ORIGINAL ARTICLE

Quantitative Nail Fold Capillary Blood Flow Using Capillaroscopy System and ImageJ Software in Healthy Individuals

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Abstract

Purpose: Measuring the blood flow velocity in capillaries is a useful method for diagnosing many diseases. Despite increasing interest in nailfold capillaroscopy, objective measures of capillary structure and blood flow have been rarely studied. This study aimed to measure the blood flow velocity along the capillary central line using capillaroscopy system, and also ImageJ software used Scale-Invariant Feature Transform (SIFT) tracking algorithms and Kalman filter for image processing.

Materials and Methods: The Red Blood Cells (RBCs) velocity in capillaries of finger nailfold was measured in 12 normal cases using a novel capillaroscopy system. The measurements of the velocity were performed at 12 points in nailfold regions by two observers separately. The image processing and automated measurement take 1-2 min per nailfold. FFmpeg software was used to convert the images format, and then the images were imported to ImageJ software and segmented. SIFT tracking algorithms and Kalman filter were used to filter noise and irregularities in the images. For reproducibility, the velocity distribution values obtained by the two performers, and Paired T-Test was used. The reliability of a measuring instrument or calculation method depends on the tools obtained using Cronbach's alpha. To assess the repeatability of the algorithm, the capillary velocity values were executed at different times with 24-hour intervals using a coefficient of variance method.

Results: The calculated RBCs velocity was in the range of 0.05-0.16 mm/s. The results based on Cronbach's alpha analysis for reliability factor was 0.97, with a good correlation among the measurements, 0.85. The average velocity (along with standard deviation) for repeatability at three different times was obtained 0.1195 ± 0.0246, 0.0974 ± 0.0221, and 0.0962 ± 0.0202 mm/s, demonstrating that there was no statistically variation between these measurements (P-value > 0.05). The velocity results for the two observers were 0.811 ± 0.392 and 0.819 ± 0.325 mm/s, indicating a good reproducibility between them (P-value = 0.959).

Conclusion: For the measurements of nailfold capillaries velocity, there was good/reasonable reliability, repeatability, and reproducibility. The results indicated a good accuracy of capillaroscopy system and ImageJ software with SIFT algorithm and Kalman filter, which can be used as an appropriate tool for determining the rate of nailfold blood flow velocity.

Keywords: Capillary Nailfold; Capillaroscopy; ImageJ Software; Scale-Invariant Feature Transform Tracking Algorithm; Kalman Filter.

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1. Introduction

Evaluation of blood flow velocity in capillaries would indicate crucial information in biomedical diagnostics of several diseases such as diabetes, Systemic Sclerosis (SSc), and coronary heart disease [1]. In addition, the capillary blood velocity, which is directly related to blood pressure is able to differentiate high blood pressure patients from normal individuals. Because, at the nailfold region, capillaries run parallel rather than perpendicular skin surface, the blood flow velocity in nailfold can be easily seen when magnified [2].

There are several methods used to measure blood flow velocity. One of them is Laser Doppler Flowmetry (LDF), however, the clinical utility of single point LDF measurements is limited due to the high spatial variability in blood flow across tissue such as the skin [1,3]. Although the full-field Laser Doppler Perfusion Imaging (LDPI) overcomes this limit, the signal-to-noise ratio is not high in this method [3]. In addition, both above-mentioned methods cannot be used to measure the Red Blood Cells (RBCs) local speed and concentration directly [4]. All in all, measuring capillary blood flow is a challenging process yet, and in many methods, the estimation of the velocity can be uncorrected, which may be due to the small number selection of vessels [5,6].

Nailfold video capillaroscopy overcomes almost all of the limitations. This method is a clinical assessment and monitoring at the microcirculatory level for RBCs [4]. Furthermore, recording of video frames series, extraction of capillary central line, intensity binarization and segmentation of individual capillary shape, the calculation of blood flow plasma gaps displacement along the capillary central line could be determined appropriately [7–10].

