PROCESSING OF LASER SPECKLE CONTRAST IMAGES TO ANALYZE THE IMPACT OF AGING ON MOVING BLOOD CELLS WHEN A LORENTZIAN VELOCITY PROFILE IS ASSUMED

A. Khalil[⋆] *A. Humeau-Heurtier*[⋆] *P. Abraham*[†] *G. Mahé*[*]

[⋆] University of Angers, LARIS–Laboratoire Angevin de Recherche en Ingénierie des Systèmes, 62 avenue Notre-Dame du Lac, 49000 Angers, France.
[†]University of Angers, Hospital of Angers, Laboratoire de Physiologie et d'Explorations Vasculaires UMR CNRS 6214-INSERM 1083, 49033 Angers cedex 01, France.
[*]Imagerie Cœur-Vaisseaux, CHU de Rennes, 35033 Rennes cedex 9, France.
adil.khalil@etud.univ-angers.fr, anne.humeau@univ-angers.fr Tel.: +33.(0)2.44.68.75.87

ABSTRACT

It has long been recognized that age alters microcirculation. The follow-up of such alterations can be performed by monitoring microvascular blood flow. Laser speckle contrast imaging (LSCI) has recently been commercialized to monitor microvascular blood flow. From laser speckle contrast images, velocity of microvascular moving scatterers (mainly red blood cells) can be computed when a profile for velocity distribution is assumed. Our goal herein is to analyze if alterations of microcirculation with age can be determined by processing experimental LSCI data. In our work a Lorentzian velocity profile is assumed and the presence of static scatterers, like skin, is taken into account. Our results show that moving scatterers velocities computed from LSCI data vary with age: blood cells velocities increase with age. Moreover, the more the static scatterers, the higher the moving scatterers velocity values. Our findings are a first step in the analysis of the impact of aging from the processing of laser speckle contrast images.

Index Terms— Laser speckle contrast imaging, Image processing, Blood flow, Lorentzian profile.

1. INTRODUCTION

Laser speckle contrast imaging (LSCI) is a noncontact and full-field optical technique for monitoring microvascular blood flow [1, 2]. LSCI is gaining an increased interest in the medical field due to the simplicity of the required instrumentation and to the high temporal and spatial resolutions of the data [3]. The principle of the LSCI technique is the following: when the tissue under study (skin) is illuminated using a coherent light (laser light), the scattered light forms a random interference pattern – called speckle – on a camera. Because of the movement of scattering particles (mainly red blood cells in the case of blood) within the tissue under study, the speckle pattern changes with time. Due to the exposure time T of the camera, a blurring of the speckle pattern is observed. This blurring is quantified through the computation of the spatial speckle contrast K_s as $K_s = \sigma_s/\langle I \rangle$ [4] where σ_s refers to the spatial standard deviation in a neighborhood around a pixel of the raw data, while $\langle I \rangle$ is the mean intensity in this neighborhood. From raw speckle data, contrast images are therefore computed as mentioned above (see an example of contrast image in Fig. 1). In the LSCI technique, if a velocity distribution for the moving scatterers is assumed, a theoretical expression of the speckle contrast K can be written as a function of the exposure time T of the camera and of the speckle correlation time τ_c. Thus, if the velocity of the scatterers is assumed to follow a Lorentzian distribution, we have [5, 6]

$$K_{Lorentzien} = \beta^{0.5} \left[\frac{\tau_c}{T} + \frac{\tau_c^2}{2T^2} \left(\exp\left(-\frac{2T}{\tau_c} \right) - 1 \right) \right]^{0.5} . \quad (1)$$

Because the correlation time τ_c is inversely proportional to the velocity of the moving blood cells as [4]

$$v_c = \frac{\lambda}{2\pi\tau_c} , \quad (2)$$

where λ is the wavelength of the laser, the contrast value K is used to determine the velocity of the moving blood cells in the microcirculation. Nevertheless, (1) does not take into account the presence of static scatterers. Moreover, only a few authors studied the theoretical expression of the speckle contrast K when the detected light is composed of *both* a *fluctuating* speckle field from moving scatterers (such as red blood cells) and of *static* reflections from static scatterers (such as skin, bones and intact skull) [7–9].

