10a) are available at http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental.

\textbf{Preprocessing}

The aim of preprocessing is to generate image sequences free of eye movements and instrumental artifacts. We analyzed and visualized the data with custom-made procedures (Igor Pro 6.1: WaveMetrics Inc., Lake Oswego, OR) to implement all analyses and visualization steps. There were three preprocessing steps:

1. \textit{Trimming}. Registration side effects are the generation of “empty lateral bands” as the images are translated/rotated and the cSLO’s central light reflex is displaced. Given that these would cause stray PCA components, we trimmed initial images so that these effects did not appear on the new region. We refer to the trimmed images here as raw images.

2. \textit{Registration}. The image sequence was aligned using a rigid body transformation to correct for translational and rotational drifts using a published multiresolution algorithm\textsuperscript{37} (implemented natively in Igor Pro).

3. \textit{Rejection of microsaccadic distortions}. In addition to fixational translational and rotational drifts, the eye undergoes microsaccadic distortions. In the images, these distortions are represented by “empty lateral bands” which are caused by translation and rotation of the images. The first PCA outcome refers to the principal components (PC0, PC1, ..., bottom left), which are a set of images representing the components found by the PCA in the input images, ranked according to variance explained. The PCA also yields as many loadings as raw images. The first three of the five PCs already provide all information. The second PCA outcome refers to the loadings (bottom right), which indicate the relative weight of each PC in a given input image.
Because of the image scan’s duration and the temporal characteristics of the microsaccades, the latter manifest themselves on approximately 10% to 20% of the images as distorted horizontal bands within the images. The affected images are easily identified on slow observation of a registered sequence. We were thus able to manually reject contaminated images. If a microsaccade (or a saccadic intrusion) was identified in the first image, it was rejected and the process repeated at the second step.

**Principal Component Analysis**

For PCA application, we assume that the image in the acquired sequences consists of several components: a static (identical across images) morphologic structure; dynamic (pulsatile) morphologic structures; eye movement–related illumination changes; further noise sources from, for example, residual translation and motion distortion. PCA takes advantage of the specific spatial patterns attributed to each of these components and its associated specific temporal signature to separate them (Fig. 1). In effect, the PCA is used here as a filter, rejecting components in the image sequence that do not provide dynamic information regarding pulsatile behavior. For a formal mathematical presentation of PCA, see Jackson.

The image sequence first was trimmed to retain the vertical stripe showing the optic nerve head and primary vessels and then was normalized by subtracting the arithmetical average of the complete sequence from each single image. The average represents static information, thus essentially retaining the anatomic information. The normalized sequence codes all the variations from this anatomic information. After this, the PCA proper is applied, implemented here as singular value decomposition. This yields the principal components (PCs) and their loadings. The PCs are spatial components (images), whereas the loadings represent the weight each PC has in a given image from the sequence.

**Outcome of PCA Filtering: A Movie**

The PCA outcome consists of a wealth of complex information. To fully understand it would require a complete model of the entire imaging process (instrument and retina). Here we restrict ourselves to the pulsatile aspects: pulsatile components were defined as showing spatial features typical of vessel movements.

The PCs are ranked according to percentage variance explained. Systematic examination of the first 20 PCs revealed that the strongest pulsatile signals were present in the first 5 to 10 PCs (Fig. 2) and that pulsatile signals were occasionally found in higher order PCs. We selected here the first five PCs and built a sequence (later referred to as a movie) based on their respective loadings. The use of more PCs was assessed but did not improve the detection of pulsating features. The average, which had been subtracted before the PCA, was added again to reproduce a typical fundus appearance.

**Observing Processed Movies**

For observation, the movies are set to loop. Movies are not likely to start and stop at the same phase in the cardiac cycle, which causes discontinuity when looping. We thus trimmed the movies to minimize discontinuities because they, like microsaccades, impair visual assessment. The movies’ playback speed proved optimal at real-time playing speed. To avoid distraction, they were observed on a dark screen.
RESULTS

Effect of Each Processing Step

We demonstrate the processing outcome in two subjects, subject 3, presenting the strongest pulsatile amplitude (Supplementary Movies S3b, S3c, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental; Fig. 3 top), and subject 6, presenting a typical pulsatile amplitude (Supplementary Movies S6b, S6c, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental; Fig. 3 bottom). (See Table 1 for a complete list of the movies.)

Each processing step successively improves the detectability of pulsatile movements. Our movies (Supplementary Movies S3c, S6c, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental) demonstrate that each step addresses a particular and well-defined limiting factor. Registration addresses the effect of fixational drifts; removal of these initially obvious distracters emphasizes the effect of microsaccades;

FIGURE 3. Effect of each processing step. This figure presents the structure of the images visible in Supplementary Movies S1b to S10b and S1c to S10c. Left to right: raw images, after registration, after microsaccade rejection, and after PCA filtering. These sequences demonstrate that each processing step isolates pulsatile features. The final PCA-filtered sequences alone are also available as Supplementary Movies S1b to S10b (http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental).