In general, the capillary blood velocity is very low for healthy individuals, however, there is no standard range for normal capillary blood velocity and also for patients with different diseases/abnormalities such as SSc, or primary open-angle glaucoma. In modern clinical practice, the morphological changes in rheumatic diseases such as SSc, dermatomyositis, and systemic lupus erythematosus are classified as normal/abnormal, or they can be divided into macroscopic stages (early, active, and late) [11]. However, subjective labeling and various observer reliability make it less suitable for monitoring the progression of the disease. Also, the morphological structural changes occur over several years, then it is not useful for tracking rapid changes in capillary flow in patients under vasoactive therapy (norepinephrine, phenylephrine, epinephrine, and vasopressin). Indeed, capillary blood flow can respond immediately to such interventions. With the development of drugs and the potential for vascular remodeling, interest in the early intervention using quantitative trend evaluation has been increased [11,12]. Although morphological features obtained from capillary images in the nailfold area have been studied in previous investigations [7,13], less attention has been paid to capillary blood velocity as a factor in the diagnosis of possible problems or diseases. In this regard, the current study aimed to calculate the capillary blood velocity of the nailfold area in healthy individuals using a computer algorithm on the images obtained by capillaroscopy. To detail, the blood flow velocity along a capillary central line was measured using capillaroscopy system, and the ImageJ program, which was used SIFT (scale-invariant feature transform) tracking algorithms and Kalman filter for image processing. ImageJ is an image analysis software widely used in the biological sciences and beyond, due to its ease of use, extensible plug-in architecture, and recordable macro language. In addition, SIFT algorithm can detect the invariant feature points used in tracking and Kalman filter can predict the target location as well.

2. Materials and Methods

2.1. Participants

The study protocol was approved by the National Ethics Committee (registration number: IR.TUMS.MEDICINE.REC.1398.673). Twelve individuals, including 6 females and 6 males, with a mean age of 31.33 ± 5.41 years (ranging 24–39 years old) participated in the current study. It should be noted that all participants were healthy and normal based on physiological criteria and had no history of underlying vascular disease and rheumatism. Written consent was obtained from each participant before the study.

2.2. Image Procedures in Capillaroscopy System

Prior to imaging, the participants were acclimatized in a temperature-controlled laboratory for 20 minutes. In addition, to take the higher image quality, the participants' fingers were soaked in oral paraffin, and then the imaging process was performed. The detail of capturing the imaging of nailfold by capillaroscopy system has been described in a previous study [11]. Briefly, the video sequences are captured in a high frame rate by the system (non-contact nailfold capilaroscope zoom pro 200x) which allows measuring the RBCs velocity. The magnification rate and pixel sizes were chosen 200x and 700 * 1300, respectively. It is notable that for more quickly capturing of sequences, the camera is mounted on a software-controlled 3-axis motorized stage. For making fully-automated measurements of capillaroscopy structure and blood flow, novel software was used for nailfold capillary image mosaics. In general, the image processing and automated measurement take 1–2 min per nailfold.

2.3. ImageJ Software

FFmpeg software was used to convert the images format to uncompressed AVI. Then, the images were imported to ImageJ software, and they were segmented using the region of interest (ROI) manager manually corresponding to capillaries' sections for post processing of the blood nailfold flow. SIFT tracking algorithms using trackmate plug-in and also Kalman filter were used to filter noise and irregularities in the images.

In Figure 1, the procedures of the current study were shown as a flowchart.

2.4. Statistical Analysis

2.4.1. Reproducibility

We examined the velocity distribution values obtained by the first and the second performer to find if they follow the normal distribution. The Kolmogorov-Smirnov sample statistical test was used to assess the normal distribution of capillary blood velocities. All analyses were performed using SPSS software package (V18, SPSS Inc., Chicago, USA).

Reproducibility statistic index (R) is a value predicted with a certain probability (for example 95%) used for the data with a normal distribution [14]. The absolute value of the difference between two individual test results measured on the same sample with the same measurement method under different conditions (different performers, devices, labs, or different times). This index can be obtained from the following Equations 1, 2 [14]:

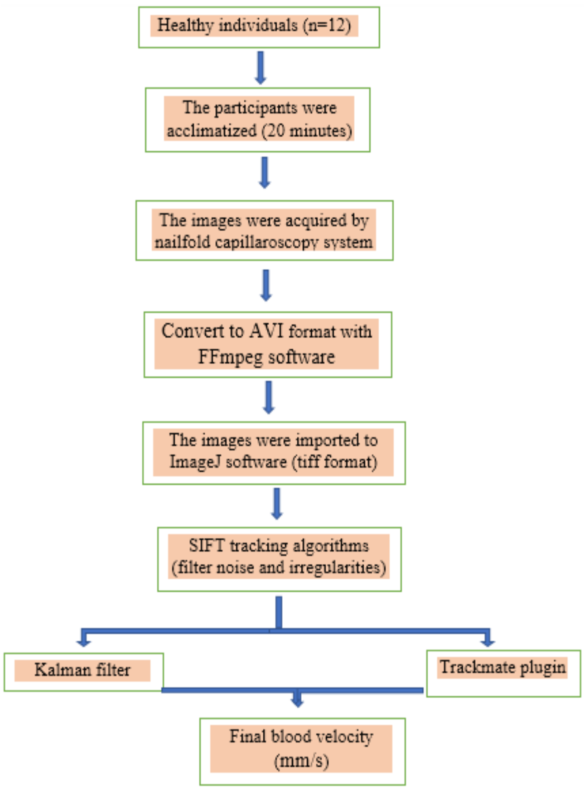

Figure 1. Flowchart scheme

$$x1 - x2 \,|\, < R \,| \tag{1}$$

$$R = t\,(p, v)\,[SQRT\ (2)\ SR] \tag{2}$$

In the above Equation, the coefficient 't' is determined according to the confidence level 'p' and the number of degrees of freedom v = n-1. 'SR' is the standard deviation of the test method and SQRT is the abbreviation for the square root.

2.4.2. Reliability

The reliability of a measuring instrument or calculation method depends on the measuring or calculating tools [1]. High-reliability value means that the measuring instrument or calculating tool has similar results under the similar conditions. In other words, this means that if we use the measuring tool for a single group of people several times and in a short period of time, there will be no significant differences in the results. To measure the reliability, we use an index called the reliability coefficient. The reliability coefficient ranges from zero (unreliable) to +1 (complete reliability). Complete reliability is seldom seen and should be viewed with skepticism if observed.

There are different methods used to calculate the reliability coefficient of measuring instruments. In this study, Cronbach's alpha test was used to evaluate the reliability of the measurements because a standard range for capillary blood velocity is not available yet.

Our proposed algorithm was executed 4 times on the same data (image series recorded from 12 volunteers) and the obtained velocities were used for performing the reliability test.

2.4.3. Repeatability

To assess the repeatability of the algorithm, we executed the capillary velocity calculation algorithm at different times with 24-hour intervals (3 different times) for all 12 series of the images. Notably, the algorithm execution conditions and input data were similar.

3. Results

The mean and standard deviation of the obtained velocity values for the two performers are illustrated in Figure 2. The statistical results of the Paired T-Test showed that the obtained velocity had not any significant differences (P-value = 0.959). Therefore, it can be noted that reproducibility is acceptable for changing the performer or experimenter.

Due to the normality of data distributions, we used reproducibility statistic index (R) too. Following Equations 1 and 2, the value of t for the 95% confidence level and the degree of freedom of 11 is equal to 2.2. Also, the SR value or standard deviation of the reproducibility of the Paired T-Test method was obtained at 0.0507. In addition, putting the values in the formula $R = t\,(p, v)\,[SQRT\,(2)\,SR]$, the value of R was equal to 0.495. The absolute value of the difference between the means of the two groups was 0.00762, which is much less than 0.495. This indicates a good and acceptable reproducibility of this method compared to changing readers or testers.

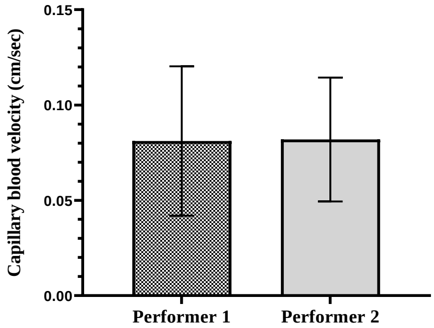

Figure 2. Mean and standard values (error bars) of capillary blood velocity obtained from the measurements of two different performers on 12 similar volunteers

For reliability, the calculated velocities obtained from the 12 series were presented 4 times of executing the calculation algorithm (Table 1). In addition, Cronbach's alpha value was 0.97, which indicates a very high reliability of the results of our velocity calculation algorithm.

