16th Mediterranean Conference on Control and Automation
Congress Centre, Ajaccio, France
June 25-27, 2008

Laser Doppler flowmetry in healthy rats: impact of isoflurane anesthetic on signal complexity

Anne Humeau, Bernard Haussy, Lionel Fizanne, Jérôme Roux, Pierre Asfar, Paul Calès, David Rousseau, and François Chapeau-Blondeau

Abstract—**Laser Doppler flowmetry (LDF) technique is an optical tool used in clinical investigations to monitor microvascular blood flow. Recent preliminary works have shown that LDF signals recorded in young healthy human subjects are weakly multifractal. Such an information is important as it could lead to a better knowledge of the underlying optical processes giving rise to the signals. In the present work, our goal is to analyze the behavior of LDF signals in anesthesia conditions. For this purpose, we herein study the possible modifications brought by isoflurane, an anesthetic commonly used in clinical practice, on the complexity of LDF signals. In order to conduct our work, twenty LDF signals from anesthetized healthy rats are processed. Anesthesia is performed by using doses of isoflurane varying between 1.5% and 3%, which leads to very light and very deep anesthesia, respectively. The signal processing approach is carried out with two different methods, a parametric generalized quadratic variation based estimation method and a Hurst rescaled range analysis. The results show that extreme doses of isoflurane lead to no distinguishable modification on the characterization of LDF signals based on the two above approaches. These findings infer that, if isoflurane changes the microvascular tissue optical properties, these modifications have no influence on LDF signals complexity measured by the two signal processing approaches used herein.**

Manuscript received January 21, 2008.

A. Humeau is with groupe esaip, 18 rue du 8 mai 1945, BP 80022, 49180 Saint Barthélémy d'Anjou cedex, France (phone: +33 (0)2 41 96 65 10; fax: +33 (0)2 41 96 65 11; e-mail: ahumeau@esaip.org) and with Laboratoire d'Ingénierie des Systèmes Automatisés (LISA), Université d'Angers, 62 avenue Notre Dame du Lac, 49000 Angers, France.

B. Haussy is with Groupe ESEO, 4 rue Merlet de la Boulaye, BP 30926, 49009 Angers cedex 01, France.

L. Fizanne and P. Cales are with Laboratoire Hémodynamique et Fibrose Hépatique (HIFIH), UFR Sciences Médicales, Pavillon Ollivier, rue Haute de Reculée, 49045 Angers cedex, France.

J. Roux is with Service commun animalerie hospitalo-universitaire, UFR Sciences Médicales, Pavillon Ollivier, rue Haute de Reculée, 49045 Angers cedex, France.

P. Asfar is with Laboratoire Hémodynamique et Fibrose Hépatique (HIFIH), UFR Sciences Médicales, Pavillon Ollivier, rue Haute de Reculée, 49045 Angers cedex, France and with Service commun animalerie hospitalo-universitaire, UFR Sciences Médicales, Pavillon Ollivier, rue Haute de Reculée, 49045 Angers cedex, France.

D. Rousseau and F. Chapeau-Blondeau are with Laboratoire d'Ingénierie des Systèmes Automatisés (LISA), Université d'Angers, 62 avenue Notre Dame du Lac, 49000 Angers, France.

I. Introduction

LASER Doppler flowmetry (LDF) is an optical method enabling the monitoring of microvascular blood flow, a very important marker of tissue health. The technique relies on the Doppler effect: when coherent light is directed toward a tissue, photons are scattered by moving objects and by static structures. If they encounter moving particles (mainly red blood cells), the Doppler effect appears, modifying the photon frequency. When the reemitted light is directed toward a photodetector, optical mixing of light frequency shifted and non frequency shifted gives rise to a stochastic photocurrent. When the concentration of moving red blood cells is low, the first moment of the photocurrent $\int \omega P(\omega) d\omega$ (where $P(\omega)$ corresponds to the power spectrum of the photocurrent) scales with the concentration of moving blood cells times their average velocity [1]. This signal is called perfusion or, more generally, LDF signal.