TABLE 1. List of Movies

<table>
<thead>
<tr>
<th>Subject</th>
<th>“Quick Tour”</th>
<th>a: Raw</th>
<th>b: Final Outcome (registered,…, PCA)</th>
<th>c: All Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Finish here</td>
<td>Movie01(a).mov</td>
<td>Movie01(b)(final).mov</td>
<td>Movie01(c)(allSteps).mov</td>
</tr>
<tr>
<td>2</td>
<td>Start here</td>
<td>Movie02(a).mov</td>
<td>Movie02(b)(final).mov</td>
<td>Movie02(c)(allSteps).mov</td>
</tr>
<tr>
<td>3</td>
<td>Continue here</td>
<td>Movie03(a).mov</td>
<td>Movie03(b)(final).mov</td>
<td>Movie03(c)(allSteps).mov</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Movie04(a).mov</td>
<td>Movie04(b)(final).mov</td>
<td>Movie04(c)(allSteps).mov</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Movie05(a).mov</td>
<td>Movie05(b)(final).mov</td>
<td>Movie05(c)(allSteps).mov</td>
</tr>
<tr>
<td>6</td>
<td></td>
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<td>Movie06(b)(final).mov</td>
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<td>Movie08(c)(allSteps).mov</td>
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<td>Movie10(b)(final).mov</td>
<td>Movie10(c)(allSteps).mov</td>
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</table>

All Supplementary Movies are available at http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental. Movies a and b allow the effects of the processing to be distinguished. Movies c demonstrate the effect of each processing step. The “quick tour” covers the observed range of pulsation amplitudes: subject 3 shows a strong pulsation, subject 6 a moderate pulsation, and subject 2 no pulsation.
Observations in Healthy Subjects

Raw and processed image sequences are provided as movies for all subjects. Figure 4 presents image samples from movies of processed data on which the location of the pulsatile feature is indicated. Locations of serpentine movements are indicated by yellow curves, and SVPs are indicated by yellow arrows. Subject 2 shows no apparent pulsation, seven subjects (subjects 1, 3, 4, 6, 7, 9, 10) show serpentine pulsations, and subjects 3 and 4 show strong pulsation. SVP is not seen in five subjects. (subjects 1, 2, 6, 7, 10). Interestingly, some subjects (subjects 1, 6, 7, 10) show no SVP but do show arterial pulsation. Three different types of SVP can be observed. The first is apparent vein diameter variations (subjects 3, 4, 5, 9), not only inside but also outside the disc, typically measuring up to 1 disc diameter. The second is complete collapse. In subject 4, a segment of the inferior hemivein lying on the cup of the disc does not present a bright central stripe but rather a dark one that disappears rhythmically, thus suggesting collapse of the vessel. The third is pulsation where the hemiveins enter the nerve head (subjects 8 and 9). When the entry point is clearly observable, the last segment of the vessel does not present its central reflex but appears uniformly dark and shows a pulsating prolongation of the dark area of the vessel, possibly originating from a lateral movement of the vessel in subject 8, where the prolongation of the vessel after entering is visible through disc translucency. In subject 9, the origin remains more elusive.

Mechanical links between arteries and veins may be deduced from subtle joint movements, as observed in four subjects: in subject 1, between the inferior temporal artery and vein, on the segments distal to the arteriovenous crossing; in subject 3, between the inferior temporal artery and vein, at both segments distal and proximal to the first crossing; in subject 4, between the superior temporal artery and vein on the nearly parallel segments distal to the first crossing; and in subject 6, between the superior temporal artery and vein on the parallel segments distal to the second crossing. These observations suggest that mechanical connections between vessels occur not only at arteriovenous crossings through common adventitial sheath but also between vessels distant by more than 1 vessel diameter.

Detailed Observations in a Strongly Pulsatile Case

Two subjects present strong pulsation and nearly all the observable features in our subjects. Figure 5 details the features observed in the processed movie (Supplementary Movie S3b, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6806/-/DCSupplemental) of one of them (subject 3). The following features are observed: (1) Serpentine movements of the principal arteries (i.e., superior and inferior temporal arteries). The movements are more pronounced where the vessels bend. (2) Spontaneous venous pulsation of the superior temporal vein outside the optic disc. (3) Pulsation of small arteries (~70-µm luminal diameter). (4) Pulsatile movement of the entire optic nerve head vasculature, suggesting a common mechanical origin. (5) Inferior temporal vein displacement at disc rim jointly with the inferior temporal artery, thus suggesting mechanical coupling between vessels. This mechanical coupling could originate from the common path of the vessels in the nerve head, from a common adventitial sheath at the arteriovenous crossing inferior to the disc, or from a connection through the nerve fiber layer. (6) Blood flow markers. Moving features inside the major vessels should not be confused with actual aggregations of erythrocytes, because PCA modifies the relationship between the raw and the filtered image. Thus, these moving features are representative of erythrocyte movements. PCA also improves the visibility of arterioles and venules that can be tracked further down the vascular tree and of choroidal vessels and vessel contours with central reflexes presenting more regular walls. All in all, this demonstrates how highly dynamic the retina is and that it is best observed after removal of distracters such as eye movements.