The monitoring of microvascular blood flow is of importance for the diagnosis of pathologies such as diabetes or hypertension, but also to analyze the impact of aging on the microvessels [10]. Thus, it has been shown that age changes

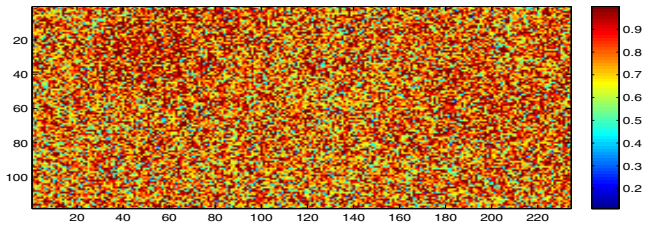

Fig. 1. Laser speckle contrast image (118×234 pixels^2) of forearm skin in a healthy subject.

morphology and quantification of the cutaneous microvasculature. However, from the best of our knowledge, such alterations have not been studied yet from LSCI data. The latter having good temporal and spatial resolutions, they could be of interest in the follow-up of age-dependent microvascular alterations.

By processing laser speckle contrast images, our goal herein is therefore to analyze if alteration of microcirculation with age can be determined from experimental LSCI data. Moreover, we take into account the presence of *both* moving and static scatterers. For this purpose, velocity of moving blood cells in the microcirculation is studied in two groups of subjects, one group is composed of young subjects while the other group is composed of elderly subjects.

In what follows, we first present the theory to compute moving scatterers velocity values in the presence of both moving and static scatterers, when a Lorentzian velocity profile is assumed. Then, the measurement procedure is described and the algorithm proposed to process the experimental laser speckle contrast images is detailed. Finally, we present our results and a discussion of our findings.

2. MATERIALS AND METHODS

2.1. Theoretical Background

As mentioned above, when the static scatterers are not taken into account and when a Lorentzian velocity distribution is assumed, the theoretical expression for the contrast K is (1) [5, 6]. Even if it has been shown that static scatterers may play an important role in the determination of moving blood cells velocity [11], there are only a few studies that proposed a theoretical expression for the contrast K in the presence of static scatterers.

Parthasarathy et al. [7] presented a model which can estimate the flow in the presence of static contributions when a Lorentzian velocity distribution is assumed. They suggested the following equation for the spatial contrast [7]

$$K\left(T,\tau_c\right)=\Bigg[\beta\rho^2\frac{\exp(-2x)-1+2x}{2x^2}+ 4\beta\rho\left(1-\rho\right)\frac{\exp(-x)-1+x}{x^2}+ v_{ne}+v_{noise}\Bigg]^{0.5}, \quad (3)$$

where $x=T/\tau_c$, T is the camera exposure time, τ_c is the correlation time and $\rho=I_f/(I_f+I_s)$ is the fraction of total light that is dynamically scattered. Moreover, β is a normalization factor to account for speckle averaging effects. v_{noise} is the constant variance due to experimental noise and v_{ne} is the constant variance due to nonergodic light [7].

Zakharov et al. [9] introduced another theoretical expression that takes into account the effect of static scatterers. The detected intensity was defined as composed of a nonfluctuating static part I_s and a dynamic part I_d. Thereby, the fraction of static scatterers to the total intensity is mentioned as $\rho=\langle I_s\rangle/(\langle I_s\rangle+\langle I_d\rangle)$. The expression of contrast is in this case written as [9]

$$K^2=\frac{2\beta}{T}\int_0^T\left[(1-\rho)\mid g_{1d}\left(\tau\right)\mid+\rho\right]^2\left(1-\tau/T\right)d\tau, \quad (4)$$

where β is the coherence factor and $g_{1d}(\tau)$ is the correlation function of the pure dynamic part.

Boas et al. [8] have proposed another formulation to compute the speckle contrast K to take into account the effect of static scatterers by assuming a Lorentzian velocity profile for the moving scatterers. They suggested [8]

$$K_{Lorentzien}=\beta^{0.5}\Bigg[\rho^2\frac{\exp(-2x)-1+2x}{2x^2}+ 4\rho(1-\rho)\frac{\exp(-x)-1+x}{x^2}+ (1-\rho)^2\Bigg]^{0.5}+C_{noise}\ , \quad (5)$$

where $x=T/\tau_c$, $\rho=I_f/(I_f+I_s)$ where I_f is the time-averaged intensity of the fluctuating dynamically scattered light, I_s is the intensity of the statically scattered light and C_{noise} is a term added to account for contrast that arises from measurement noise such as shot noise or camera readout noise [8]. For a general point of view, we propose to use (5) to determine the velocity of moving scatterers computed from LSCI data when a Lorentzian velocity profile for the moving scatterers is assumed. Furthermore, to determine the possible effect of static scatterers on the velocity values of moving scatterers (red blood cells), four cases of ρ are analyzed in our work: $\rho=1$, $\rho=0.9$, $\rho=0.8$ and $\rho=0.7$.