Recent preliminary works conducted on LDF data have shown that perfusion signals recorded on young healthy human subjects are weakly multifractal [2], and that aging can lead to a reduced multifractality [3]. Such an information is important as it could lead to a better knowledge of the underlying optical processes giving rise to the signals. The results could also be used to model the peripheral cardiovascular system.

In this paper, our goal is to analyze the behavior of LDF signals in anesthesia conditions, more precisely when anesthesia is induced by isoflurane. Isoflurane is an halogenated volatile anesthetic commonly used in clinical practice and for which pharmacology [4] and mode of action have been reviewed [5]. We herein focus our work on the possible modifications brought by the anesthetic on the *complexity* of LDF signals. More precisely, the question to answer here is: do LDF signals recorded in two different isoflurane-induced anesthesia conditions (light and deep anesthesia) have the same complexity? In order to conduct our study, two different signal processing methods, a parametric generalized quadratic variation (GQV) based estimation method, and a Hurst rescaled range analysis (R/S), are proposed. To our knowledge, these two methods have never been applied on LDF signals recorded during isoflurane-induced anesthesia. The influence of the

978-1-4244-2505-1/08/$20.00 ©2008 IEEE

depth of anesthesia is studied by using doses of isoflurane varying between 1.5% and 3% (very light and very deep anesthesia, respectively). As in other applications [6-8], rats are used in our work because experiments in rats are generally easier to conduct than in humans. Moreover, the *in vivo* circulatory effects of isoflurane in rats are well characterized [9-12] and are similar to those in humans [13-15].

The present paper is organized as follows: the measurement procedure used to record the LDF signals in rats is detailed in the following section. Then, the two processing methods proposed herein are introduced. Afterwards, the results are presented and discussed. Finally, we end the paper with a conclusion.

II. Measurement Procedure

Twenty Sprague Dawley rats were analyzed. Procedures for the maintenance and use of the experimental animals were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1996). Isoflurane was administered to split the group of rats into two. The first group (10 rats) corresponds to rats with a light anesthesia: dose of isoflurane between 1.5% and 1.9%. The second group (10 rats) corresponds to rats with a dose of isoflurane between 2.4% and 3% (deep anesthesia) [16], [17]. For the blood perfusion signal acquisition, the LDF probe was connected to a laser Doppler flowmeter (PF5000 Master, Periflux, Perimed, Sweden) and positioned on the thigh of the rat placed in the prone position. The wavelength of the laser Doppler flowmeter was of 780 nm and the signals were recorded for 16 min 40 s with a frequency sampling of 32 Hz. LDF signals were assessed in arbitrary units (a.u.) because the LDF technique can yet not measure absolute perfusion. Signals recorded during light and deep anesthesia are shown in Figs. 1 and 2, respectively.

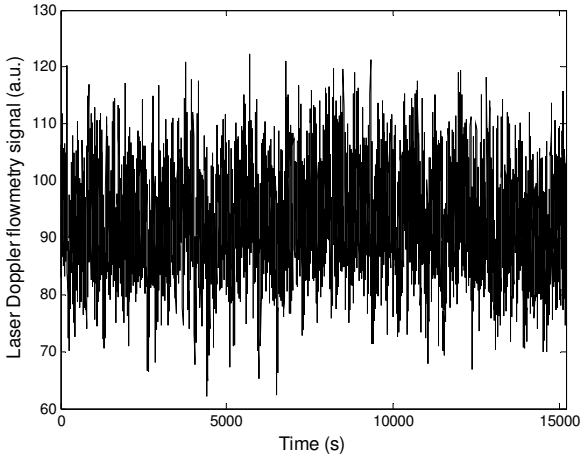

Fig. 1. Skin laser Doppler flowmetry signal recorded on a healthy rat, during light anesthesia.

