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Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation

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Mathura, Keshen R., Karlijn C. Vollebregt, Kees Boer, Jurgen C. de Graaff, Dirk T. Ubbink, and Can Ince. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. *J Appl Physiol* 91: 74–78, 2001.—Orthogonal polarization spectral (OPS) imaging is a new clinical technique for observation of the microcirculation of organ surfaces. For validation purposes, we compared OPS images of the nailfold skin with those obtained from conventional capillary microscopy at rest and during venous occlusion in 10 male volunteers. These images were computer analyzed to provide red blood cell velocity and capillary diameters of the same nailfold capillaries at rest and during venous occlusion. Results showed that OPS images provided similar values for red blood cell velocity and capillary diameter as those obtained from capillary microscopy images. OPS imaging, however, provided significantly better image quality, as shown by comparison of image contrast between OPS imaging and capillary microscopy. This made image analysis better and easier to perform. It is anticipated, therefore, that OPS imaging will become a new and powerful technique in the study of the human microcirculation in vivo because it can be used on human internal organs.

nailfold; intravital; validation; erythrocyte; contrast; orthogonal polarization spectral imaging

RECENTLY, OUR LABORATORY APPLIED orthogonal polarization spectral (OPS) imaging for the first-time observation of the human brain microcirculation during surgery (9). Since then, we have applied this new technique in a large number of clinical scenarios and were able to observe single-file movement of erythrocytes, as well as sticking and rolling leukocytes (15). OPS imaging is implemented in a small handheld device. The objective of the device can be sterilized with a sterile cover and drape. It can be easily used, therefore, in various clinical settings and on organ surfaces not accessible to intravital microscopy settings. Validation of OPS imaging, however, needs to be performed to demonstrate that OPS images are as accurate as the current standard in human microcirculatory studies, which is capillary microscopy of the human nailfold. For many years, information concerning the human microcirculation was obtained by use of intravital microscopy for the diagnosis and treatment of peripheral vascular disease, diabetes, hypertension, and other vascular diseases (6, 19–21). Capillary microscopy (capillaroscopy) has been the only technique available for study of the human circulation at the microscopic level in vivo. The capillary microscopy setup consists of an intravital microscope, which restricts its use in humans to the skin and other easy accessible sites like the lip and the bulbar conjunctiva (6).

The OPS imaging device uses the absorption of hemoglobin to visualize the microcirculation using a polarized light technique (9). Polarized lighting techniques have been used to visualize the microcirculation in humans as well as in animals (9, 18). It is expected that OPS imaging may perform better in similar settings as used for capillaroscopy because of the polarized light technique.

The present study was conducted to compare OPS imaging with conventional capillaroscopy for imaging the nailfold of 10 healthy male volunteers. Comparisons between the two techniques were made of the red blood cell velocity (RBCV) and diameter at rest and during venous occlusion. Differences in image contrast between the images produced by capillaroscopy and those of OPS imaging were analyzed and compared with each other. Initial results of this study have been presented elsewhere (22).

METHODS AND SUBJECTS

Technique. The capillaroscopy setup consisted of a Wild-Leitz 3M stereomicroscope and a Leitz Wetzlar 100-W mercury lamp with infrared filter. Incident illumination was achieved by using a Leitz Ploemopak 2.1 system equipped with a Leitz Pol-cube. Leitz objectives of ×4 (numerical aperture = 0.12; on-screen magnification, ×225) and ×10 (numerical aperture = 0.30; on-screen magnification, ×566) were used. Image capture was achieved by a PULNiX TM-6 CN black and white charge-coupled device camera (PULNiX America) positioned in the intermediate image plane. This setup has been used by Ubbink et al. (20, 21) in their studies on critical lower limb ischemia. Images were recorded using

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the ×10 objective. Contrast was compared using the ×10 and ×4 objectives.