2.2. Measurement Procedure

The prior written consent was obtained from all subjects before the implementation of this study which was conducted in accordance with the declaration of Helsinki. In this study, we included 14 subjects subdivided into two groups. The first group was composed of 7 subjects (4 men and 3 women); in this first group, all the subjects were less than 30 years old. The second group was composed of 7 subjects (2 men and 5 women); all were more than 45 years old. The recordings were performed in a quiet room without any air movement [12] and with a controlled temperature [13], while the subjects were in the lying position. The ventral face of the forearm was studied. A laser speckle contrast imager PeriCam PSI System (Perimed, Sweden) having a laser wavelength of 785 nm was used. In this imager, the camera exposure time T is set at 6 ms. Moreover, the distance between the laser head to forearm skin was adjusted to 15 ± 1 cm [14] which gave images with a resolution around 0.45 mm.

Experimental data were obtained during three physiological states: 1) 2 min at rest, 2) 3 min of vascular occlusive (biological zero) [15], obtained by inflating an arm cuff to 220 mmHg; 3) 5 min of post-occlusive reactive hyperaemia. All the contrast images were stored in a computer for an off-line analysis. We have computed and compared moving blood cells velocities from regions of interest (ROI) of different sizes [16]: 1×1 pixels^2, 3×3 pixels^2, 5×5 pixels^2, and 7×7 pixels^2. For each case 1 min of recording at rest, 1 min of recording during biological zero, and 3 s during reactive hyperaemia peak have been processed.

Statistical analyses were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium). Using Wilcoxon test we compared velocity values obtained for young subjects with the ones obtained for the elderly subjects. For each statistical analysis, a p value < 0.05 was considered significant.

3. RESULT AND DISCUSSION

Fig. 2 presents the theoretical relationship between speckle contrast K and the ratio of the speckle correlation time τ_c to camera exposure time T for a Lorentzian profile with different values of ρ. From this Figure, we observe that the pattern of the curves is similar for all the ρ values chosen. However, for the lowest value τ_c/T shown, the contrast K becomes higher when the ρ value decreases: K goes from 0.1 (for $\rho = 1$) to more than 0.3 (for $\rho = 0.7$). These differences are due to the static scatterers: the more the static scatterers, the higher the value of the contrast K for τ_c/T close to zero.

The mean velocities of moving scatterers for $\rho = 1$, $\rho = 0.9$, $\rho = 0.8$ and $\rho = 0.7$ are shown in tables 1 to 4, respectively. For each table, mean results for the three physiological states are shown: rest (mean value on 1 min), vascular occlusive (biological zero; mean value on 1 min) and post-occlusive reactive hyperaemia (mean value on 3 s at peak). Moreover, values obtained for the different ROI sizes (1×1 pixels^2, 3×3 pixels^2, 5×5 pixels^2, and 7×7 pixels^2) are detailed for the two populations: young and elderly subjects.

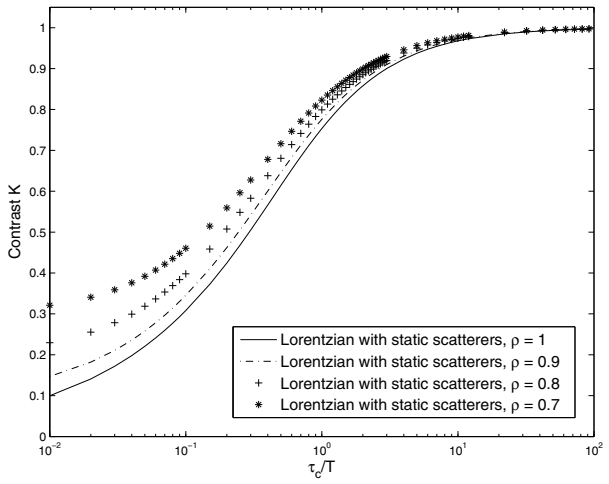

Fig. 2. Theoretical relationship between speckle contrast K and the ratio of the speckle correlation time τ_c to camera exposure time T for a Lorentzian profile with different values of ρ. β is equal to 1 for each curve. See text for details.

