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A new complimentary web-based tool for manual analysis of microcirculation videos: validation of the Capillary Mapper against the current gold standard AVA 3.2

Running title: A web-based tool for microcirculatory analysis

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Abstract

OBJECTIVE: The aim of the current study was to compare a newly developed web-based freely accessible software program for manual analysis of the microcirculation, the Capillary Mapper (CM), with AVA3.2 software (AVA; MicroVision Medical B.V., Amsterdam, the Netherlands), which is the current gold standard for analysis of microcirculation videos.

METHODS: A web-based software program was developed, which enables manual analysis of videos of the microcirculation to be carried out according to recommendations of the 2018 consensus conference. A set of 50 high quality microcirculation videos was analyzed with AVA and CM with respect to total vessel density, perfused vessel density, proportion of perfused vessels, and the microvascular flow index.

RESULTS: Comparison of the mean values derived from manual analysis with CM and AVA revealed no significant differences in microcirculatory variables. Analysis according to Bland and Altman revealed an acceptable bias between manual analysis with the CM and AVA for all variables tested with sufficient limits of agreement. The analysis of intraclass correlation showed “excellent” agreement for all microcirculatory variables analyzed.

CONCLUSIONS: The newly developed CM was successfully validated for manual analyses of microcirculation videos against the current gold standard, the software AVA 3.2.

KEYWORDS: Microcirculation; analysis; software; validation.

List of Abbreviations

AVA, Automated Vascular Analysis;
AVI, audio video interleave;
CCtools, CytoCamTools;
CM, Capillary Mapper;
HVM, hand-held vital microscopes;
ICC, intraclass correlation coefficient;
IDF, incident dark field;
LOA, limits of agreement;
MFI, microvascular flow index;
PPV, proportion of perfused vessels;
PVD, perfused vessel density;
QS, quantizer scale;
SDF, sidestream dark field;
SVG, scalable vector graphics;
TVD, total vessel density.

Introduction

Disturbances of the microcirculation are of crucial relevance in the development of organ dysfunction and are associated with increased mortality.[1, 2] Whereas former technologies required the application of a dye to visualize the microcirculation for a limited time period, newly developed computer-controlled image sensor-based HVM can visualize the microcirculation directly and continuously, and are therefore finding widespread use in experimental and clinical research.[3, 4] In 2007 a consensus conference made the first recommendations for the analysis of microcirculation videos.[5] A second consensus on the assessment of sublingual microcirculation in critically ill patients was published recently.[4] However, the analysis of microcirculatory videos is still dependent on the individual investigator, because available programs allow only manual or semi-manual analyses. This risk of bias would be eliminated with software for automatic analysis, but such programs have so far proven insufficiently accurate on testing.[6, 7] In addition, the few commercially available software packages that have been validated are quite expensive.[3, 5, 8] Users have therefore turned to standard image processing programs lacking specific

validation.[9] Because of its usability and reasonable analysis time, the commercial software AVA 3.2 (Microvision Medical B.V., Amsterdam, the Netherlands) is most commonly used for manual analysis of microcirculation videos and frequently taken as the gold standard.[6, 8]

As a consequence, there is an imminent need for freely accessible software for the standardized analysis of microcirculation videos, according to the recommendations of the consensus conference, to promote this crucial area of research and allow more widespread use of bedside microvascular monitoring. The Capillary Mapper 1.3 (http://capillary-mapper.uni-muenster.de/), a web-based freely accessible software program for the manual analysis of the microcirculation, was developed to meet this demand. The aim of the current study was to compare the Capillary Mapper 1.3 with the AVA 3.2 software, which is currently the gold standard for manual analysis of microcirculation videos according to the latest recommendations of the consensus conference.[4, 5]

Materials and Methods

Software development of Capillary Mapper 1.3

The primary objective of the software development was the programming of a complimentary web-based software for fast and reliable manual analysis of microcirculation videos. Such videos, recorded by modern HVM, tend to have high data volumes. For a web-based approach, these videos therefore needed to be converted into a browser playable file format. An algorithm for automatic conversion of microcirculation videos into a browser playable format using H.264 codecs (FFMPEG free software project) was therefore developed. However, most common browsers are not able to replay videos, which have been converted by lossless compression (i.e. where the original video data can be reconstructed almost perfectly from the compressed data). To overcome this issue, a QS value of 6 was chosen for the compression of the video files (the range of the QS of the H.264 codec is 0-51, whereby 0 is lossless, 23 is standard, and 51 is worst quality). According to experienced analyzers of microcirculation videos, no visible reduction in video quality was apparent with these settings. Even the granularity of the videos was preserved during the conversion, thereby retaining fine movements and changes in brightness.