A CYTOSCAN E-II Backfocus type device (Cytometrics, Philadelphia, PA) was used for OPS imaging. For positioning purposes, the device was attached to the stage of the capillaroscope by a holder. This holder was custom made at the Department of Instrumentation of the Academic Medical Center of the University of Amsterdam and allowed the device to be positioned on the same platform as the capillaroscope. This allowed for easy switching between OPS imaging and the capillaroscope. For OPS imaging, a ×5 objective (on-screen magnification of ×326) was used during all measurements. The ×10 (on-screen magnification of ×650) and ×5 OPS imaging objectives were used for contrast measurements. All data were recorded on a Sony DSR-20P digital video recorder and visualized on a Sony PVM 97 black and white monitor.

Subjects. For this study, 10 nonsmoking healthy male volunteers were used. None of the subjects used any medication at the time of the study, and all refrained from drinking coffee for at least 2 h before the measurements. Mean age was 22.9 ± 1.4 (SD) yr.

Protocol. The subjects were seated with their arms slightly bent at heart level and resting on a pillow. The fingers were stabilized by embedding them gently in a mass of clay. The temperature in the air-conditioned room was between 23 and 25°C. By random selection, it was decided which device was to be used first. During the measurements using OPS imaging, the capillaroscopy setup was turned completely off and vice versa. A drop of paraffin oil was used to make the skin more translucent and to reduce reflection. From the nailfold of the fourth digit of the nondominating hand, three capillaries were chosen based on their visibility and contrast. These three capillaries were as far apart from each other as possible. We compared the RBCV at rest ($RBCV_r$) and during venous occlusion ($RBCV_{vo}$) to test the response of the nailfold microcirculation to a defined physiological stimulus. This test was chosen because of its good reproducibility (16). Each capillary was recorded for 2 min to measure $RBCV_r$ and capillary diameter at rest. Then a cuff around the upper arm of the subject was inflated to 50 mmHg for venous occlusion, and for 3 min thereafter images were recorded for the measurement of the $RBCV_{vo}$ and capillary diameter during venous occlusion. After these measurements, the cuff was deflated. From nine subjects, the two capillaries that had the best contrast in both the capillaroscopy and OPS image were selected for contrast ratio calculation, because almost all subjects had at least one capillary that was suboptimal in terms of contrast.

A thermocouple connected to a digital thermometer (Keithley 871A, Keithley Instruments) was taped proximal to the nailfold on the skin and used to monitor the skin temperature continuously. The measurements were excluded if the skin temperature changed >2°C. In a separate experiment, the influence of emitted light from both setups on the temperature was measured using a thermocouple connected to the digital thermometer. The tip of the thermocouple was put in the light of the device and kept there until a stable temperature was recorded. This temperature was compared with that of the room temperature measured using the same thermocouple and thermometer as described above. The door was closed during all experiments as described above. Written, informed consent was obtained from all subjects. Approval from the local Academic Medical Center Medical Ethics Committee was obtained for this study.

Analysis. The RBCV and capillary diameters were analyzed by using a specialized software program for the analysis of video sequences of the microcirculation (CapImage, Dr. Zeintl Software Engineering, Heidelberg, Germany) (12). The program uses the line-shift diagram method for measuring RBCV (5, 12). Capillary diameter was calculated by measuring the diameter of the erythrocyte column inside the arterial limb of the capillary loop. Contrast ratios were measured with the use of the ImageJ program (developed at the US National Institutes of Health). To compare image contrast, an oval region of interest (size, 5 × 10 pixels) was selected inside the vessel as well as outside the vessel adjacent to it. The region of interest inside the vessel was selected in the bend of the venous limb for each capillary. The mean pixel value of the region of interest was then measured by ImageJ. The mean pixel value of the region inside the vessel was the I_{min}, and the region outside the vessel was the I_{max} (the pixel value of black = 1 and white = 255). The ratio of these values $[(I_{max} - I_{min})/(I_{max} + I_{min})]$ of capillaroscope and OPS imaging were then compared as a method for measuring contrast. Best image contrast was achieved before the image was made for contrast comparison. The ratios were calculated for the ×10 and ×4 objectives of the capillaroscope and the ×5 and ×10 objectives of the OPS imaging device. The ×10 objective of the capillaroscope and the ×5 objective of the OPS imaging device were used for the velocity, diameter, and temperature measurements.