Table 2 shows the correlation values between the data of different iterations. It is obvious that there is a high

Table1. Capillary blood velocity values obtained from executing the proposed algorithm 4 times on the same data (image series recorded from 12 volunteers)

Volunteer No.	Capillary blood velocity (mm/sec)			
	First run	Second run	Third run	Fourth run
1	0.1847	0.1066	0.1176	0.1195
2	0.0535	0.0676	0.0673	0.0702
3	0.0907	0.0934	0.0911	0.0961
4	0.0864	0.0804	0.0785	0.0801
5	0.0943	0.0808	0.0951	0.0978
6	0.0856	0.0925	0.0981	0.0921
7	0.1128	0.1093	0.1165	0.1117
8	0.0771	0.0745	0.0863	0.0767
9	0.1121	0.1133	0.1014	0.0955
10	0.0904	0.0896	0.0778	0.0903
11	0.0901	0.0773	0.0907	0.0909
12	0.1603	0.1587	0.1495	0.1535

Table 2. Correlation values between the data of different iterations of the algorithm for obtaining capillary blood velocity values

	Inter-Item Correlation Matrix			
	Itteration1	**Itteration2**	**Itteration3**	**Itteration4**
Itteration1	1.000	0.958	0.958	0.853
Itteration2	0.958	1.000	0.961	0.902
Itteration3	0.958	0.961	1.000	0.849
Itteration4	0.853	0.902	0.849	1.000

Table 3. The calculated capillary blood velocity values for three iterations of our proposed algorithm with 24-hour intervals

Volunteers No.	Capillary blood velocity (mm/sec)		
	First iteration	**Second iteration**	**Third iteration**
1	0.1195	0.1580	0.1584
2	0.0702	0.0745	0.0692
3	0.0961	0.0945	0.0889
4	0.0801	0.0894	0.0764
5	0.0978	0.0819	0.0770
6	0.0921	0.0770	0.0874
7	0.1087	0.1126	0.1124
8	0.0767	0.0858	0.0834
9	0.0975	0.1021	0.0942
10	0.0823	0.0756	0.0897
11	0.0909	0.0855	0.0786
12	0.1435	0.1445	0.1538
Mean (SD)	0.1195 (0.0246)	0.0974 (0.0221)	0.0962 (0.0202)

SD: Standard Deviation

correlation (more than 0.85) between all the data and this also approves the high reliability of the calculated results.

The results related to repeatability values for all three iterations are presented in Table 3. The Coefficient of Variance (% CV), which is the result of dividing the standard deviation value by the mean value, was used to evaluate the repeatability. The coefficient of repeatability or variance changes was calculated using the following Equation [15]:

$$CV\ (\%) = \frac{Standard\ deviation}{Mean} \times 100 \quad (3)$$

CV values were obtained for different iterative data for each individual; the values are illustrated in Table 4. According to the obtained values, CV% value is less than 10% for most of the data related to different volunteers and between 10 and 15% for 5 of them. Also, the average value of CV% was equal to 8.43%, indicating good repeatability of the capillary blood velocity calculating algorithm.

Due to the normal distribution of the data in all 3 iterations, repeated measurements statistical test was used to investigate, and it has been shown that there is no statistical difference between the data of 3 different iterations results. For different methods, the P-value of repeated measurements tests was less than 0.623, indicating no significant difference between different iteration of the algorithm for capillary blood velocity calculating, and our proposed algorithm has high repeatability.

Table 4. The mean, standard error, and %CV calculated values of capillary blood velocity for each volunteer

Volunteers No.	Capillary blood velocity (mm/sec)		
	Average	**Standard Error**	**%CV**
1	0.1372	0.0194	14.18
2	0.0705	0.0038	5.40
3	0.0924	0.0051	5.57
4	0.0835	0.0051	6.11
5	0.0853	0.0112	13.12
6	0.0873	0.0089	10.23
7	0.1124	0.0036	3.16
8	0.0774	0.0081	10.50
9	0.1007	0.0027	2.73
10	0.0825	0.0070	8.49
11	0.0907	0.0051	5.63
12	0.1587	0.0255	16.08
Mean	0.0982	0.0088	8.43

4. Discussion

In morphological investigations into the capillaries of the nailfold area, it is necessary to prepare one or more images with acceptable magnification (100 to 600 times). In the present study, a series of capillary images were used to obtain capillary blood velocity. A computer algorithm was also designed to estimate capillary blood velocity by processing and analyzing a series of images. Indicators related to the performance of this computer algorithm such as reproducibility, reproducibility, and reliability were also assessed in this study. All in all, this is the first study which has been performed in Iran for indicating blood flow velocity in normal individuals using capillaroscopy system. In addition, the present study is different in terms of the studied parameters and also in the used technique compared to previous studies, in a way that, no studies are using ImageJ software for image processing captured with capillaroscopy system of capillary nailfold.