Amplitude of the Serpentine Arterial Pulsation

The amplitude of the displacement was estimated in the seven subjects presenting arterial pulsation. Displacement was measured on the vessel segments that showed the strongest lateral movement, as represented on Figure 4 by orange bars. The vessel intensity profiles over a 3-pixel-thick section were extracted across time (Fig. 6). The displacement of the artery was then estimated using the displacement of the maximum of the central reflex of the vessel. Because the movement amplitudes are of the order of 1 pixel (11 µm × 11 µm), quantization required super-resolution. The intensity profile signal was up-
DISCUSSION

We present an image-processing chain for fundus image sequences that registers the images, rejects microsaccade contamination, and, with the help of a PCA, segregates the dynamic from the static properties of the retinal image. In this initial small sample population, the dynamics revealed even minute pulsatile features, demonstrating strong pulsatile dynamics in the retina.

Our results suggest improvements in data acquisition for further investigation. The image rate of nine images per second is a little low for capturing the rapid rise in pressure during a systole. Furthermore, given that clinical observations are made in mydriasis anyway, visible illumination could be used (e.g., red-free), thus capturing more information from superficial layers and less from the choroid. An independent component analysis capable of isolating functional signals and removing pulsation could complement the PCA; however we found the present approach to be sufficient.

In this investigation we observed spontaneous venous pulsation in 5 of 10 (50%) subjects, whereas previous studies reported 75% to 90% ophthalmoscopically detectable venous pulsations. Our lower incidence is not likely to have been due to our processing approach because examining our data before PCA processing revealed the same proportion of SVP. Further research could be directed at examining age and other factors affecting the frequency of SVPs.

The etiology of spontaneous venous pulsation is still under debate, especially considering the synchronicity of the arterial and venous pulsations. Better understanding of SVP etiology, combined with a better observation technique, would improve its use as a marker for elevated intracranial pressure or papilledema. So far, explanations have invoked pressure gradients across the lamina cribrosa, and have invoked pressure gradients across the lamina cribrosa.

The present method is easy to implement in any fundus imaging system that allows the acquisition of sequential digital images. PCA is standard in mathematical software packages and computable on modern personal computers. Our implementation requires <10 seconds for the construction of one movie. Furthermore, because the cardiac cycle is obvious in the movie, there is need to simultaneously record ECG, thus allowing noncontact recording.

The observer’s visual system is the last element in the imaging chain. This processing system was designed considering the following psychophysical phenomena:

1. The “mudsplash effect.” Approximately 1 of 10 images is distorted by microsaccades. While a movie is scrutinized, microsaccades seriously disrupt the perception of pulsation through the mudsplash effect. This means that any sudden interruption in image flow, such as brief global or local flashing features, induces “change blindness,” which interferes with coherence-based motion detection. Thus, even obvious changes occurring around “splash time” go unnoticed by the observer.

2. Peripheral movement detection. Because motion detection falls off more slowly with eccentricity than with acuity, a strong impression of overall pulsation is perceived on processed movies, even without specific fixation.

3. Pattern matching. Looped viewing of the processed movies enables the observer to construct an internal
model of the moving structures, making it possible to detect increasingly finer details.

The pulsation of arteries has implications for imaging applications. Modern imaging techniques, such as adaptive optics and frequency domain–optical coherence tomography, are progressing toward cellular resolution.52–54 Eye movement compensation has been demonstrated recently,55 but our results suggest that pulse-gated acquisition may also be necessary. The present technique could be used together with the retinal vessel analyzer56 to rapidly identify locations at which vessel diameters can be measured. Finally, a number of techniques, such as angiography, oximetry, and intrinsic imaging, could benefit from our approach because they all are affected by pulsatile artifacts.

Arterial pulsation analysis may offer new applications. Differing pulsatile features may reveal arteriosclerotic segments, as suggested by Swan and Bailey,25 and a surrogate marker of the eye’s pulse arterial pressure and, consequently, of the brain can be obtained by tracking lateral vessel displacements (Fig. 6).

We have demonstrated that the present technique allows straightforward observation of minute pulsatile features, enabling the visualization and appraisal of dynamic changes in retinal microstructures. It reveals that retinal structures can be more dynamic than is usually assumed, and it may help in the visualization and investigation of retinal and intracranial diseases in an unprecedented manner. Finally, it demonstrates the involvement of perceptual mechanisms for dynamic fundus examination and retinal imaging.

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References