Our results show that, for a given ROI size and a given ρ value, at rest and during the biological zero, the velocities found for young subjects are lower than the ones obtained for elderly subjects (see Tables 1 to 4). Nevertheless, these differences are not statistically significant. From the litterature, the value of basal blood flow (product of velocity by concentration of moving blood cells) for young and elderly subjects remains debatable (see, e.g., [17, 18]). However, during reactive hyperaemia, for a given ROI size and a given ρ value, we find statistically significant differences between the velocities obtained for young subjects and the ones obtained for older subjects (see Tables 1 to 4). The mean velocities obtained for the elderly group are higher than the ones obtained for the young people. This may be due to the stiffness of vessels that increases with age. Stiffness being higher for aged people, the revasculatisation of vessels after the vascular occlusion (post-occlusive reactive hyperaemia) may lead to higher moving blood cells velocity values. Furthermore, to the best of our knowledge, the changes of the velocity of moving scatterers at rest, during biological zero and reactive hypereamia changes with ρ values have not been studied yet.

We also demonstrate that, for the two populations, the velocity of moving scatterers increases while the value of ρ decreases (see Tables 1 to 4). Therefore, our results stress an effect of static scatterers (like skin) over the velocity of moving scatterers. Moreover, when the results for the different ROI sizes are analyzed, we observe that the velocity values obtained from a single pixel are higher than the ones obtained

from ROI sizes of $3\times$ 3, 5×5, 7×7 pixels^2; see Tables 1 to 4. This could be due to the signal-to-noise ratio that increases for larger ROI sizes on LSCI data [16]. Larger ROI sizes have not been tested due to the computational time needed to process the data.

4. CONCLUSION

From above, our main findings are:

- static scatterers play an important role in the determination of moving blood cells velocity values.
- velocity values of moving scatterers differ with age: they are higher for elderly people compared to the ones obtained for young people. The results are statistically significant during post-occlusive reactive hyperaemia. This may be due to stiffness of vessels that increases with age. The impact of age on microvascular blood flow (stiffness of vessels, among others) could therefore be quantified by processing LSCI data.

Finally, from the best of our knowledge, this study is the first one that analyzes the effect of aging from LSCI data by computing red blood cells velocities.

REFERENCES

[1] A.F. Fercher and J.D. Briers, "Flow visualization by means of single exposure speckle photography," *Opt. Comm.*, vol. 37, pp. 326–330, 1981.

[2] A. Humeau-Heurtier, E. Guerreschi, and G. Mahé, "Relevance of laser Doppler and laser speckle techniques for assessing vascular function: State of the art and future trends," *IEEE Trans. Biomed. Eng.*, vol. 60, pp. 659–666, 2013.

[3] L. M. Richards, S. M. Shams Kazmi, J. L. Davis, K. E. Olin, and A. K. Dunn, "Low-cost laser speckle contrast imaging of blood flow using a webcam," *Biomed. Opt. Express*, vol. 4, pp. 2269–2283, 2013.

[4] J.D. Briers and S. Webster, "Laser speckle contrast analysis (LASCA): a nonscanning, full-field technique," *Biomed. Opt.*, vol. 1, pp. 174–179, 1996.

[5] D.D. Duncan, S.J. Kirkpatrick, and J.C. Gladish, "What is the proper statistical model for laser speckle?," *Proc. SPIE*, vol. 6855, pp. 685502–1–685502–7, 2008.

[6] J.C. Ramirez-San-Juan, R. Ramos-Garcia, I. GuizarIturbide, G. Martinez-Niconoff, and B. Choi, "Impact of velocity distribution assumption on simplified laser speckle imaging equation," *Opt. Express*, vol. 16, pp. 3197–3203, 2008.

[7] A.B. Parthasarathy, W.J. Tom, A. Gopal, X.J. Zhang, and A.K. Dunn, "Robust flow measurement with multi-exposure speckle imaging," *Opt. Express*, vol. 16, pp. 1975–1989, 2008.

[8] D.A. Boas and A.K. Dunn, "Laser speckle contrast imaging in biomedical optics," *J. Biomed. Opt.*, vol. 15, pp. 011109–1–011109–12, 2010.

[9] P. Zakharov, A.C. Völker, M.T. Wyss, F. Haiss, N. Calcinaghi, C. Zunzunegui, Buck A., F. Scheffold, and B. Weber, "Dynamic laser speckle imaging of cerebral blood flow," *Opt. Express*, vol. 17, pp. 13904–13917, 2009.

[10] L. Li, S. Mac-Mary, J.M. Sainthillier, S. Nouveau, O. de Lacharriere, and P. Humbert, "Age-related changes of the cutaneous microcirculation in vivo," *Gerontology*, vol. 52, pp. 142–153, 2006.

[11] A. Khalil, A. Humeau-Heurtier, P. Abraham, and G. Mahé, "Comparative study to analyze the effect of aging on microvascular blood flow by processing laser speckle contrast images when Lorentzian and Gaussian velocity profiles are assumed for moving scatterers," *Accepted to the 4th International Conference on Image Processing Theory, Tools and Applications (IPTA), Paris, 2014*.