For manual analysis, a graphical user interface was developed including a drawing function based on SVG. These SVG polygons enable the length and diameter of drawn capillaries to be determined. The quality of capillary flow (classified as absent, intermittent, sluggish, or continuous following the recommendation of the consensus conference[4]) was attached to the SVG polygons as a file attribute. As video material is based on pixels, it is possible to calculate the capillary length (using the center-line

of the capillaries). Finally, the microcirculatory parameters recommended by the consensus conference are calculated and saved in a database.

Analysis of microcirculation using Capillary Mapper 1.3

Basic principles of microcirculatory analysis were recently described in detail by Massey et al.[3] The analysis report of the Capillary Mapper is based on the recommendations of the latest consensus conference.[4] In short, analysis of videos of the microcirculation is carried out as follows: After logging in to the welcome page (http://capillary-mapper.uni-muenster.de/; each user is given a personal user account), and it is possible to upload microcirculatory videos, which are automatically converted into a browser playable format. Before uploading, it is necessary to specify the spatial calibration for each video (manual entry, e.g. x-axis: 1 pixel=0.66 µm; y-axis: 1 pixel=0.66 µm). Uploaded videos are replayed on a graphical user interface, which enables videos of the microcirculation to be analyzed (Figure 1 shows a screenshot of the graphical user interface of the Capillary Mapper 1.3). Depending on the image resolution, navigation elements of the analysis are arranged variably around the video (e.g. a higher video resolution leads to a larger video image in the browser). It is therefore possible to analyze different video resolutions as long as the spatial resolution is known. For determination of TVD, PVD and PPV, capillaries are drawn in by hand using a computer mouse or other suitable input device (e.g. drawing pen) and the flow is then classified as absent, intermittent, sluggish, or continuous. The $MFI_{quadrant}$ is taken as the average of the predominant flow in each of the four quadrants in the video.[10] This, in turn, is used to obtain the heterogeneity index, which is calculated as the highest flow value in the quadrants of the $MFI_{quadrant}$ minus the lowest flow value, divided by the mean flow (=$MFI_{quadrant}$).[5, 11]
Besides the quadrant-based MFI ($MFI_{quadrant}$), the Capillary Mapper automatically calculates a MFI (MFI_{vessel}) for each individual vessel. The flow in each vessel is thereby multiplied by its length and MFI_{vessel} is calculated as the mean of the products divided by the total vessel length of all individual vessels in the video.
As suggested by the 2018 consensus conference, the option of assessing videos according to the Microcirculation Image Quality Score of Massey et al. is included in the analysis menu of the Capillary Mapper.[12]
The Capillary Mapper analysis results are continuously displayed in real-time in a table below the video (see Figure 2. A user manual for the Capillary Mapper 1.3 is supplied as supplemental digital content; see Supporting Information 1).