Statistical analysis. All results were analyzed by using a Student's paired *t*-test. A *P* value of <0.05 was considered significant. To graphically compare the results of both techniques, they were plotted in a Bland-Altman plot (3, 4). The limits of agreement were defined as the mean difference ± 2 SD of the difference, according to Bland and Altman (3, 4). Linear regression was also calculated for each Bland-Altman plot to test for systematic errors. The regression coefficient and *P* value are given for each plot. All data are represented as means ± SD, unless stated otherwise.

RESULTS

The main result of this study was that OPS imaging is comparable to conventional capillaroscopy in measuring $RBCV_r$, $RBCV_{vo}$, and capillary diameter. Comparison in image contrast shows that images made by OPS imaging have better contrast than conventional capillaroscopy.

A total of 30 capillaries in the 10 subjects were studied. It was possible to measure the $RBCV_r$ of 21 capillaries and $RBCV_{vo}$ of 22 capillaries using capillaroscopy. OPS imaging allowed measurement of the $RBCV_r$ of all 30 capillaries and the $RBCV_{vo}$ of 28 capillaries. Failure to measure $RBCV_r$ or $RBCV_{vo}$ was due to failure of CapImage to correct for movement of the finger because there was insufficient contrast. CapImage failed to correct for movement in nine capillaroscopy images for both $RBCV_r$ and $RBCV_{vo}$. Movement correction failed in two OPS images for measuring $RBCV_{vo}$. There was no significant difference ($P = 0.32$) between the $RBCV_r$ measured by the capillaroscope (0.77 ± 0.24 mm/s) and OPS imaging (0.74 ± 0.28 mm/s), as shown in Fig. 1, which is also reflected in the Bland-Altman plot (Fig. 2). There was no significant difference ($P = 0.11$) between the $RBCV_{vo}$ measured by the capillaroscope (0.19 ± 0.14 mm/s) and OPS imaging (0.15 ± 0.091 mm/s) as shown in Figs. 1 and 3. The $RBCV_{vo}$ was significantly lower than $RBCV_r$, both for capillaroscopy ($P < 0.0001$) and for

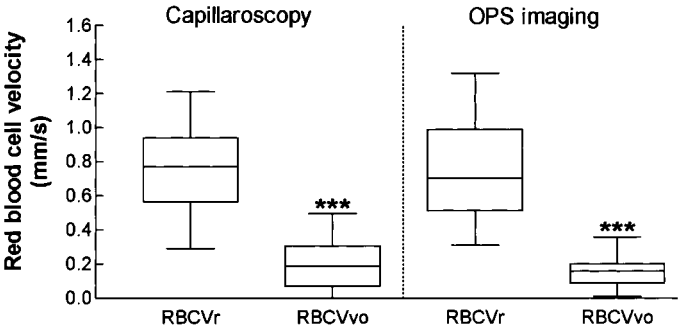

Fig. 1. Box-and-whisker plots showing the measured red blood cell velocity (RBCV) at rest ($RBCV_r$) and during venous occlusion ($RBCV_{vo}$). The boxes in the plots represent the 25th and 75th percentiles. The lines in the boxes represent the 50th percentile. The whiskers represent the highest and lowest value in the data. $RBCV_{vo}$ was significantly lower than $RBCV_r$, both for capillaroscopy and orthogonal polarization spectral (OPS) imaging, $^{***}P < 0.0001$.