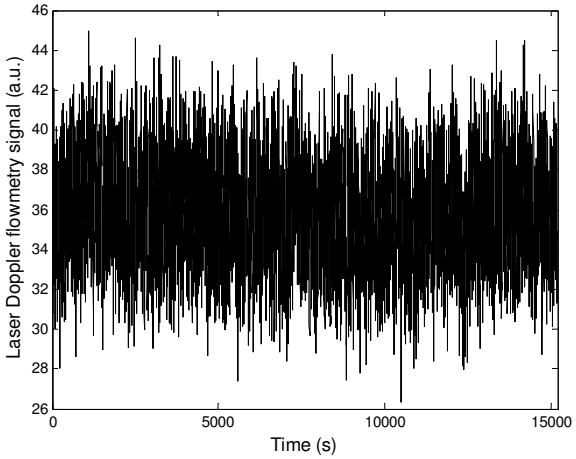

Fig. 2. Skin laser Doppler flowmetry signal recorded on a healthy rat, during deep anesthesia.

III. Processing Methods

Two signal processing methods are proposed herein to characterize the complexity of LDF signals during light and deep anesthesia in rats. The first one corresponds to a parametric GQV based estimation method that leads to the computation of Hölder exponents. The second one refers to a R/S method that leads to the computation of Hurst exponents.

A. Hölder exponents and parametric generalized quadratic variation based estimation method

The rapid changes in a time series are called singularities and a characterization of their strength is obtained with the Hölder exponents [18]. The Hölder exponent $h(x_0)$ of a function f at the point x_0 is the highest h value so that f is Lipschitz at x_0. There exists a constant C and a polynomial $P_n(x)$ of order n so that for all x in a neighborhood of x_0 we have [19-21]

$$\left|f(x)-P_n(x-x_0)\right| \le C\left|x-x_0\right|^h . \quad (1)$$

Moreover, the Hausdorff dimension of the set where the Hölder exponent is equal to h is [19]

$$D(h)=\dim_H\left\{x \middle| h(x)=h\right\} . \quad (2)$$

Monofractal signals are homogeneous: they have the same scaling properties throughout the entire signal; therefore, they are indexed by a single global exponent, called Hurst exponent, which suggests that they are stationary from viewpoint of their local scaling properties [22]. However, multifractal signals can be decomposed into many subsets characterized by different local Hurst exponents that quantify the local singular behavior and relate the local scaling of the time series [22]. Therefore, signals are considered as

multifractal when a "broad" range of Hölder exponents is found, whereas a "narrow" range implies monofractality. Multifractal signals are more complex and inhomogeneous than monofractal ones [2], [23].

In our work, each one of the twenty LDF signals is first processed with a parametric GQV based estimation method (method described in Refs. [2], [24], [25]) to obtain its Hölder exponents. For that purpose, we use the FracLab v2.0 tool [26], and we take into account 15200 pointwise Hölder exponents for each time series.

B. R/S analysis

The second method used herein is the R/S analysis. The latter method was introduced by Hurst and applied to the fractal analysis by Mandelbrot. It is a well known way to compute Hurst exponents [27]. The main steps of the algorithm for a time series x_i, $1 \le i \le n \le N$ are (N is the number of samples in the signal)

- Computation of the mean of n terms

$$\langle x \rangle_n = \frac{1}{n} \sum_{i=1}^{n} x_i \,. \tag{3}$$

- Calculation of the sum of the differences between one term and the mean

$$X(i,n) = \sum_{u=1}^{i} \left[x_u - \langle x \rangle_n \right]. \tag{4}$$

- Evaluation of the range of those differences

$$R(n) = \max_{1 \le i \le n} X(i,n) - \min_{1 \le i \le n} X(i,n). \tag{5}$$

- Computation of the standard deviation for n terms

$$S(n) = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(x_i - \langle x_n \rangle \right)^2} \,. \tag{6}$$

- Introduction of Hurst exponent (H)

$$R(n) / S(n) \approx (n/2)^H \,. \tag{7}$$

The value of the Hurst exponent is obtained with a least square linear fit.

When applying this method on the LDF signals, we can see that they can be cut into sets of 500 or 1000 points without corrupting them; a chi square test shows that, considering sets of 500 or 1000 points, the Gaussian character of the repartition of their values is preserved. Herein, the evaluation of Hurst exponents is therefore made considering sets of 500 points from 15200 points of each time series.