Comparison of microcirculatory analyses by AVA 3.2 and Capillary Mapper 1.3

A set of 50 videos, each 5 seconds in length with a frame rate of 25 per second, was randomly chosen from a large database of high quality videos[12] of ovine conjunctival and sublingual microcirculation including sheep in a healthy state as well as in septic or hemorrhagic shock (approval numbers of the Animal Care Committee of the State Government of North-Rhine Westphalia 84-02.04.2015.A555 and 84-02.04.2012.A297). All videos were recorded with a computer-controlled image sensor-based HVM (CytoCam®, Braedius medical, Huizen, the Netherlands) and afterwards exported in AVI file container format (video resolution 720 x 480 pixel), which is necessary to analyze videos with AVA 3.2 software.[8] Analyses of the videos were performed with Capillary Mapper 1.3 and AVA 3.2 by an experienced examiner using the same values for spatial calibration (x-axis: 1 pixel=1.3625 µm; y-axis: 1 pixel=1.3635 µm). The examiner was blinded with respect to the pathological condition of the individual animal. Values for TVD, PVD, PPV, and $MFI_{quadrant}$ were obtained for microvessels, which are defined as vessels with a diameter <20 µm and include arterioles, capillaries, and venules, with both software solutions.[4] In addition, the time for the actual analysis (without time requested for loading, stabilization or converting the video) was assessed. MFI_{Vessel} and heterogeneity index were not compared, as AVA 3.2 software does not cover these parameters.

Statistical methods

Statistical analysis was performed with IBM SPSS statistics software version 24 (IBM, Armonk, New York, USA). All data are presented as mean with standard deviation unless otherwise stated.
Variables were tested to confirm the equality of variances by Levene's test, and the Kolmogorov-Smirnov test was used to confirm normal distribution. Subgroups of compromised and non- compromised microcirculation were formed using an arbitrary cut-off value of PPV <95% in videos analyzed with the gold standard AVA 3.2. Comparisons between groups were made using the t-test for independent groups. Comparisons for agreement between the two analysis software programs were made using intraclass correlation and calculating ICC. The ICC are presented with 95% confidence intervals as a measure of dispersion.[13] The values of the ICC can theoretically range from 0 to 1, a higher value indicating less variance between the analyses with the two software options. According to Cicchetti et al., agreement was characterized as "poor" for values below 0.40, as "fair" between 0.40 and 0.59, as "good" between 0.60 and 0.74 and as "excellent" for values greater 0.74.[14] In addition, agreement was analyzed following the suggestions of Bland and Altman and Bland-Altman-plots were drawn.[15] Bland-Altman plots are constructed by plotting the mean difference of the two values (AVA 3.2 and Capillary Mapper 1.3) for each video against the average of those two values. The mean bias (95% confidence interval) was calculated as well as the LOA as 1.96-fold of the standard deviation of

the mean bias. In addition, percentage error was calculated (1.96 standard deviation of the mean bias for both software programs divided by the mean of the reference method).[16] Recently Carsetti et al. assumed that new software to determine microcirculatory variables can be considered interchangeable when the percentage error does not exceed >30 %.[7, 16] Asymptotic, two-sided *P*-values smaller than 0.05 were taken as statistically significant.

Results

The comparison of the mean values derived from manual analysis with Capillary Mapper 1.3 and AVA 3.2 software showed no significant differences with respect to microcirculatory variables in the current sample of videos (n=50; Table 1). Time required for analysis was significantly shorter using the Capillary Mapper 1.3. In subgroups of non-compromised and compromised microcirculation, no significant differences in microcirculatory variables were found between groups. Analysis time was significantly shorter in both subgroups using the manual analysis with Capillary Mapper 1.3 (Table 1).

Results of the intraclass correlation between Capillary Mapper 1.3 and AVA 3.2

The analysis of intraclass correlation showed “excellent”[14] agreement for all microcirculatory variables analyzed in all videos (n=50) as well as in the subgroups of non-compromised and compromised microcirculation (Table 2). Figure 3 shows scatterplots of the PVD and TVD analyzed with AVA 3.2 plotted against the analysis results of the Capillary Mapper 1.3.

Bland-Altman analysis between Capillary Mapper 1.3 and AVA 3.2

Analysis according to Bland and Altman[15] revealed an acceptable bias between manual analysis with the Capillary Mapper 1.3 and AVA 3.2 for all variables tested and subgroups with sufficient LOA (Table 3). Figure 4 presents the respective Bland-Altman plots for TVD and PVD (n=50). Percentage error of microcirculatory variables and groups did not exceed the cut-off of >30 % for interchangeability of two methods to determine microcirculatory variables.[7, 16]

Discussion

The main outcome of the current study was the successful validation of the newly developed complimentary web-based tool, Capillary Mapper 1.3, for manual analyses of microcirculation videos against the current gold standard software AVA

3.2. Analyses performed with Capillary Mapper 1.3 showed excellent agreement (defined as values greater than 0.74 according to Cicchetti et al.[14]) with respect to variables recommended by the consensus conference under healthy and pathological conditions. Notably, time needed for actual analysis was significantly shorter using the Capillary Mapper 1.3 compared to analysis with AVA 3.2.