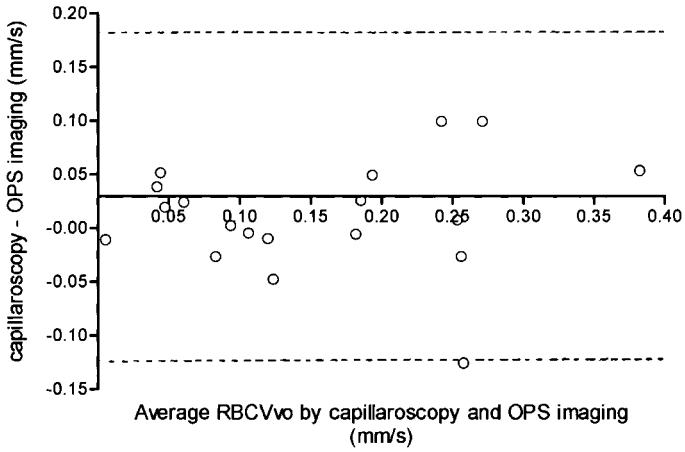

Fig. 3. Bland-Altman plot of the measured $RBCV_{vo}$. Dashed lines represent ±2 SD.

OPS imaging ($P < 0.0001$), as shown in Fig. 1. The plots (Figs. 2 and 3) also show that the differences were equally distributed across the range of values measured, and there were no significant systematic differences (linear regression: $r = 0.25$, $P = 0.28$ for $RBCV_r$; $r = 0.43$, $P = 0.05$ for $RBCV_{vo}$).

The mean rest diameter measured from the capillaroscopy images was 11.2 ± 1.6 vs. 11.2 ± 2.0 μm for OPS imaging. The capillary diameter increased significantly (capillaroscopy, $P = 0.0045$; OPS imaging, $P = 0.0086$) during venous occlusion as measured both for capillaroscopy and OPS imaging (Fig. 4). The mean capillary diameter during venous occlusion was 12.0 ± 1.6 μm for capillaroscopy and 12.1 ± 1.8 μm for OPS imaging. There were no significant differences in capillary diameter measured by capillaroscopy or by OPS imaging either during rest ($P = 0.80$) or during venous occlusion ($P = 0.43$). The Bland-Altman plots for capillary diameter (Figs. 5 and 6) show an equal distribution and no significant systematic differences (linear regression: $r = 0.33$, $P = 0.089$ for capillary diameter at rest; $r = 0.17$; $P = 0.41$ during venous occlusion).

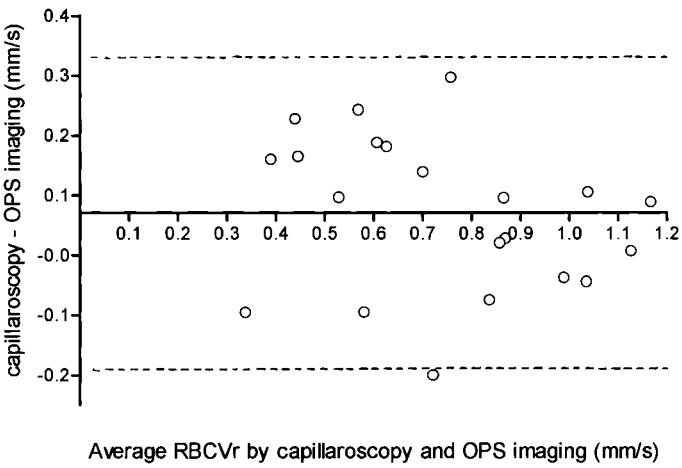

Fig. 2. Bland-Altman plot of the measured $RBCV_r$. Dashed lines represent ±2 SD.

The temperature of the skin proximal to the nailfold did not change >1°C during all experiments. Temperature measurements revealed that the light emitted by the capillaroscopy setup increased the temperature of the illuminated surface by 0.8°C. No increase in illuminated surface temperature was found using OPS imaging.