Flow and SIFT optical algorithms were used in the present work, in which an image is often measured with its neighbors. SIFT method is one of the optical flow methods which is capable to record a large amount of displacement in the image frames and is also less sensitive to noise.

Several studies used multiple variables and regressions to examine their relationship with the type of disease/ problem [11,16]. For example, in 2019, Cousins *et al.* [16] examined the blood flow of resting nailfold vessels in Primary Open-Angle Glaucoma (POAG). In their study, multiple logistic regressions were used to investigate the relationship between nailfold vascular capillary blood flow and POAG. Finally, it was stated that the reduction of the capillary flow of resting vessels in POAG is independent of variables such as blood pressure and pulse [16]. In another study, Berks *et al.* [11] used structural measurements, capillary flow, and a combined measurement to separate patients with SSc from those with primary Raynaud's phenomenon (PRP) and healthy controls. It was reported that both structural and blood flow measurements can help distinguish patients with SSc from those with PRP and healthy individuals. If the morphological parameters are combined with the capillary blood velocity, it could be a more powerful tool for differentiating between patients and healthy individuals in multivariate analysis.

The reproducibility and reliability of the capillary blood flow velocity estimation method in the present investigation were acceptable (Cronbach's alpha equal to 0.97). In a study by Dinsdale *et al.* [17], the reproducibility capillaroscopy imaging was obtained from the imaging of 41 patients (26 patients with SSc and 15 patients with PRP) and 10 healthy individuals. The images were randomly evaluated by experienced observers. The results of their study showed that the estimation of reproducibility between inter- and intra-tests was, respectively, equal to 0.97 and 0.9, for overall image grades, 0.92 and 0.65 for vessel density, 0.91 and 0.79 for mean vessel width, and 0.68 and 0.56 for the presence of giant capillaries.

In the present study, the reproducibility was investigated based on the change of the test performers, and no significant difference was observed between the results of different testers. Hofstee *et al.* [18], found similar results in terms of reproducibility, however, in their study, the reproducibility between and within the observers of the qualitative and quantitative parameters used in the evaluation of capillary of nailfold vessels images. They have declared that all the quantitative and qualitative parameters have high reproducibility between and within the observers.

In the recent studies [8,19–22], in most cases, the velocity of capillary RBCs using capillaroscopy was determined by measuring the displacement of gaps within the plasma. In these studies, it was assumed that the capillary width was much smaller than the capillary length mainly due to the insufficient spatial and temporal resolution. In other words, only the RBC displacement along the capillary was evaluated but the lateral displacement of RBC was ignored. In a study by Watanabe *et al.* [8] and also in the present study, capillary blood velocity was calculated in both axially along the capillary and laterally, and absolute blood velocity was reported by processing sequential images.

It has been shown that there are large differences in the reported values of nailfold capillary blood, even among healthy individuals [8]. In Bollinger *et al.*'s [23] study, the highest level of measured capillary blood velocity in 5 normal individuals was reported 3.47 mm/s at rest, however, it was reported 19.4 mm/s in a study by Watanabe *et al.* [8]. According to our findings, the maximum capillary blood velocity reached 1.6 mm/s. The reason for the difference between the available findings is probably due to the differences in the choice of capillaries in which the blood velocity is measured and the computer technique that estimates the blood velocity.