As long as the complex reality of the microcirculation is encoded in rectangular pixels in videos, all values derived from a video will tend to be approximate rather than exact. Precision of microcirculatory analysis is therefore crucially dependent on the spatial resolution of an imaging technology. In this context, van Elteren et al. demonstrated the superiority of modern IDF computer-controlled image sensor-based HVM over SDF technology.[17] Indeed, this simplistic interrelationship facilitated the approach to the calculation of microcirculatory variables recommended by the consensus conference[4] , i.e. use of the Capillary Mapper 1.3 with addition of hypotenuses. However, despite the robustness of this method of calculating microcirculatory variables, the provision of microcirculation videos in a web-based tool required compromises regarding data handling and processing (as described above). Thus, the need arose for validation of the method against the current gold standard, AVA 3.2, comparing video by video with the same spatial calibration. Overall, in the chosen sample of 50 videos the interclass correlation shows an "excellent" level of agreement[14], while the analysis according to Bland and Altman[15] reveals an acceptable bias between the two sets of measurements.

Despite narrow LOA for all analyzed microcirculatory variables, Bland-Altmann plots and scatterplots (figures 3 and 4) reveal slight variability in single measurements. This variability could be explained by the principle applied for determination of microcirculatory variables: Values are calculated from a manual analysis of microcirculatory videos. This means that vessels are detected visually and drawn by hand (in the case of AVA 3.2 supported by an algorithm, but still depending on an examiner – and therefore termed semi-manual). This naturally accounted for some variability between the two measurement methods, although the same experienced examiner conducted the analyses in both cases. In other words, the variability observed may be a reflection of the manual detection method used as much as a possible inaccuracy in software performance.[7, 10, 18, 19] Notably, against the background of previous studies on the inter- and interrater variability of microcirculatory analyses, the low variability between the two methods in the present study would seem acceptable. In this context, Carsetti et al. compared semi-manual analyses with AVA 3.2 and an automated analysis using CytoCamTools 1.7.12 (CCtools; Braedius medical, Huizen, The Netherlands) and interpreted a much larger bias between TVD by CCtools and TVD by AVA 3.2 as comparable between software (mean bias was 2.20 $mm \cdot mm^{-2}$ with LOA of -4.39 to 8.7 vs. -0.48 $mm \cdot mm^{-2}$ with LOA of -2.70 to 1.75 in the present study). In addition, percentage errors of all the microcirculatory variables analyzed in the current study lie far below the cut-off

value for interchangeability between two microcirculatory measurement methods (>30%) reported in the literature.[7]

In the present study, a subgroup analysis of compromised and non-compromised microcirculation was conducted to evaluate differences in software performance under pathological conditions. In both subgroups (compromised and non-compromised), interclass correlation showed a high agreement between the two software options, supported by a small bias in Bland-Altmann plots with acceptable LOA and acceptable percentage error.[7] The possible risk of a reduced quality of analysis, where microcirculation is compromised, for example in septic or hemorrhagic shock, could therefore be excluded. In contrast, automatic microcirculation analysis software was recently shown to fail to discriminate between microcirculation in healthy animals and under hemorrhagic shock.[6]