IV. Results and Discussion

The results obtained with the parametric GQV based estimation method for the two states of anesthesia are presented in Table I, and illustrated in Figs. 3 and 4. Table I is computed as follows: for each of the twenty time series, we determine for the Hölder exponents, the minimum, maximum, range, mean, and standard deviation values. Then, for each group of rats, an average for the ten minimum values obtained is computed; idem for the maximum, range, mean, and standard deviation (see Table I). In order to compare the results with known multifractal data, we generate a multifractional Brownian motion (multifractal signal) [28] and compute 15200 Hölder exponents from the latter signal (see Table I).

From Table I, we can see that the minima, maxima, ranges, means, and standard deviations are very similar (or even equal) for light and deep anesthesia. The complexity of the signals from the two groups of rats can therefore be considered as equivalent. This also leads us to the proposition that a full range of use for isoflurane (a dose of isoflurane lower than 1.5% is not enough to anesthetize the rats, and a dose higher than 3% is too much to keep the rats alive) leads to the same characteristics in terms of complexity for LDF signals. Moreover, we can see that Hölder exponents ranges for light and deep anesthesia are much lower than the Hölder exponents range for the mBm

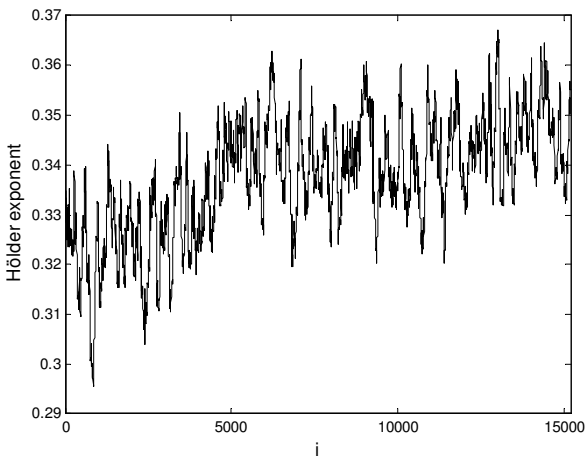

Fig. 3. Hölder exponents from the signal presented in Fig. 1.

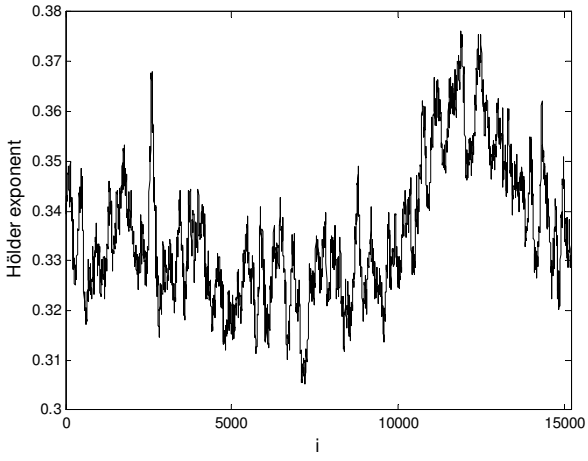

Fig. 4. Hölder exponents from the signal presented in Fig. 2.

TABLE I
VALUE FOR THE MINIMUM, MAXIMUM, RANGE, MEAN, AND STANDARD DEVIATION OF THE HÖLDER EXPONENTS COMPUTED FOR SKIN LASER DOPPLER FLOWMETRY (LDF) SIGNALS RECORDED ON LIGHT AND DEEP ISOFLURANE-INDUCED ANESTHETIZED RATS, AND FOR A MULTIFRACTAL SIGNAL (mBm).

LDF signals	Minimum value	Maximum value	Range	Mean value	Standard deviation
Light anesthesia	0.29	0.38	0.09	0.33	0.01
Deep anesthesia	0.30	0.38	0.08	0.34	0.01
mBm	0.29	0.71	0.42	0.51	0.13

TABLE II
VALUE FOR THE MEAN AND STANDARD DEVIATION OF THE HURST EXPONENTS COMPUTED FOR SKIN LASER DOPPLER FLOWMETRY (LDF) SIGNALS RECORDED ON LIGHT AND DEEP ISOFLURANE-INDUCED ANESTHETIZED RATS.