Regarding the small diameter size of the capillaries, if we want to estimate the required capillary time resolution, we have to follow the order of plasma gap displacement obtained in previous research with a maximum speed of 10 mm/s in consecutive frames with a frame spacing of 0.02 seconds. The calculated size of plasma gaps shifts in capillary vessels is about 0.2 mm and this amount is almost ten folds larger than that of a normal capillary artery. The normal visible length of an arterial or venous by capillaroscopy is about 0.2-0.25 mm and the visibility of the plasma gap in the apical is significantly reduced, therefore, measuring the RBC rate of 10 mm/s is so difficult and requires high temporal resolution cameras. The reported capillary blood velocity is generally less than 1.0 mm/s using conventional video capillaroscopy, or the maximum reported velocity is 3.47 mm/s [23], and these values are consistent with the results of our study (0.0811 ± 0.0392 mm/s). However, it is unknown that capillary blood velocities of the order of 10 mm/s, which are rarely observed, can only be obtained with high temporal resolution devices or can be obtained with conventional capillaroscopy at the present stage.

Capillary blood velocity is also obtained by the other methods such as optical flow without using the SIFT method and laser Doppler [24,25]. For example, in a study by Wu *et al.* [25], the RBC velocity of 12 vessels was examined at three measured locations (arterial, curved, and venous) over 45 seconds of vessel occlusion-flow mode. There were four stages of circulatory conditions: rest, pre-occlusion, post-occlusion, and release. The results of both approaches showed that the speed difference between the three sites was not significant. The RBC velocity distribution pattern was also reported. The blood velocity in the expressed states obtained from the optical flow method was 79.3 ± 11.7, 84.9 ± 10.9, 53.8 ± 13.9, and 84.8 ± 15.9 μm/s, for rest, obstruction, post-occlusion, and in a release, respectively. In another study [24] using a Doppler laser, the capillary blood velocity of the nailfold area was examined in 20 healthy volunteers (10 men and 10 women). The mean velocity of RBCs within the capillaries was 0.47 ± 0.37 mm/s. Different results of the methods showed a variation in blood velocity, which means that the methods can affect the blood velocity measurements.

One of the limitations in the present study is the lack of comparisons between the results of tracking algorithms and the lack of a standard value or range for capillary blood velocity in the nailfold area for normal individuals. In addition, the type of video capillary device with a specific spatial and temporal resolution was one of the limitations.

5. Conclusion

The proposed method in this research, which was the use of ImageJ software with SIFT tracking algorithm and Kalman filter on capillary images of blood vessels in nailfold area using capillaroscopy system, had excellent reliability and reproducibility. Although there is a contradiction among the previously reported capillary blood velocity values, the velocity values obtained using this method for healthy individuals are consistent with some of the previous studies. The reason for contradiction is the wide range of capillary blood velocity values in different studies due to the differences in the study area as well as the technique of detecting blood velocity. Therefore, it seems that for each study technique, it is necessary to estimate the blood velocity of normal individuals.

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References

1- Gurov I, Volkov M, Margaryants N, Pimenov A, Potemkin A. "High-speed video capillaroscopy method for imaging and evaluation of moving red blood cells.", *Opt Lasers Eng*;104:244–51, (2018).

2- Smith V, Thevissen K, Trombetta AC, Pizzorni C, Ruaro B, Piette Y, *et al.* "Nailfold capillaroscopy and clinical applications in systemic sclerosis.", *Microcirculation.*; 23(5):364–72, (2016).

3- Allen J, Howell K. "Microvascular imaging: techniques and opportunities for clinical physiological measurements.", *Physiol Meas*.;35(7):R91, (2014).

4- Daly SM, Leahy MJ. "‘Go with the flow’: a review of methods and advancements in blood flow imaging.", *J Biophotonics*;6(3):217–55. (2013).

5- Shih T-C, Zhang G, Wu C-C, Hsiao H-D, Wu T-H, Lin K-P, *et al.* "Hemodynamic analysis of capillary in finger nail-fold using computational fluid dynamics and image estimation.", *Microvasc Res*;81(1):68–72, (2011).

6- Mugii N, Hasegawa M, Hamaguchi Y, Tanaka C, Kaji K, Komura K, *et al.* "Reduced red blood cell velocity in nail-fold capillaries as a sensitive and specific indicator of microcirculation injury in systemic sclerosis." *Rheumatology*; 48(6):696–703, (2009).

7- Cutolo M, Pizzorni C, Sulli A. "Capillaroscopy." *Best Pract Res Clin Rheumatol*;19(3):437–52, (2005).

8- Watanabe M, Matsubara M, Sanada T, Kuroda H, Iribe M, Furue M. "High speed digital video capillaroscopy: nailfold capillary shape analysis and red blood cell velocity measurement." *J Biomech Sci Eng*;2(2):81–92, (2007).