Overall, the time taken for analysis with the Capillary Mapper 1.3 was significantly shorter for all videos and also for subgroups of compromised and non-compromised microcirculation compared to the gold standard AVA 3.2. A few points may have contributed to this time saving. First, in contrast to AVA 3.2, the Capillary Mapper 1.3 allows capillary drawing and concurrent flow characterization while the videos are still running. Second, the diameter can be preselected for a selected capillary region with Capillary Mapper 1.3, because the diameter of small capillaries is almost constant in sections before branching. With AVA 3.2, on the other hand, the diameter of each capillary needs to be chosen separately. Third, during the development of Capillary Mapper emphasis was placed on usability; for example, diameter and flow can be adjusted by keyboard shortcuts, allowing additional time saving. Nevertheless, analysis took twice as long with the Capillary Mapper 1.3 than with automatic CCTools, as recently reported.[7] However, as long as automatic analysis is associated with the current significant lack of accuracy and the results show considerable bias[6, 7], the Capillary Mapper represents a valid alternative to the gold standard AVA 3.2. Another advantage of the Capillary Mapper over current automatic analysis tools is its universal applicability. Certain capillary regions with a very dark background (e.g. kidney or intestinal villi microcirculation[20]) may be almost inaccessible to current automated analysis tools. In addition, by not requiring software installation, this approach may allow more flexibility in microcirculatory research and teaching. Of note, it is possible to use the Capillary Mapper 1.3 on tablet computers. Using fingers to draw capillaries further accelerates manual analysis, thereby potentially enabling “eyeballing” of microcirculatory analyses.[3, 20] However, this hypothesis needs to be verified in future investigations.

There are some limitations regarding the present study, which should be acknowledged. First, the study was designed to compare two different software solutions for microcirculatory analysis with one single experienced examiner, who examined each video with the respective software. It may be argued that one examiner is insufficient. However, the interrater reliability in microcirculatory analyses using manual software is generally high, even when examiners are novice users.[9, 19]

Furthermore, since the current study found no relevant bias between the two software packages, we assumed interrater reliability to be sufficient. Second, although the Capillary Mapper 1.3 provides microcirculatory variables for capillaries (which were defined as vessels <10 µm in diameter[4]), microvessels (<20 µm in diameter) and all vessels (≥20 µm in diameter), the current study focused primarily on microvessels. Thus, the results of the current study may not be fully applicable to capillaries and larger vessels. However, since values for microvessels are calculated from the data of all vessels using an arbitrary cut-off value for diameter (in the current study set at <20 µm), data for capillaries and all vessel can be assumed to be robust.
It should also be mentioned that for the current study, microcirculatory videos of sheep were randomly chosen from a video database and analyses with the Capillary Mapper and AVA3.2 were checked for consistency. To further demonstrate the ability of the newly developed Capillary Mapper to detect and replicate established findings in disease states and trace the time course of changes, we analyzed sample videos of the microcirculation in sheep in hemorrhagic shock and after volume therapy with the Capillary Mapper (see Supporting Information 2).

Conclusions

The newly developed Capillary Mapper 1.3 was successfully validated for manual analyses of microcirculation videos against the current gold standard, the software AVA 3.2. Analyses performed with Capillary Mapper 1.3 showed excellent agreement with respect to variables recommended by the consensus conference under healthy and pathological conditions. Notably, the time needed for actual analysis was significantly shorter using the Capillary Mapper 1.3 than with AVA 3.2. As a web-based approach with complimentary availability and equivalence to the gold standard, Capillary Mapper 1.3 presents a tool of great potential for future research into the microcirculation in health and disease.

Perspectives

Techniques for bedside monitoring of the microcirculation are currently being introduced into clinical practice. The Capillary-Mapper, a web-based freely accessible software package based on current recommendations for analysis of the microcirculation, was developed to achieve more flexibility in research and teaching within this field. In this study, the newly developed Capillary-Mapper was successfully validated for manual analyses of microcirculation videos against the current gold standard.

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Competing interests

Michael Hessler received reimbursement of travel expenses from Astellas Pharma. Tim-Gerald Kampmeier received travel reimbursements and honoraria as a consultant from Fresenius Kabi Germany. Sebastian Rehberg received reimbursement of travel expenses from Orion Pharma and Astellas Pharma and is Medical Advisor for Fresenius Kabi Germany. Can Ince has developed SDF imaging and is listed as inventor on related patents commercialized by MicroVision Medical (MVM) under a license from the Academic Medical Center (AMC) as well as the software platform AVA described in this paper. He has no connection to MVM in any form. Braedius Medical, a company owned by a relative of Can Ince, has developed and designed a hand-held microscope called CytoCam-IDF imaging. Can Ince has no financial connection to Braedius Medical of any sort, i.e., has never owned shares, or received consultancy or speaker's fees from Braedius Medical. The remaining authors have disclosed that they do not have any conflicts of interest.