The contrast ratio of the capillary microsocope ×4 objective was 0.098 ± 0.031 AU and that of the ×10 objective was 0.16 ± 0.038 AU. The contrast ratio of the ×5 objective of the OPS imaging device was 0.15 ± 0.067 AU and that of the ×10 objective was 0.24 ± 0.067 AU. The images acquired by OPS imaging ×5 and ×10 objectives had a significantly higher contrast ratio than the images obtained by capillaroscopy using the ×4 and ×10 objectives, as shown in Fig. 7.

DISCUSSION

The present study has shown that the values of microcirculatory parameters from the human nailfold

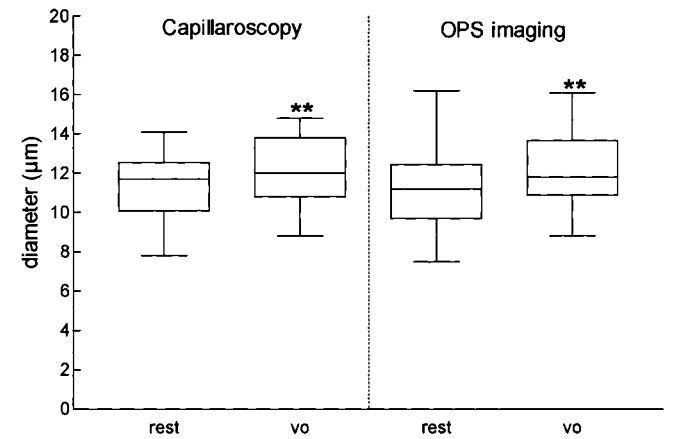

Fig. 4. Box-and-whisker plots showing the measured diameters at rest and during venous occlusion (vo). The boxes represent the 25th and 75th percentiles. The lines in the boxes represent the 50th percentile. The whiskers represent the highest and lowest values in the data. Capillary diameter increased significantly during vo for both capillaroscopy ($^{**}P = 0.0045$) and OPS imaging ($^{**}P = 0.0086$).

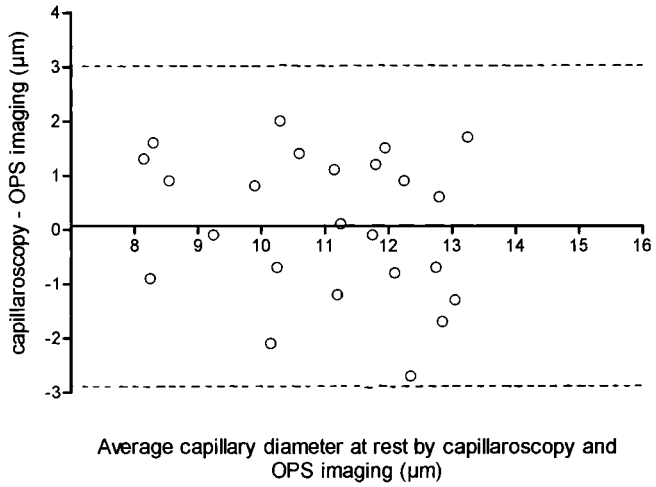

Fig. 5. Bland-Altman plot of the measured capillary diameter at rest. Dashed lines represent ±2 SD.

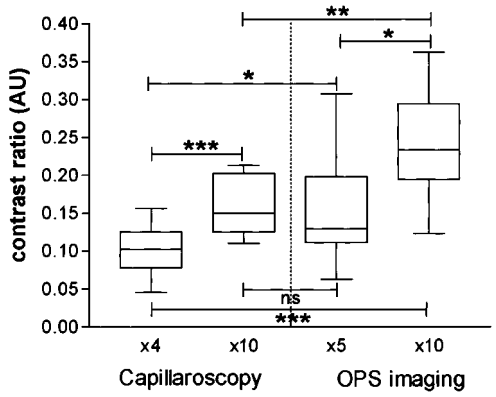

Fig. 7. Box-and-whisker plots showing the calculated contrast ratios. Boxes in the plots represent the 25th and 75th percentiles. The lines in the boxes represent the 50th percentile. The whiskers represent the highest and lowest values in the data. Significant differences: $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$. AU, arbitrary units; ns, not significant.

obtained by OPS imaging are comparable to those obtained by capillaroscopy. Images obtained by OPS imaging, however, had significantly better contrast (Fig. 7). This enhanced contrast gave better quality images for computer analysis, resulting in less failure to correct for movement by the CapImage software. Therefore, OPS imaging performed better in circumstances with suboptimal variables than did capillaroscopy.