9- Gurfinkel YI, Suchkova OV, Sasonko ML, Priezzhev AV. "Implementation of digital optical capillaroscopy for quantifying and estimating the microvascular abnormalities in type 2 diabetes mellitus.", *In: Saratov Fall Meeting 2015: Third International Symposium on Optics and Biophotonics and Seventh Finnish-Russian Photonics and Laser Symposium (PALS). International Society for Optics and Photonics*; p. 991703, (2016).

10- Gurfinkel YI, Sasonko ML, Priezzhev AV. "Digital capillaroscopy as important tool for early diagnostics of arterial hypertension. In: Saratov Fall Meeting 2014: Optical Technologies in Biophysics and Medicine XVI; Laser Physics and Photonics XVI; and Computational Biophysics.", *International Society for Optics and Photonics*; p. 944804, (2015).

11- Berks M, Dinsdale G, Murray A, Moore T, Manning J, Taylor C, *et al.* "Automated structure and flow measurement—a promising tool in nailfold capillaroscopy.", *Microvasc Res*;118:173–7, (2018).

12- Murray AK, Moore TL, Manning JB, Taylor C, Griffiths CE, Herrick AL. "Noninvasive imaging techniques in the assessment of scleroderma spectrum disorders.", *Arthritis Care Res*.;61(8):1103–11, (2009).

13- Cutolo M, Sulli A, Pizzorni C, Accardo S. "Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis." *J Rheumatol*;27(1):155–60, (2000).

14- McNutt M. "Reproducibility.", *American Association for the Advancement of Science*; (2014).

15- Men SJ, Chen C-L, Wei W, Lai T-Y, Song SZ, Wang RK. "Repeatability of vessel density measurement in human skin by OCT-based microangiography.", *Skin Res Technol*;23(4):607–12, (2017).

16- Cousins CC, Chou JC, Greenstein SH, Brauner SC, Shen LQ, Turalba AV, *et al.* "Resting nailfold capillary blood flow in primary open-angle glaucoma.", *Br J Ophthalmol*;103(2):203–7, (2019).

17- Dinsdale G, Moore T, O’Leary N, Berks M, Roberts C, Manning J, *et al.* "Quantitative outcome measures for systemic sclerosis-related microangiopathy–reliability of image acquisition in nailfold capillaroscopy.", *Microvasc Res*;113:56–9, (2017).

18- Hofstee HM, Serne EH, Roberts C, Hesselstrand R, Scheja A, Moore TL, *et al.* "A multicentre study on the reliability of qualitative and quantitative nail-fold videocapillaroscopy assessment.", *Rheumatology*; 51(4):749–55, (2012).

19- Gasser P. "Capillary blood cell velocity in finger nailfold: Characteristics and reproducibility of the local cold response.", *Microvasc Res*;40(1):29–35, (1990).

20- Butti P, Intaglietta M, Reimann H, Holliger CH, Bollinger A, Anliker M. "Capillary red blood cell velocity measurements in human nailfold by videodensitometric method.", *Microvasc Res*;10(2):220–7, (1975).

21- Boss CH, Schneuwly P, Mahler F. "Evaluation and clinical application of the flying spot method in clinical nailfold capillary TV-microscopy.", *Int J Microcirc Clin Exp*;6(1):15–23,b(1987).

22- Mahler F, Saner H, Boss C, Annaheim M. "Local cold exposure test for capillaroscopic examination of patients with Raynaud’s syndrome.", *Microvasc Res*;33(3):422–7, (1987).

23- Bollinger A, Butti P, Barras J-P, Trachsler H, Siegenthaler W. "Red blood cell velocity in nailfold capillaries of man measured by a television microscopy technique.", *Microvasc Res*;7(1):61–72, (1974).

24- Stücker M, Baier V, Reuther T, Hoffmann K, Kellam K, Altmeyer P. "Capillary blood cell velocity in human skin capillaries located perpendicularly to the skin surface: measured by a new laser Doppler anemometer.", *Microvasc Res*;52(2):188–92, (1996).

25- Wu C-C, Zhang G, Huang T-C, Lin K- P. "Red blood cell velocity measurements of complete capillary in finger nail-fold using optical flow estimation.", *Microvasc Res*;78(3):319–24, (2009).