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Legends to figures

Figure 1 Screenshot of the graphical user interface of the Capillary Mapper

Microcirculatory videos are replayed in the center of a graphical user interface. Flow states are color-coded in the video, ranging from red: hyperdynamic to light rose: no flow. For details, please see the user manual for the Capillary Mapper 1.3 (Supporting Information 1).

Figure 2 Screenshot of the Capillary Mapper analysis results section

Results are continuously displayed in real-time in a table below the video. For details, please see the user manual for the Capillary Mapper 1.3 (Supporting Information 1).
Abbreviations: HI: heterogeneity index; $MFI_{quadrant}$: microvascular flow index by quadrants; MFI_{vessel}: microvascular flow index by vessels; PVD: perfused vessel density; PPV: proportion of perfused vessels; QS: Microcirculation Image Quality Score; TVD: total vessel density.

Figure 3 Scatterplots of microcirculatory variables

Values for Total Vessel Density (TVD; A. n=50) and Perfused Vessel Density (PVD; B. n=50) measured with AVA 3.2 were plotted against values calculated with Capillary Mapper 1.3. Dotted lines indicate optimal agreement. Continuous lines show regression lines.

Figure 4 Bland-Altman plots for Total Vessel Density (TVD; A) and Perfused Vessel Density (PVD; B; each n=50).

Continuous line represents the mean difference whereas upper and lower dashed lines represent the limits of agreement (LOA; equivalent to ±1.96 standard deviation [SD] of mean difference).

Legends to tables

Table 1 Microcirculatory variables and analysis time

* indicates a statistically significant difference between groups.
Abbreviations: $MFI_{quadrant}$: microvascular flow index; PVD: perfused vessel density; PPV: proportion of perfused vessels; Time: mean time for analysis of a single video with the respective software package (Capillary Mapper 1.3 or AVA 3.2); TVD: total vessel density.

Table 2 Intraclass correlation of microcirculatory variables.

Abbreviations: CI: confidence interval; ICC: intraclass correlation coefficient; $MFI_{quadrant}$: microvascular flow index; PVD: perfused vessel density; PPV: proportion of perfused vessels; TVD: total vessel density.

Table 3 Bland-Altman analysis between Capillary Mapper 1.3 and AVA 3.2 and percentage error

Abbreviations: LOA: limits of agreement; PVD: perfused vessel density; PPV: proportion of perfused vessels; TVD: total vessel density

Supporting Information

Supporting Information 1

User manual for the Capillary Mapper 1.3 with commentated screenshots of the web page.

Supporting Information 2

Analysis of microcirculatory videos of sheep in hemorrhagic shock and after resuscitation with the Capillary Mapper.

Table 1 Microcirculatory variables and analysis time

All videos (n=50)			
Variable [Unit]	**Capillary Mapper**	**AVA 3.2**	***P*-value**
TVD [mm/mm^2]	17.5 ± 4.2	18.0 ± 4.2	0.575
PVD [mm/mm^2]	15.2 ± 5.6	15.5 ± 5.8	0.769
PPV [%]	86.5 ± 22.8	85.6 ± 23.2	0.844
$MFI_{quadrant}$	2.9 ± 0.4	2.8 ± 0.4	0.525
Time [min]	6.7 ± 2.2	8.9 ± 3.0	<0.001 *
Subgroup: Non-compromised microcirculation (n=22)			
TVD [mm/mm^2]	18.2 ± 3.5	18.8 ± 3.7	0.572
PVD [mm/mm^2]	17.8 ± 3.6	18.4 ± 3.7	0.556
PPV [%]	97.9 ± 1.6	97.9 ± 1.8	0.942
$MFI_{quadrant}$	3.0 ± 0.3	3.0 ± 0.2	0.364
Time [min]	6.3 ± 1.8	7.9 ± 2.3	0.011 *
Subgroup: Compromised microcirculation (n=28)			
TVD [mm/mm^2]	17.0 ± 4.7	17.4 ± 4.5	0.773
PVD [mm/mm^2]	13.1 ± 6.0	13.2 ± 6.11	0.958
PPV [%]	77.5 ± 27.5	75.9 ± 27.4	0.822
$MFI_{quadrant}$	2.7 ± 0.4	2.7 ± 0.4	0.739
Time [min]	6.9 ± 2.5	9.8 ± 3.3	0.001 *