LDF signals	Mean value	Standard deviation
Light anesthesia	0.76	0.04
Deep anesthesia	0.73	0.04

signal (see Table I). This means that LDF signals recorded on isoflurane-induced anesthetized healthy rats (light and deep anesthesia) do not show a degree of complexity similar to that of a multifractional Brownian motion.

The results given by the R/S method are shown in Table II, and illustrated in Figs. 5 and 6. For each group of rats, the average of the average of the Hurst exponent and the average of the standard deviation of the Hurst exponent are computed. The Hurst exponent, in both cases, presents very small variations, pointed out by the small value of the standard deviation (see Table II). One of the main characteristics of the multifractal signals is that they can be decomposed into subsets with different local Hurst exponents, which is not the case here. Therefore, the LDF signals processed in our work are homogeneous with respect to the Hurst exponent. Furthermore, the Hurst exponents

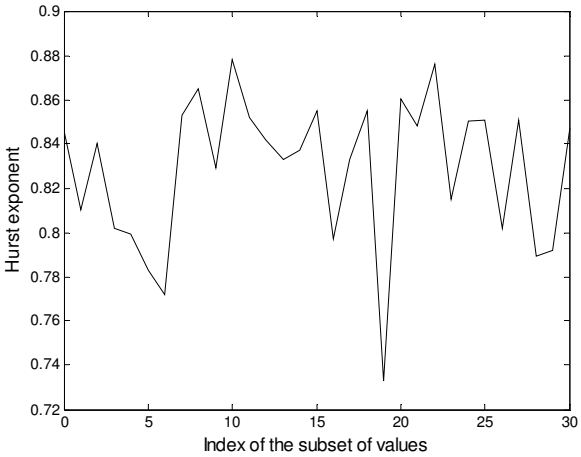

Fig. 5. Variations of Hurst exponent considering sets of 500 points from the signal presented in Fig. 1.

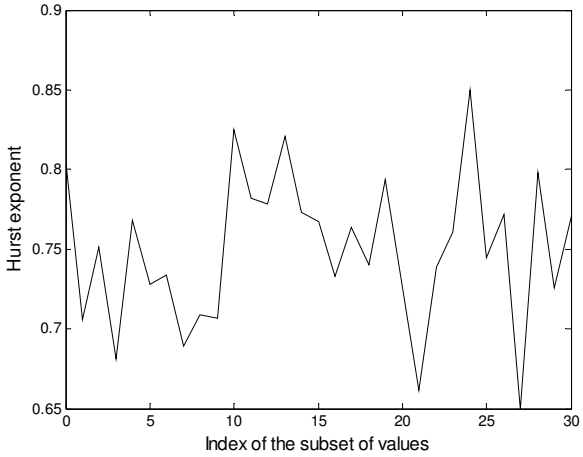

Fig. 6. Variations of Hurst exponent considering sets of 500 points from the signal presented in Fig. 2.

values being higher than 0.5, the fluctuations in the LDF dynamics exhibit correlated behavior.

The conclusions obtained with the R/S method are in accordance with the ones given by the parametric GQV based estimation method: low or high doses do not lead to significant discernible change in LDF signals complexity. Moreover another complexity study, conducted by our group in isoflurane-induced anesthetized healthy rats, has shown that multifractal spectra (which correspond to another signal processing approach to analyze signal complexity) lead to the same findings as those obtained in the present paper [29].

V. CONCLUSION

LDF technique is an optical tool able to bring information on microvascular blood flow and optical properties of tissues. Complexity of LDF signals in healthy rats is herein examined during isoflurane-induced anesthesia. Two different signal processing approaches are proposed. The results obtained with the two methods show that extreme doses of isoflurane (between 1.5% and 3%) do not lead to distinguishable modification on complexity of LDF signals. These findings infer that, if isoflurane changes the microvascular tissues optical properties (this question is currently being analysed in our group), these modifications have no influence on LDF signals complexity measured by the two signal processing approaches used herein. Further work is now needed in order to analyze if pathological conditions modify these results. Moreover, because the *in vivo* circulatory effects of isoflurane in rats are similar to those in humans, we can hypothesize that our results could be transposable to healthy human signals.