[12] G. Mahé, S. Durand, A. Humeau, G. Leftheriotis, P. Rousseau, and P. Abraham, "Air movements interfere with laser speckle contrast imaging recordings," *Lasers Med. Sci.*, vol. 27, pp. 1073–1076, 2011.

[13] P. Abraham, M. Bourgeau, M. Camo, A. Humeau-Heurtier, P. Rousseau, S. Durand, and G. Mahe, "Effect of skin temperature on skin endothelial function assessment," *Microvasc. Res.*, vol. 88, pp. 56–60, 2013.

[14] G. Mahé, F. Haj-Yassin, P. Rousseau, A. Humeau, S. Durand, G. Leftheriotis, and P. Abraham, "Distance between laser head and skin does not influence skin blood flow values recorded by laser speckle imaging," *Microvasc. Res.*, vol. 82, pp. 439–442, 2011.

[15] G.B. Tee, A.H. Rasool, A.S. Halim, and A.R. Rahman, "Dependence of human forearm skin post occlusive reactive hyperemia on occlusion time," *Pharmacol. Toxicol. Methods*, vol. 50, pp. 73–78, 2004.

[16] A. Humeau-Heurtier, G. Mahe, S. Durand, D. Henrion, and P. Abraham, "Laser speckle contrast imaging: Multifractal analysis of data recorded in healthy subjects," *Med. Phys.*, vol. 39, pp. 5849–5856, 2012.

[17] R. Ogrin, P. Darzins, and Z. Khalil, "Age-related changes in microvascular blood flow and transcutaneous oxygen tension under basal and stimulated conditions," *J. Gerontol. Ser. A-Biol. Sci. Med. Sci.*, vol. 60, pp. 200–206, 2005.

[18] G.A. Tew, M. Klonizakis, and J.M. Saxton, "Effects of ageing and fitness on skin-microvessel vasodilator function in humans," *Eur. J. Appl. Physiol.*, vol. 109, pp. 173–181, 2010.

Table 1. Mean velocity results (μm/s) of moving scatterers computed from LSCI data over different ROI sizes and for $\boldsymbol{\rho = 1}$ (see text). Three physiological states are analyzed: 1 min at rest, 1 min during biological zero and 3 s during a reactive hypereamia peak. The results are obtained from two groups (young and elderly) of seven subjects each, when a Lorentzian velocity profile is assumed. *means statistically significant with results obtained from the elderly group for the same ROI size (see text for details).

ROI (pixels^2)	1×1		3×3		5×5		7×7	
group	young	elderly	young	elderly	young	elderly	young	elderly
Rest	43.7	47.8	39.0	42.4	38.7	42.0	37.9	42.0
Biological zero	20.0	20.7	14.1	15.7	14.0	15.5	14.8	15.5
Hyperaemia	89.6*	129.0	77.1*	109.0	76.7*	107.0	76.4*	107.0

Table 2. Same as Table 1, but for $\boldsymbol{\rho = 0.9}$ (see text for details).

ROI (pixels^2)	1×1		3×3		5×5		7×7	
group	young	elderly	young	elderly	young	elderly	young	elderly
Rest	51.8	57.0	45.7	49.8	45.3	49.3	44.4	49.3
Biological zero	23.1	23.9	16.0	17.9	15.8	17.6	16.8	17.6
Hyperaemia	110.6*	167.3	93.5*	134.8	92.8*	133.2	92.5*	132.4

Table 3. Same as Table 1, but for $\boldsymbol{\rho = 0.8}$ (see text for details).

ROI (pixels^2)	1×1		3×3		5×5		7×7	
group	young	elderly	young	elderly	young	elderly	young	elderly
Rest	65.1	72.6	55.5	60.7	53.7	60.1	53.7	59.9
Biological zero	27.4	28.4	18.6	20.7	18.4	20.5	19.4	20.4
Hyperaemia	156.1*	281.3	122.1*	185.9	120.8*	182.4	120.2*	181.1

Table 4. Same as Table 1, but for $\boldsymbol{\rho = 0.7}$ (see text for details).

ROI (pixels^2)	1×1		3×3		5×5		7×7	
group	young	elderly	young	elderly	young	elderly	young	elderly
Rest	92.3	110.6	71.6	79.0	70.5	77.8	68.8	77.5
Biological zero	34.4	35.6	22.1	24.7	21.8	24.3	23.2	24.3
Hyperaemia	312.9*	942.3	187.7*	344.3	183.5*	323.6	181.7*	317.9