** indicates a statistically significant difference between groups.*
Abbreviations: $MFI_{quadrant}$: microvascular flow index; PVD: perfused vessel density; PPV: proportion of perfused vessels; Time: mean time for analysis of a single video with the respective software package (Capillary Mapper 1.3 or AVA 3.2); TVD: total vessel density.

Table 2 Intraclass correlation of microcirculatory variables.

All videos			
Variable [Unit]	**Number**	**ICC [95% CI]**	**Agreement**
TVD [mm/mm^2]	50	0.968 [0.968-0.990]	Excellent
PVD [mm/mm^2]	50	0.990 [0.982-0.994]	Excellent
PPV [%]	50	0.992 [0.986-0.996]	Excellent
$MFI_{quadrant}$	50	0.943 [0.900-0.968]	Excellent
Subgroup: Non-compromised microcirculation			
TVD [mm/mm^2]	22	0.982 [0.901-0.994]	Excellent
PVD [mm/mm^2]	22	0.980 [0.896-0.994]	Excellent
PPV [%]	22	0.869 [0.682-0.946]	Excellent
$MFI_{quadrant}$	22	0.809 [0.548-0.920]	Excellent
Subgroup: Compromised microcirculation			
TVD [mm/mm^2]	28	0.977 [0.951-0.989]	Excellent
PVD [mm/mm^2]	28	0.989 [0.975-0.995]	Excellent
PPV [%]	28	0.990 [0.979-0.996]	Excellent
$MFI_{quadrant}$	28	0.948 [0.889-0.976]	Excellent

Abbreviations: CI: confidence interval; ICC: intraclass correlation coefficient; $MFI_{quadrant}$: microvascular flow index; PVD: perfused vessel density; PPV: proportion of perfused vessels; TVD: total vessel density.

Table 3 Bland-Altman analysis between Capillary Mapper 1.3 and AVA 3.2 and percentage error

All videos				
Variable [Unit]	**Number**	**Mean bias [95% CI]**	**LOA**	**percentage error [%]**
TVD [mm/mm^2]	50	-0.48 [-0.80-(-0.15)]	-2.70-1.75	12.4
PVD [mm/mm^2]	50	-0.33 [-0.66-(-0.01)]	-2.58-1.91	14.4
PPV [%]	50	0.91 [-0.24-2.06]	-7.01-8.83	9.3
$MFI_{quadrant}$	50	0.05 [-0.00-0.10]	-0.17-0.41	12.6
Subgroup: Non-compromised microcirculation				
TVD [mm/mm^2]	22	-0.63 [-0.97-(-0.29)]	-2.13-0.88	7.9
PVD [mm/mm^2]	22	-0.65 [-1.01-(-0.29)]	-2.24-0.95	8.7
PPV [%]	22	-0.04 [-0.55-0.48]	-2.32-2.24	2.3
$MFI_{quadrant}$	22	0.07 [-0.02-0.15]	-0.31-0.44	12.3
Subgroup: Compromised microcirculation				
TVD [mm/mm^2]	28	-0.36 [-0.89-0.17]	-3.02-2.3	15.3
PVD [mm/mm^2]	28	-0.09 [-0.59-0.42]	-2.65-2.48	19.5
PPV [%]	28	1.65 [-0.37-3.68]	-8.58-11.89	13.5
$MFI_{quadrant}$	28	0.04 [-0.03-0.10]	-0.31-0.38	13.0

Abbreviations: LOA: limits of agreement; PVD: perfused vessel density; PPV: proportion of perfused vessels; TVD: total vessel density.