REFERENCES

[1] A. Humeau, W. Steenbergen, H. Nilsson, and T. Strömberg, "Laser Doppler perfusion monitoring and imaging: novel approaches," *Med. Biol. Eng. Comput.*, vol. 45, pp. 421—435, 2007.

[2] A. Humeau, F. Chapeau-Blondeau, D. Rousseau, M. Tartas, B. Fromy, and P. Abraham, "Multifractality in the peripheral cardiovascular system from pointwise Hölder exponents of laser Doppler flowmetry signals," *Biophys. J.*, vol. 93, pp. L59—L61, 2007.

[3] A. Humeau, F. Chapeau-Blondeau, D. Rousseau, W. Trzepizur, and P. Abraham, "Multifractality, sample entropy, and wavelet analyses for age-related changes in the peripheral cardiovascular system: preliminary results," *Med. Phys*., vol. 35, pp. 717—723, 2008.

[4] E. I. Eger, "Pharmacology of isoflurane," *Br. J. Anaesth.*, vol. 56, pp. 71S—99S,1984.

[5] G. Pocock and C. D. Richards, "Excitatory and inhibitory synaptic mechanisms in anaesthesia," *Br. J. Anaesth.*, vol. 71, pp. 134—147, 1993.

[6] H. F. Zhang, K. Maslov, M. Sivaramakrishnan, G. Stoica, and L. V. Wang, "Imaging of hemoglobin oxygen saturation variations in single vessels *in vivo* using photoacoustic microscopy," *Appl. Phys. Lett.,* vol. 90, 053901, 2007.

[7] C. P. Cheney, A. Wig, R. H. Farahi, A. Gehl, D. L. Hedden, T. L. Ferrell, D. Ji, R. Bell, W. J. McBride, and S. O'Connor, "*In vivo* real-time ethanol vapor detection in the interstitial fluid of a Wistar rat using piezoresistive microcantilevers," *Appl. Phys. Lett.*, vol. 90, 013901, 2007.

[8] S. M. Kim, F. Hatami, J. S. Harris, A. W. Kurian, J. Ford, D. King, G. Scalari, M. Giovannini, N. Hoyler, J. Faist, G. Harris, "Biomedical terahertz imaging with a quantum cascade laser," *Appl. Phys. Lett.*, vol. 88, 153903, 2006.

[9] N. Kokita, T. Stekiel, M. Yamazaki, Z. J. Bosnjak, J. P. Kampine, and W. J. Stekiel, "Potassium channel-mediated hyperpolarization of mesenteric vascular smooth muscle by isoflurane," *Anesthesiology,* vol. 90, pp. 779—788, 1999.

[10] M. Yamazaki, T. A. Stekiel, Z. J. Bosnjak, J. P. Kampine, and W. J. Stekiel, "Effects of volatile anesthetic agents on in situ vascular smooth muscle transmembrane potential in resistance- and capacitance-regulating blood vessels," *Anesthesiology*, vol. 88, pp. 1085—1095, 1998.

[11] P. F. Conzen, B. Vollmar, H. Habazettl, E. J. Frink, K. Peter, and K. Messmer, "Systemic and regional hemodynamics of isoflurane and sevoflurane in rats," *Anesth. Analg.*, vol. 74, pp. 79—88, 1992.

[12] M. W. Crawford, J. Lerman, M. Pilano, H. Orrego, V. Saldivia, and F. J. Carmichael, "Haemodynamic and organ blood flow responses to sevoflurane during spontaneous ventilation in the rat: a dose-response study," *Can. J. Anaesth.*, vol. 39, pp. 270—276, 1992.