The $RBCV_r$ measured by both techniques were not significantly different. There was, however, a large variability in the results of both techniques (Fig. 2), probably because of the large variability in RBCV caused by flow motion. It is known that the $RBCV_r$ in capillaries shows spontaneous fluctuations with a frequency of 4–11 cycles/min (7, 8, 17). These fluctuations are due to changes in blood pressure, changes in smooth muscle cell tonus in the arterioles, or baroreceptor regulation (2, 8, 10). The capillaroscopy setup that we used showed an increase in temperature of the illuminated surface, whereas OPS imaging did not. The temperature of the skin is a major influence on RBCV (1, 6). It is, however, uncertain whether this increase of 0.8°C can influence the RBCV enough to be significant. The present study found no significant difference between the RBCV measured using capillaroscopy and that using OPS imaging.

The 2 SD range on the $RBCV_{vo}$ Bland-Altman plot was narrower than the 2 SD range of the $RBCV_r$ Bland-Altman plot, indicating that the difference in RBCV measured by the two techniques is less during venous occlusion. This is also reflected by the narrower range of the $RBCV_{vo}$ data compared with that of the $RBCV_r$ in the box-and-whisker plots in Fig. 1. These findings are in agreement with the observations by Rosen et al. (16).

There were no significant differences in capillary diameter either during rest or during venous occlusion using either technique (Fig. 4). The Bland-Altman plots show evenly distributed data. Venous occlusion caused an increase in capillary diameter compared with rest diameter. An increase in capillary diameter during venous occlusion has been described before using FITC-labeled albumin (13).

The great advantage that OPS imaging has over capillaroscopy is its applicability on organ surfaces, where not only capillaries but also complete microcirculatory networks can be studied. Because the OPS imaging device is built into a small handheld device, it is more practical than a conventional capillaroscope and can be used anywhere, including during surgery. This way, our laboratory has applied OPS imaging during neurosurgery and in the intensive care unit and to examine the rectal microcirculation during inflammatory bowel disease (11, 14, 15). It is anticipated, therefore, that OPS imaging will open the way to study the human microcirculation much more extensively than has been previously possible, because results from the present study show that OPS imaging provides images of good quality from which quantitative microcirculatory data can be obtained. The better contrast of OPS imaging gives a better signal quality for

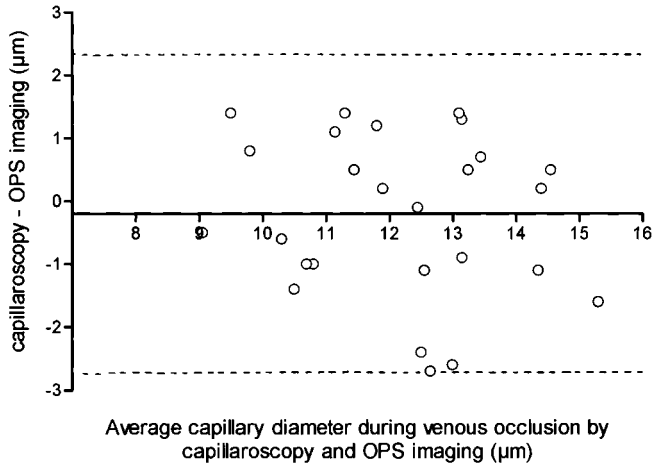

Fig. 6. Bland-Altman plot of the measured capillary diameter during venous occlusion. Dashed lines represent ±2 SD.

computer analysis, making quantification of images more precise.

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