[13] T. Kazama and K. Ikeda, "The comparative cardiovascular effects of sevoflurane with halothane and isoflurane," *J. Anesthes.*, vol. 2, pp. 3—68, 1988.

[14] D. A. Holaday and F. R. Smith, "Clinical characteristics and biotransformation of sevoflurane in healthy human volunteers," *Anesthesiology*, vol. 54, pp. 100—106, 1981.

[15] W. C. Stevens, T. H. Cromwell, M. J. Halsey, E. I. Eger, T. F. Shakespeare, and S. H. Bahlman, "The cardiovascular effects of a new inhalation anesthetic, forane, in human volunteers at constant arterial carbon dioxide tension," *Anesthesiology*, vol. 35, pp. 8—16, 1971.

[16] L. Fizanne, B. Fromy, M. P. Preckel, D. Sigaudo-Roussel, and J. L. Saumet, "Effect of isoflurane on skin-pressure-induced vasodilation," *J. Vasc. Res.*, vol. 40, pp. 416—422, 2003.

[17] A. Humeau, L. Fizanne, J. Roux, P. Asfar, P. Cales, D. Rousseau, and F. Chapeau-Blondeau, "Laser Doppler flowmetry signals to quantify effects of isoflurane on the peripheral cardiovascular system of healthy rats," *Appl. Phys. Lett.*, vol. 91, 263901, 2007.

[18] Z. R. Struzik, "Determining local singularity strengths and their spectra with the wavelet transform," *Fractals*, vol. 8, pp. 163—179, 2000.

[19] J. F. Muzy, E. Bacry, and A. Arneodo, "Multifractal formalism for fractal signals: the structure-function approach versus the wavelet-transform modulus-maxima method," *Phys. Rev. E*, vol. 47, pp. 875—884, 1993.

[20] J. F. Muzy, E. Bacry, and A. Arneodo, "The multifractal formalism revisited with wavelets," *Int. J. Bifurcation Chaos Appl. Sci. Eng.*, vol. 4, pp. 245—302, 1994.

[21] N. Scafetta, L. Griffin, and B. J. West, "Hölder exponent spectra for human gait," *Physica A*, vol. 328, pp. 561—583, 2003.

[22] P. Ch. Ivanov, L. A. N. Amaral, A. L. Goldberger, S. Havlin, M. G. Rosenblum, Z. R. Struzik, and H. E. Stanley, "Multifractality in human heartbeat dynamics," *Nature*, vol. 399, pp. 461—465, 1999.

[23] J. L. Del Rio-Correa and A. Munoz-Diosdado, "Further study of the asymmetry for multifractal spectra of heartbeat time series," *Conf. Proc. IEEE Eng. Med. Biol. Soc.,* vol. 1, pp. 1450—1453, 2006.

[24] J. Istas and G. Lang, "Quadratic variations and estimation of the local Hölder index of a Gaussian process," *Ann. Inst. Henri Poincaré*, vol. 33, pp. 407—436, 1997.

[25] A. Ayache and J. LevyVehel, "On the identification of the pointwise Hölder exponent of the generalized multifractional Brownian motion," *Stochastic Processes and their Applications*, vol. 111, pp. 119—156, 2004.

[26] J. Lévy Véhel and P. Legrand, "Signal and Image processing with FracLab", in *FRACTAL04, Complexity and Fractals in Nature, 8th International Multidisciplinary Conference,* Vancouver, Canada, April 4-7, 2004.

[27] A. Muñoz-Diosdado, "A non linear analysis of human gait time series based on multifractal analysis and cross correlations," *J. Phys.: Conference Series*, vol. 23, pp. 87—95, 2005.

[28] R. F. Peltier and J. Lévy Véhel, "Multifractional Brownian motion: definition and preliminary results," INRIA research report n°2645, Le Chesnay (France), 1995.

[29] A. Humeau, L. Fizanne, J. Roux, P. Asfar, P. Cales, D. Rousseau, F. Chapeau-Blondeau, "Linear and nonlinear analyses of laser Doppler flowmetry signals recorded during isoflurane-induced anesthesia in healthy rats," submitted.